



EPA Document No.
822D21001

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EXTERNAL PEER REVIEW DRAFT
Proposed Approaches to the Derivation of a
Draft Maximum Contaminant Level Goal for
Perfluorooctanoic Acid (PFOA)
(CASRN 335-67-1) in Drinking Water

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(CASRN 335-67-1) in Drinking Water

Prepared by:

U.S. Environmental Protection Agency
Office of Water (4304T)
Health and Ecological Criteria Division
Washington, DC 20460

EPA Document Number: EPA 822D21001

NOVEMBER 2021

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Acknowledgments

This document was prepared by the Health and Ecological Criteria Division, Office of Science and Technology, Office of Water (OW) of the U.S. Environmental Protection Agency (EPA). The agency gratefully acknowledges the valuable contributions of EPA scientists from the OW, Office of Research and Development (ORD), the Office of Children’s Health Protection (OCHP), and the Office of Land and Emergency Management (OLEM). OW authors for the document include Brittany Jacobs, PhD; Casey Lindberg, PhD; Kelly Cunningham, MS; Barbara Soares, PhD; Greg Miller, MS; Ruth Etzel, MD, PhD; Colleen Flaherty, MS. ORD authors of the document include J. Michael Wright, ScD; Elizabeth Radke, PhD; Michael Dzierlenga, PhD, Todd Zurlinden, PhD; Jacqueline Weinberger; Thomas Bateson, ScD; Hongyu Ru, PhD; Kelly Garcia, MPH. An OCHP author for the document includes Chris Brinkerhoff, PhD. EPA scientists who provided valuable contributions to the development of this document from OW include Adrienne Keel, MS; Joyce Donohue, PhD; Amanda Jarvis, MS; James R. Justice, MS; from ORD include Timothy Buckley, PhD; Peter Egeghy, PhD; Elaine Cohen Hubal, PhD; Paul Schlosser, PhD; from OLEM includes Stiven Foster, MS. The agency gratefully acknowledges the valuable executive direction provided by Elizabeth (Betsy) Behl, PhD (OW); Susan Euling, PhD (OW); Kristina Thayer, PhD (ORD); Viktor Morozov, PhD (ORD).

The systematic review work included in this assessment was prepared in collaboration with ICF under the U.S. EPA Contracts EP-C-16-011 (Work Assignment Nos. 4-16 and 5-16) and PR-OW-21-00612 (TO-0060). ICF and subcontractor authors of the assessment include Samantha Snow, PhD; Sorina Eftim, PhD; Wren Tracy, MHS; Ryan Cronk, PhD; Kezia Addo, PhD; Barrett Allen, BS; Carlye Austin, PhD; Robyn Blain, PhD; Meredith Clemons, MPH; Hannah Eglinton, BA; Rebecca Gray, MPH; Joanna Greig, PhD; Jessica Jimenez, BA; Madison Lee, MPH; Cynthia Lin, PhD; Alex Lindahl, MPH; Melissa Miller, MPH; Rachel O’Neal, MSPH; Ashley Pepperiell, PhD; Mia Peng, MPH; Lisa Prince, PhD; Courtney Rosenthal, MS; Amanda Ross, MEd; Karen Setty, PhD; Raquel Silva, PhD; Joanne Trgovcich, PhD; Janielle Vidal, BA; Pradeep Rajan, PhD (subcontractor).

ICF contributors to this assessment include Lauren Browning, MS; Caelen Caspers, BS; Laura Charney, MPH; Kathleen Clark, BA; Sarah Colley, MSPH; Grace Cooney, BA; Katie Duke, PhD; Lauren Fitzharris, MPH; Caroline Foster, MS; Jeremy Frye, MSLS; Anthony Hannani, MPH; Pam Hartman, MEH; Cara Henning, PhD; Audrey Ichida, PhD; Caroline Jasperse, MPS; Kaedra Jones, MPH; Michele Justice, MA; Denyse Marquez Sanchez, BA; Alicia Murphy, BS; Kimberly Osborn, BS; Lucas Rocha Melogno, PhD; Johanna Rochester, PhD; Alessandria Schumacher, BA; Jennifer Seed, PhD; Sheerin Shirajin, BA; Connie Xiong, MEM; Maricruz Zarco, MPH.

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Acronyms and Abbreviations

17-OHP	17-hydroxyprogesterone	AUC _{avg,pup,gest,lact}	AUC normalized per day during gestation/lactation
ABCG2	ATP-binding cassette transporter G2	AUC _{avg,pup,lact}	AUC normalized per day during lactation
aBMD	aerial bone mineral density	AUC _{avg,pup,total}	AUC normalized per day over entire study
ACD	anterior chamber death	BBB	blood brain barrier
ACTH	adrenocorticotrophic hormone	BCRP	breast cancer resistance protein
ADHD	attention deficit hyperactivity disorder	BDI	Beck Depression Inventory
ADME	absorption, distribution, metabolism, excretion	BDI-II	Beck Depression Inventory-II
AFFF	aqueous film forming foam	BMC	bone mineral content
AGD	anogenital distance	BMD	benchmark dose
AIC	Akaike information criterion	BMD ₁₀	dose corresponding to a 10% change in response
ALP	alkaline phosphatase	BMDL	benchmark dose lower limit
ALSPAC	Avon Longitudinal Study of Parents and Children	BMDL ₁₀	dose level corresponding to the 95% lower confidence limit of a 10% change
ALT	alanine aminotransferase	BMDS	Benchmark Dose Software
AMH	anti-Müllerian hormone	BMI	body mass index
APFO	ammonium perfluorooctanoate	BMR	benchmark response
apoB	apolipoprotein B	BRIEF	Behavior Rating Inventory of Executive Function
ApoC-III	apolipoprotein C-III	BSID-II	Bayley Scales of Infant Development
aPPT	activated partial thromboplastin time	BUN	blood urea nitrogen
ASD	autism spectrum disorder	BWT	birth weight
ASQ	Ages and Stages Questionnaire	C _{7,avg}	average concentration over final week of study
AST	aspartate aminotransferase	CAD	coronary artery disease
	ATSDR Agency for Toxic Substances and Disease Registry	CalEPA	California EPA
AUC	area under the curve	CAR	constitutive androstane receptor
AUC _{avg,dam,gest}	AUC normalized per day during gestation		
AUC _{avg,pup,gest}	AUC normalized per day during gestation		

CAS	Chemical abstracts service	C _{max}	maximum blood concentration
CASRN	Chemical Abstracts Service Registry Number	C _{max,dam}	maximum maternal concentration during gestation
C _{avg}	average blood concentration	C _{max,pup,gst}	maximum fetal concentration during gestation
C _{avg_pup_gst}	C _{avg} calculated during gestation	C _{max,pup,lact}	maximum fetal concentration during lactation
C _{avg_pup_lact}	C _{avg} calculated during lactation		
C _{avg_pup_gst_lact}	C _{avg} calculated during gestation and lactation	CNS	central nervous system
CBCL	Child Behavior Checklist	COPD	chronic obstructive pulmonary disease
CCL	Contaminant Candidate List	CSF	cancer slope factor
CCPT-II	Connors' Continuous Performance Test-II	CSM	cholestyramine
CDC	Centers for Disease Control and Prevention	CVD	cardiovascular disease
cDNA	complementary DNA	DBP	diastolic blood pressure
C-F	Carbon-fluorine	DDE	dichlorodiphenyl dichloroethane
CH	congenital hypothyroidism	DFI	DNA fragmentation index
CHARGE	Childhood Autism Risk from Genetics and Environment	DHEA	dehydroepiandrosterone
CHD	coronary heart disease	DHEAS	dehydroepiandrosterone sulfate
CHECK	Children's Health and Environmental Chemicals in Korea	DNA	deoxyribonucleic acid
CHEF	Children's Health and the Environment in the Faroes	DNBC	Danish National Birth Cohort
CHF	congestive heart failure	DPP	Diabetes Prevention Program
CHO	Chinese hamster ovary	DPPOS	Diabetes Prevention Program and Outcomes Study
CI	Confidence interval	DWI	drinking water intake
CIMT	carotid intima-media thickness test	E2	Estradiol
CKD	Chronic kidney disease	EFSA	European Food Safety Authority
CL _R	renal clearance	eGFR	estimated glomerular filtration rate
Cl _{total}	renal clearance for men and women older than 50	ENT1	equilibrative nucleoside transporter
		EPA	Environmental Protection Agency
		ER	estrogen receptor

F ₁	first generation	HFPO	hexafluoropropylene oxide
F ₂	second generation		
FCC	Fernald Community Cohort	Hib	<i>Haemophilus influenza</i> type b
FDA	Food and Drug Administration	HOMA-B	Homeostatic Model Assessment of Beta-Cell Function
FEV ₁	forced expiratory volume in one second	HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
FR _α	folate receptor alpha		
FSH	follicle stimulating hormone	HOME	Health Outcomes and Measures of the Environment
FT3	free triiodothyronine		
FT4	free thyroxine	HPA	hypothalamic-pituitary-adrenal
FTI	free thyroxine index		
FTOH	fluorotelomer alcohol	HPT	hypothalamic-pituitary-thyroid
FVC	forced vital capacity		
FXR	Farnesoid X receptor	HR	hazard ratio
GBCA	Genetic and Biomarker study for Childhood Asthma	HRL	health risk limit
		HSA	human serum albumin
GD	gestation day	HUMIS	Norwegian Human Milk Study
GF	glomerular function		
GFR	glomerular filtration rate	IARC	International Agency for Research on Cancer
GGT	γ-glutamyltransferase		
GI	gastrointestinal	IBD	inflammatory bowel disease
GM	geometric mean	ID	intellectual disability
GSD	geometric standard deviation	IDL	intermediate-density lipoprotein
HAWC	Health Assessment Workplace Collaborative	IGF-1	insulin-like growth factor 1
HbA1c	hemoglobin A1c	IgE	immunoglobulin E
HDL	high-density-lipoprotein cholesterol	IgM	immunoglobulin M
		IHD	ischemic heart diseases
HED	human equivalent dose	INMA	Spanish Environment and Childhood (Infancia y Medio Ambiente)
HEK-293	human embryonic kidney		
HERO	Health and Environmental Research Online	INUENDO	Biopersistent Organochlorines in Diet and Human Fertility cohort
HESDs	Health Effects Support Documents		
HFMD	hand, foot, and mouth disease	IPCS	International Programme on Chemical Safety
		IQ	intelligence quotient

IQR	interquartile range	MCDI	MacArthur
IRIS	Integrated Risk Information System		Communicative Development Inventories for Infants
IUFD	intrauterine fetal death		
IV	intravenous	MCLG	Maximum Contaminant Level Goal
IVD	in vitro digestion method		
K _{oc}	organic carbon-water partitioning coefficient	MDI	Mental Development Index
K _{ow}	octanol-water partition coefficient	MDR1	p-glycoprotein
k ₁₂	intercompartment transfer rate	Me-PFOA-AcOH or MeFOSAA	2-(N-Methyl-perfluorooctane sulfonamido) acetic acid
k _a	absorption rate		
K _d	disassociation constant	MIREC	Maternal-Infant Research on Environmental Chemicals
K _i	metabolic inhibition constant		
K _m	Michaelis constant	MMR	measles, mumps, and rubella
K _{mem/w}	membrane/water partition coefficients	MOA	mode of action
LBW	low birth weight	MoBa	Norwegian Mother, Father, and Child Cohort Study
LCT	Leydig cell tumors		
LD	Lactation day	MPAH	2-(N-methyl-PFOA) acetate
LDL	low-density lipoprotein		
L-FABP	liver fatty acid binding protein	m-PFOA	branched isomers of PFOA
LH	luteinizing hormone	MRL	minimum reporting level
LHWA	Little Hocking Water Association	mRNA	messenger ribonucleic acid
LIFE	Longitudinal Investigation of Fertility and the Environment	MRP2	multi-drug resistance-associated protein 2
LINC	Linking Maternal Nutrition to Child Health	MRPs	multidrug resistance-associated proteins
LOAEL	lowest-observed-adverse-effect level	MS	multiple sclerosis
LOD	limit of detection	NCCA	National Coastal Condition Assessment
LOQ	limit of quantification	NCI	National Cancer Institute
L-PFOA	linear isomers of PFOA	NHANES	National Health and Nutrition Examination Survey
LTRI	lower respiratory tract infection	NMR	nuclear magnetic resonance
M/P	milk/plasma	NOAEL	no-observed-adverse-effect level

NPDWR	National Primary Drinking Water Regulation	PFBS PFCAs	perfluorobutane sulfonate perfluoroalkyl carboxylic acids
NRSA	National Rivers and Streams Assessment	PFDA	perfluorodecanoic acid
NTCP	sodium-taurocholate cotransporting polypeptide	PFDoDA PFHpA	perfluorododecanoic acid perfluoroheptanoic acid
NTP	National Toxicology Program	PFHxA PFHxS	perfluorohexanoic acid perfluorohexanesulfonate
OATP1a1	organic anion transporters polypeptide 1a1	PFNA PFOA	perfluorononanoic acid perfluorooctanoic acid
OATPs	organic anion transporting polypeptides	PFOS	perfluorooctane sulfonic acid
OATs	organic anion transporters	PFUnDA	perfluoroundecanoic acid
OECD	Organisation for Economic Co-operation and Development	P _{ion}	passive anionic permeability
OR	ddds Ratio	PK	pharmacokinetic
ORD	Office of Research and Development	pKa	negative base-10 logarithm of acid dissociation constant (K _a)
OSS	Oslo Severity Score	PLCO	Prostate, Lung, Colorectal, and Ovarian Screening Trial
OST	Office of Science and Technology	P _{milk}	maternal milk: blood partition coefficient
P ₀	parental generation	PND	postnatal day
PACT	pancreatic acinar cell tumors	PNW	postnatal week
PAD	peripheral artery disease	POD	point of departure
PBET	physiologically based extraction test	POD _{HED}	point of departure human equivalent dose
PBPK	physiologically-based pharmacokinetic	POI	premature ovarian insufficiency
PC	Partition coefficient	POPUP	Persistent Organic Pollutants in Uppsala Primiparas Study
PCOS	polycystic ovarian syndrome	POUNDS-Lost	Prevention of Obesity Using Novel Dietary Strategies-Lost
PECO	Populations, Exposures, Comparator, and Outcome	PPAR	peroxisome proliferator activated receptor
PEF	peak expiratory flow rate	PPAR α	peroxisome proliferator-activated receptor alpha
PFAA	perfluoroalkyl acids	ppm	parts per million
PFAS	per- and polyfluoroalkyl Substances	PPT	prothrombin time
PFBA	perfluorobutanoic acid		

PR	progesterone receptor	SHBG	sex hormone binding globulin
PSA	prostate-specific antigen		
PTB	preterm birth	SMBCS	Shanghai Minhang Birth Cohort Study
PWS	public water system		
PXR	pregnane X receptor	SMR	standardized mortality ratios
Q1	quartile one		
Q2	quartile two	SRBC	sheep red blood cells
Q3	quartile three	SRS	Social Responsiveness Scale
Q4	quartile four		
QA	quality assurance	SWAN	Study of Women’s Health Across the Nation cohort
R ₀	baseline risk	T3	triiodothyronine
r ⁰ _{milk}	starting milk consumption rate	T4	thyroxine
r ¹ _{milk}	week 1 milk consumption rate	TA	thyroid antibody
		TC	total cholesterol
r ² _{milk}	week 2 milk consumption rate	TDS	Total Diet Study
		TgAb	thyroglobulin antibodies
r ³ _{milk}	week 3 milk consumption rate	TiAb	title-abstract
		T _{max}	time to C _{max}
RCC	renal cell carcinoma	TPOAb	thyroid peroxidase antibody
RCM	ratio of cord blood to maternal blood concentrations		
		TRR	total reactive residues
		TSCA	Toxic Substances Control Act
RFC-1	reduced folate carrier 1		
RfD	reference dose	TSCATS	Toxic Substance Control Act Test Submissions
R _{fm}	fetus:mother concentration ratio		
		TSH	thyroid stimulating hormone
r ⁱ _{milk}	milk consumption rate for the i th week of lactation	TTEs	transplacental efficiencies
RR	risk ratio; relative risk	TTR	transthyretin
RSC	relative source contribution	UBM	Unified BARGE method
		UCMR3	Third Unregulated Contaminant Monitoring Rule
SAB	Science Advisory Board		
SBP	systolic blood pressure		
SD	Sprague Dawley	UF	uncertainty factors
SD	Standard deviation	UF _A	interspecies UF
SDQ	Strengths and Difficulties Questionnaire	UF _D	database UF
		UF _H	intraspecies UF
SDWA	Safe Drinking Water Act	UF _L	LOAEL-to-NOAEL extrapolation UF
SES	socioeconomic status		
SGA	small for gestational age	UF _{TOT}	total uncertainty factors
		URL	uniform resource locator

U.S. EPA	United States Environmental Protection Agency	WCST	Wisconsin Card Sorting Test
UV	ultraviolet	WHO	World Health Organization
V _d	volume of distribution	WIAT-II	Wechsler Individual Achievement Test-II
VI	Visual impairment	WTC	World Trade Center
VLDL	very low-density lipoproteins		
V _{max}	maximum rate of transport		
VMWM	Virtual Morris Water Maze		
WBHGB	whole blood hemoglobin		

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, *EPA's Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* that derives oral toxicity values and a relative source contribution (RSC) for perfluorooctanoic acid (PFOA). This paper is being submitted for scientific review by the EPA SAB along with three other documents:

- *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) 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 PFOS draft document, develop a number of values, including toxicity values, that could be used 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 PFOA and PFOS. Prior to rule proposal, 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, perfluorooctane sulfonic acid (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 extremely persistent in the human body and the environment {Calafat, 2007, 1290899; Calafat, 2019, 5381304}. Due to their widespread use, physicochemical properties, persistence, and bioaccumulation potential, many 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 based on structure, 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 PFOA Under SDWA

1.1.1 History of PFOA 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). PFOA is included on the third CCL (CCL 3) {U.S. EPA, 2009, 1508321} and on the fourth CCL (CCL 4) {81 FR 81099; U.S. EPA, 2016, 6307617}.

After they were listed on the CCL 3 in 2009, EPA initiated developing the *Health Effects Support Document for Perfluorooctanoic Acid (PFOA)* and one for another PFAS, PFOS, 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 health effects support documents (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 PFOA {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, 3982042}.

SDWA requires EPA to make regulatory determinations for at least five CCL contaminants every 5 years. EPA must begin 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 uses 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* {U.S. EPA, 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 draft document is to derive updated chronic oral RfD, cancer slope factor (CSF) if relevant data are available (as needed), and a draft relative source contribution (RSC) for PFOA for SAB review. These toxicity values and RSC values build upon the information provided in the 2016 PFOA HESD {U.S. EPA, 2016, 3603365} and Health Advisory {U.S. EPA, 2016, 3982042}, respectively. EPA will incorporate SAB feedback to finalize the values derived in this assessment and subsequently derive an MCLG for the NPDWRs for PFOA and PFOS.

Secondary purposes of this document, which support the primary purpose, 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 POD for chronic toxicity value derivation on the basis of several study design considerations.
- Describe study evaluations conducted for epidemiological and animal toxicological studies on 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 for PFOA.
- 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 point of departure (POD) and extrapolated from the POD to an RfD. For PFOA, 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 PFOA are summarized below.

Step 1: Evaluate the data to identify and characterize endpoints related to exposure to PFOA. 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 (see 4.1.3 for additional detail). A number of dose-metrics across life stages are selected for simulation in a mouse, rat, monkey, or human. Concentrations of PFOA in blood are 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. 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 screening 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% allows for potential unidentified sources even when exposure data from other 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 screening MCLG on a case-by-case basis. The drinking water intake (DWI) used to calculate screening MCLGs should 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 PFOA for SAB review. Prior to rule proposal, EPA will incorporate SAB feedback into analyses that are used to establish an MCLGs and NPDWRs for PFOA.

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 that dose) 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

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.

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

1.3 Chemical Identity

PFOA is a completely fluorinated organic synthetic acid that was used in the United States primarily as an aqueous dispersion agent in the manufacture of fluoropolymers and in a variety of water-, oil-, and stain-repellant products. PFOA is a strong acid that is generally present in solution as the perfluorooctanoate anion. It is water soluble and mobile in water, with an estimated log K_{oc} of 2.06. PFOA is stable in environmental media because it is resistant to environmental degradation processes, such as biodegradation, photolysis, and hydrolysis. In water, no natural degradation has been demonstrated, and dissipation is by advection, dispersion, and sorption to particulate matter. PFOA has low volatility in ionized form but can adsorb to particles and be deposited on the ground and into water bodies. Because of its persistence, it can be transported long distances in air or water, as evidenced by detections of PFOA in arctic media and biota, including in polar bears, ocean-going birds, and fish found in remote areas {Lindstrom, 2011, 1290802; Smithwick, 2006, 1424802}. Physical and chemical properties and other reference information for PFOA are provided in Table 1. Please see the 2016 PFOA Health Advisory {U.S. EPA, 2016, 3982042} for additional details on the chemical and physical properties (Section 2.1) and environmental fate of PFOA (Section 2.3).

Table 1. Chemical and Physical Properties of PFOA

Property	Perfluorooctanoic Acid; Experimental Average (Experimental Range) ^a	Source (# of References Supporting Reported Value)
Chemical Abstracts Service Registry Number (CASRN) ^b	335-67-1	EPA CompTox Chemicals Dashboard
Chemical Abstracts Index Name	2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid	
Synonyms	PFOA; Pentadecafluoro-1-octanoic acid; Pentadecafluoro-n-octanoic acid;	EPA CompTox Chemicals Dashboard

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.

Property	Perfluorooctanoic Acid; Experimental Average (Experimental Range) ^a	Source (# of References Supporting Reported Value)
Chemical Formula	Octanoic acid, pentadecafluoro-; Perfluorocaprylic acid; Pentadecafluorooctanoic acid; Perfluoroheptanecarboxylic acid C ₈ HF ₁₅ O ₂	EPA CompTox Chemicals Dashboard
Molecular Weight	414.07 g/mol	EPA CompTox Chemicals Dashboard
Color/Physical State	White powder (ammonia salt)	{Lewis, 2004, 9642153}
Boiling Point	190 °C (188–199 °C)	EPA CompTox Chemicals Dashboard (17)
Melting Point	56.1 °C (47.5–59.5 °C)	EPA CompTox Chemicals Dashboard (20)
Vapor Pressure	0.952 mm Hg (0.0165–10 mm Hg) ^c	EPA CompTox Chemicals Dashboard (13)
Henry's Law Constant	1.92×e ⁻¹⁰ atm-m ³ /mol (predicted)	EPA CompTox Chemicals Dashboard (predicted)
pK _a	3.15 (2.50–3.80)	EPA CompTox Chemicals Dashboard (2)
K _{oc}	2.06 L/kg of organic carbon	{Higgins, 2006, 5084923}
K _{ow}	3.10 (1.92–3.60)	EPA CompTox Chemicals Dashboard (5)
Solubility in Water	0.0137 mol/L (0.00821–0.0250 mol/L)	EPA CompTox Chemicals Dashboard (15)

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

^a Unless otherwise noted.

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

^c Dependent on temperature.

1.4 Occurrence Summary

Data from the third Unregulated Contaminant Monitoring Rule (UCMR 3) are currently the best available national 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) and are currently the only nationally representative finished water dataset for PFOA and PFOS. 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

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 (U.S. EPA, 2021).

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 considering 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 (0.07 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. However, the extent of these changes is unknown. 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}. The finished water data available from fifteen states collected since UCMR 3 showed that there were at least 29 PWSs where the summed concentrations of PFOA and PFOS exceeded the EPA HRL. The agency 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 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.

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.

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 PFOA {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 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 Health Effects Support Document (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 PFOA 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} by EPA 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 PFOA Health Effects Systematic Review

Table 2 describes the PECO inclusion criteria used to screen the literature in this assessment.

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>Chemical Abstracts Service (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;</p>

PECO Element	Inclusion Criteria
	<p>perfluorocaprylic acid; pentadecafluorooctanoic acid; perfluoroheptanecarboxylic acid; octanoic acid, pentadecafluoro- PFOS (CAS number 1763-23-1). Other names: perfluorooctane sulfonate, perfluorooctanesulfonic acid, perfluorooctane sulfonic acid, perfluorooctane sulphonate, perfluorooctanyl sulfonate, heptadecafluorooctane-1-sulphonic, Heptadecafluoro-1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid</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> <p>Comparator Human: A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) of PFOA or PFOS, or exposure to PFOA or PFOS for shorter periods of time. Case reports and case series will be tracked as “potentially relevant supplemental information.”</p> <p>Animal: A concurrent control group exposed to vehicle-only treatment or untreated control.</p> <p>Outcome All health outcomes (both cancer and noncancer).</p> <p>PBPK Models Studies describing PBPK models will be included</p>

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 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 populations) 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,
- National Center for Toxicological Research,
- 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="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=(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-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-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 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 **Error! Reference source not found..**

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 1 and 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 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; maximum rate of transport (V_{max}), Michaelis constant (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> <p>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).</p>

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 **Error! Reference source not found.**) 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).</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>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	<p>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.</p>

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 {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 HERO: 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, 388 were considered to meet PECO eligibility criteria (Table 2) and included information on PFOA. The studies meeting PECO criteria at the full-text level included 350 epidemiological studies, 25 animal studies, and 44 PBPK studies. Additional details of the literature search and screening process are shown in Figure 3.

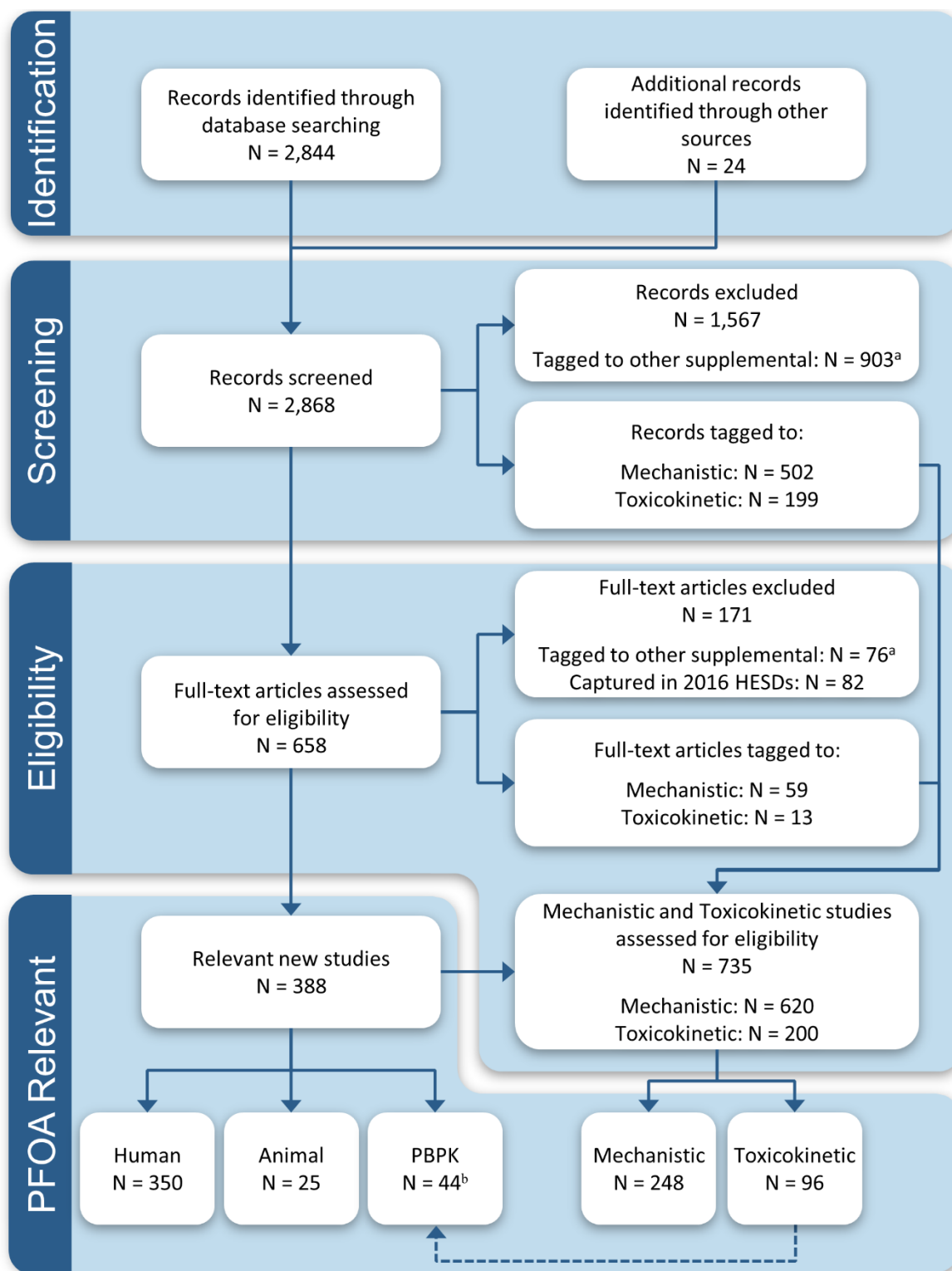


Figure 3. Summary of Literature Search and Screening Process for PFOA

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

^b Number 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 PFOA

Of the 349 epidemiological studies that met the inclusion criteria, most studies had a cohort ($n = 152$) study design. Of the remaining studies, 138 had a cross-sectional design, 33 had a case-control design, and 26 had other study designs (i.e., nested case-control). Epidemiological studies were categorized into 18 health systems. Most studies reported on the metabolic ($n = 72$), developmental ($n = 63$), cardiovascular ($n = 61$), or female reproductive systems ($n = 54$). Additional study details are shown in Figure 4.

Health System	Study Design				Grand Total
	Case-control	Cohort	Cross-sectional	Other	
Cancer	3	3	2	5	13
Cardiovascular	3	13	39	6	61
Dermal	0	1	0	0	1
Developmental	3	43	15	2	63
Endocrine	1	7	17	8	33
Gastrointestinal	1	6	0	0	7
Hematologic	0	0	7	1	8
Hepatic	1	5	13	4	23
Immune	4	25	12	3	44
Metabolic	7	34	26	5	72
Musculoskeletal	0	1	6	2	9
Nervous	3	26	5	3	37
Ocular	0	0	1	0	1
Renal	0	5	17	2	24
Reproductive, Male	0	7	13	1	21
Reproductive, Female	9	21	22	2	54
Respiratory	1	4	1	0	6
Other	0	3	3	0	6
Grand Total	33	152	138	26	349

Figure 4. Summary of Epidemiology Studies of PFOA^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 PFOA

Of the 32 animal studies that met the inclusion criteria, most studies had either short-term ($n = 15$) or developmental ($n = 12$) study designs. Of the animal studies, most reported on mice ($n = 26$). The mice studies included short-term ($n = 13$), developmental ($n = 12$), and subchronic ($n = 1$) study designs. The remaining studies reported on rats ($n = 6$) using subchronic, short-term, chronic, or reproductive study designs or monkeys ($n = 1$) using a chronic study design. Animal studies were categorized into 15 health systems. Most studies reported on the hepatic ($n = 21$), reproductive ($n = 18$), whole body ($n = 17$), or developmental ($n = 13$) systems. Additional study details are shown in Figure 5.

Health System	Study Design & Species								Grand Total
	Subchronic		Short-term		Chronic		Developmental	Reproductive	
	Mouse	Rat	Mouse	Rat	Monkey	Rat	Mouse	Rat	
Cancer	0	0	0	0	0	2	0	0	2
Cardiovascular	0	0	1	1	0	2	2	0	6
Developmental	0	0	0	0	0	1	11	1	13
Endocrine	0	0	1	1	0	2	2	1	7
Gastrointestinal	0	0	0	0	1	2	0	0	3
Hematologic	0	0	0	1	0	1	0	0	2
Hepatic	1	1	9	2	0	2	6	1	21
Immune	1	0	3	1	0	2	1	1	9
Metabolic	0	0	0	1	0	2	2	0	5
Musculoskeletal	0	0	0	0	0	0	1	0	1
Nervous	0	0	2	0	0	1	3	1	7
Renal	0	0	1	1	0	2	1	1	6
Reproductive	1	1	3	1	0	2	9	1	18
Respiratory	0	0	0	1	0	1	0	0	2
Whole Body	1	1	7	1	0	2	4	1	17
Grand Total	1	1	13	2	1	2	12	1	32

Figure 5. Summary of Toxicology Studies of PFOA^a

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

^aStudy counts include key studies from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}.

3.2 Toxicokinetics

3.2.1 ADME

Due to strong carbon-fluorine bonds, PFOA is stable to metabolic and environmental degradation. It also is resistant to metabolic biotransformation. Thus, the toxicity and pharmacodynamics of the parent compound is the concern. Because of its impact on cellular receptors and proteins, it possesses the ability to impact the biotransformation of dietary constituents, intermediate metabolites, and other xenobiotic chemicals by altering enzyme activities and transport kinetics. PFOA is known to activate peroxisome proliferator activated receptor (PPAR) pathways by increasing transcription of mitochondrial and peroxisomal lipid metabolism, sterol, and bile acid biosynthesis and retinol metabolism genes. Based on transcriptional activation of many genes in peroxisome proliferator activated receptor alpha (PPAR α)-null mice, however, the effects of PFOA involve far more than activation of PPAR and consequent peroxisome proliferation. The data indicate that it also can activate the constitutive androstane receptor (CAR), farnesoid X receptor (FXR), and pregnane X receptor (PXR) and metabolic activities linked to these nuclear receptors. Activation of these receptors could impact the toxicokinetics of PFOA itself {Andersen, 2008, 3749214}.

PFOA is not readily eliminated from humans and other primates. Toxicokinetic profiles and the underlying mechanism for half-life differences are not completely understood, although many of the differences appear to be related to elimination kinetics and factors that control membrane transport. Thus far, three transport families appear to play a role in PFOA absorption, distribution, and excretion: organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and multidrug resistance-associated proteins (MRPs) {Klaassen and Aleksunes 2010, 9641804; Launay-Vacher, 2006, 9641802}. The transporters are critical for GI absorption, uptake by the tissues, and excretion via bile and the kidney. These transport systems are located at the membrane surfaces of the kidney tubules, intestines, liver, lungs, heart, blood brain barrier (BBB), blood placental barrier, blood testes barrier (BTB), and mammary glands where they function to protect the organs, tissues, and fetus through active removal of foreign

compounds {Ito and Alcorn, 2003, 9641803; Klaassen and Aleksunes 2010, 9641804, Zaïr, 2008, 9641805}. However, luminal transporters in the kidney may cause reuptake of PFOA from the proximal tubule resulting in decreased excretion from the body {Weaver, 2009, 2010072}. This would cause PFOA to persist in the body over time. Transporters have also been identified that may facilitate uptake and reuptake of PFOA from the gut {Ruggiero, 2021, 964180}.

There are differences in transporters across species, sexes, and individuals. In addition, more PFOA-specific information is available about the OAT and OATP families than about the MRPs. These limitations have hindered the development of PK models for use in predicting effects in humans based on the data from animal studies.

3.2.1.1 Absorption

Absorption data are available in laboratory animals for oral, inhalation, and dermal exposures, and extensive data are available from humans demonstrating the presence of PFOA in serum. In vitro absorption data indicate that uptake is influenced by pH, temperature, and concentration as well as OATP activity. Detailed study descriptions of literature informing absorption of PFOA in humans and animals are provided in Section D.1.

3.2.1.1.1 Cellular Uptake

The absorption process requires transport from the external environment across the interface of the gut, lung, or skin. Uptake in cells cultured in vitro is fast and saturable, consistent with a role of transporters. Cellular transfection of cells with vectors coding for organic ion transporters have confirmed their role in uptake of PFOA {Kimura, 2017, 3981330; Nakagawa, 2007, 3981330; Nakamura, 2009, 2919342; Yang, 2009, 2919328; Yang, 2010, 2919288}. Several studies suggest involvement of OATs, OATPs, and MRPs in enterocytes in the uptake of PFOA {Klaassen and Aleksunes 2010, 9641804; Zaïr et al. 2008, 9641805}. Few studies have been conducted on the intestinal transporters for PFOA in humans or laboratory animals, although one study supports a role for OATPs in PFOA uptake by immortalized intestinal cells {Kimura, 2017, 3981330}. Most of the research has focused on transporters in the kidney that are relevant to excretion and were carried out using cultured cells transfected with the transporter proteins.

In addition to facilitated transport, there is evidence supporting passive diffusion in cells cultured in vitro {Yang, 2009, 2919328} and in placenta in vivo {Zhang, 2013, 3859792}. Since PFOA 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 {2020, 6505873} determined membrane/water PCs ($K_{\text{mem/w}}$) for PFOA and examined possible permeation into cells by measuring the passive anionic permeability (P_{ion}) through planar lipid bilayers. In this system, the PCs 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

PFOA phospholipophilicity influences absorption through the GI tract, lungs, or skin is unknown.

3.2.1.1.2 Absorption and Bioavailability in Humans and Animals

In vivo, PFOA is well absorbed following oral exposure. Supporting this is evidence that PFOA is present in serum of humans after exposure to contaminated drinking water {e.g., Xu, 2020, 6781357; Worley, 2017, 3859800}. Studies on male rats administered PFOA by gavage using a single or multiple dose regimen estimated dose absorption of at least 92.3% {Gibson, 1979, 9641813; Cui, 2010, 2919335}. In rats, the time to reach the maximum PFOA plasma concentration (T_{max}) following oral exposure is very fast and varies by sex {Kim, 2016, 3749289; Dzierlenga, 2019, 5916078}. For example, the study by Kim and colleagues estimated T_{max} after a single oral dose of 1 mg/kg to be 1.44 hours in female rats versus 2.07 days in males.

Recent studies confirm that bioavailability of PFOA after oral exposure is very high in rats. Comparison of serum concentrations after oral dosing ranged from 82–140% of levels measured after intravenous (IV) dosing, which may reflect increased reabsorption by intestinal transporters by the oral route relative to the IV route of exposure {Kim, 2016, 3749289; Dzierlenga, 2019, 5916078}. Bioavailability of PFOA appears to be modified by diet. Using in vitro and in vivo (BALB/c mice) systems, Li et al. {2015, 2851033} found that PFOA bioavailability is strongly influenced by diet, with high fat diets associated with reduced absorption. The authors suggest an important factor influencing PFOA bioaccessibility is colloidal stability in intestinal solutions.

Limited data also support PFOA absorption through both inhalation {Hinderliter, 2006, 135732} and dermal routes {Fasano, 2005, 3749187; O'Malley, 1981, 4471529; Kennedy, 1985, 3797585}.

3.2.1.2 Distribution

3.2.1.2.1 PFOA Binding to Blood Fractions and Serum Proteins

Detailed study descriptions of literature informing distribution of PFOA 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 the blood cell constituents, PFOA preferentially accumulates in platelets over red blood cells and leukocytes {De Toni, 2020, 6316907}. Within human blood fractions, PFOA accumulates to 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 PFOA concentrations in plasma, serum, and whole blood were 1.90, 1.60 and 0.93 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 PFOA.

PFOA is distributed within the body by noncovalently binding to plasma proteins. Many studies have investigated PFOA interactions with human serum albumin (HSA) {Wu et al., 2009, 536376; MacManus-Spencer et al., 2010, 2850334; Qin et al., 2010, 3858631; Salvalaglio et al., 2010, 2919252; Weiss et al., 2009, 534503; Luebker et al., 2002, 1291067; L. Zhang et al., 2013, 5081488; Cheng and Ng, 2018, 5024207; Gao et al., 2019, 5387135; Yue et al., 2016, 3479514}.

In vitro analyses found that plasma proteins can bind 97–100% of the PFOA in plasma from humans, cynomolgus monkeys, and rats {Kerstner-Wood et al., 2003, 4771364}.

HSA is the primary PFOA binding protein in plasma {Han, 2003, 5081471} and intermolecular interactions are mediated through van der Waals forces and hydrogen bonds {MacManus-Spencer, 2010, 2850334; Chen, 2020, 6324256}. Beesoon and Martin {2015, 2850292} determined that linear PFOA molecule bound more strongly to calf serum albumin than the branched chain isomers in the order of 4m < 3m < 5m < 6m (iso) < linear. PFOA-mediated conformational changes may also interfere with albumin's ability to transport its natural ligands and drugs {Wu, 2009, 536376} such as fatty acids, thyroxine (T4), Warfarin, indole, and benzodiazepine.

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

Other plasma proteins that bind PFOA, albeit with lower affinity than HSA, include low-density lipoproteins (LDLs), alpha-globulins (alpha-2-macroglobulin), gamma-globulins, transferrin, and fibrinogen {Kerstner-Wood et al., 2003, 4771364}. PFOA also binds serum thyroid hormone transport protein, transthyretin (TTR), causing up to a 50% inhibition of T4 binding to TTR {Weiss, 2009, 534503}. In contrast to serum proteins, little is known regarding PFOA binding to proteins in the gut. One study found that PFOA can bind to and cause a conformational change in pepsin {Yue, 2016, 3479514}, though it is unclear whether PFOA-pepsin interactions impact absorption or distribution from the gut to other compartments in the body.

3.2.1.2.2 PFOA Binding to Subcellular Fractions, Intracellular Proteins, and Transporters

Han et al. {2005, 5081570} observed a sex-dependent subcellular distribution of PFOA in the liver and kidney of male and female rats necropsied 2 hours after oral gavage dosing. The proportion of PFOA in the liver cytosol of female rats was almost twice that of the male rats. They hypothesized that females might have a greater amount than males of an unknown liver cytosolic binding protein with an affinity for perfluorinated acids. In the kidney, the subcellular distribution did not show the sex difference seen with the liver; however, the protein-bound fraction for the males (42%) was about twice that for the females (17%). In another study {Zhang 2020, 6316915} PFOA preferentially distributed to cytosol followed by nuclei and mitochondria in human colorectal cancer cells, human lung epithelial cells, and human normal liver cells. Within liver cells, PFOA binds to the liver fatty acid binding protein (L-FABP) through polar and hydrophobic interactions {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.

PFOA interactions with various protein transporters play a role in the tissue uptake of orally ingested PFOA. The transporters are located at the interface between serum and a variety of tissues (e.g., liver, kidneys, lungs, heart, brain, testes, ovaries, placenta, and uterus) {Klaassen, 2010, 9641804}. The liver is an important uptake site for PFOA. OATPs and MRPs, at least one OAT, and the sodium-taurocholate cotransporting polypeptide (NTCP)—a hepatic bile uptake transporter—have been identified at the boundary of the liver at the portal blood and/or the canalicular membranes within the liver {Kim, 2003, 9641809; Kusuvara and Sugiyama, 2009, 9641810; Zair, 2008, 9641805}. Transporters responsible for PFOA transport across the placenta are not well understood though preliminary studies examining transporter expression identified OAT4 as a candidate receptor {Kummu, 2015, 3789332}. The expression of 9 transporter genes were found to vary at different stages of gestation changes {Li, 2020, 6505874}, though direct experimental evidence for these transporters in mediating transfer of PFOA to the fetus is lacking.

3.2.1.2.3 Tissue Distribution in Humans and Animals

Evidence from human autopsy and surgical tissues demonstrates that PFOA distributes to a wide range of tissues, organs, and matrices throughout the body. Although blood and liver are major sites of PFOA accumulation {Olsen, 2001, 9641811}, recent findings confirm PFOA accumulation to variable levels in brain and cerebral spinal fluid in patients {Fujii et al., 2015, 2816710; Wang, 2018, 5080654}, thyroid gland {Pirali, 2009, 757881}, and reproductive tissues {Kang, 2020, 6356899}. In a study of autopsy tissues collected in the first 24 hours after death, Pérez et al. {2013, 2325349} measured PFOA levels in tissue samples (liver, kidney, brain, lung, and bone) and found PFOA primarily in the bone (60.2 ng/g), lung (29.2 ng/g), liver (13.6 ng/g), and kidney (2.0 ng/g), with levels below the limit of detection (LOD) in the brain.

Most experimental animal studies were conducted in rats and mice by oral dosing. Studies in primates were limited to measurements in blood and liver {Butenhoff 2002, 1276161; Butenhoff, 2004, 3749227}. PFOA primarily distributes to serum, liver, lungs, and kidney in rats across a range of dosing regimens and durations {Ylinen, 1990, 5085631; Kemper, 2003, 6302380; NTP, 2020, 7330145; NTP, 2019, 5400977} and mice {Lau, 2006, 1276159; Lou, 2009, 2919359; Burkemper, 2017, 3858622; Li, 2017, 4238518; Guo, 2019, 5080372}. Sex-specific differences in PFOA levels were observed in several rat studies. For example, in a 28-day study {NTP, 2019, 5400977}, males exhibited higher plasma concentrations than females across all dose groups even though females were administered a 10-fold higher dose of PFOA, suggesting that female rats excrete PFOA more efficiently than males. Sex-specific differences were less striking in studies conducted in mice compared to rats {Lau, 2006, 1276159; Lou, 2009, 2919359}.

Liver PFOA levels are regulated, at least in part, by PPAR α . In human and rodent hepatocytes, PPAR α activation induces expression of genes involved in lipid metabolism and cholesterol homeostasis. PFOS and PFOA structurally resemble fatty acids and are well-established ligands of PPAR α in the rat and mouse liver. As PPAR α agonists, PFOS and PFOA can induce the β -oxidation of fatty acids, induce fatty acid transport across the mitochondrial membrane, decrease hepatic very low-density lipoprotein (VLDL)-triglyceride and apolipoprotein B (apoB) production, and promote lipolysis of triglyceride-rich plasma lipoproteins {Fragki, 2020, 8442211}. The liver can transport PFOA from hepatocytes to bile ducts that is mediated at least partly by PPAR α {Minata, 2010, 1937251}. PFOA levels were significantly lower in PPAR α

null mice than wild-type mice exposed to doses of 25 and 50 $\mu\text{mol/kg}$, supporting a role for PPAR α in PFOA clearance in the liver {Minata, 2010, 1937251}.

Studies administering radiolabeled PFOA demonstrate the range of tissue distribution in rats {Kemper, 2003, 6302380} and mice {Burkemper, 2017, 3858622; Bogdanska, 2020, 6315801} that include the central nervous system (CNS), cardiovascular, gastrointestinal, renal, immune, reproductive, endocrine, and musculoskeletal systems. PFOA crossed the BBB in males an order of magnitude more efficiently than in females {Ylinen, 1990, 5085631}. Fujii and colleagues {2015, 2816710} found that while a relatively small amount of PFOA was measured in the brains of mice (0.1%), it is noteworthy that PFOA can cross the BBB in healthy animals. In mice, Burkemper et al., {2017, 3858622} observed the highest PFOA levels in bone, liver, and lungs. Bogdanska et al. {2020, 6315801} also observed PFOA in testes of C57BL/6 mice at levels similar to those observed in epididymal fat and in intestines. In BALB/c mice exposed to PFOA for 28 days, PFOA levels in the testes increased with increasing dose {Zhang, 2014, 2850230} and accumulated in the epididymis of BALB/c mice in a dose-dependent manner {Lu, 2016, 3981459}.

Fujii and colleagues {2015, 2816710} observed that perfluoroalkyl carboxylic acids (PFCAs) (C6 and C7) were excreted rapidly through urine, while longer chains ($\geq\text{C8}$) accumulated in the liver. Moreover, PFAS with longer chain lengths were found to exhibit increasing affinity for serum and L-FABPs. The authors suggest that lipophilicity driven by chain length may account for the distribution patterns of PFAS, which is consistent with the high levels of PFOA accumulation in serum and liver. These large sequestration volumes of PFOA observed in the liver seem to be attributable to the liver's large binding capacity in mice.

3.2.1.2.4 Distribution During Reproduction and Development

Many recent studies quantified distribution of PFOA from pregnant females to fetuses and from mothers to infants. Distribution from mother to fetus has been confirmed by measuring PFOA levels in placenta, cord blood, and amniotic fluid. The ratio of PFOA in placenta relative to maternal serum (R_{PM}) ranged between 0.326 to 0.460 {Zhang, 2013, 3859792; Chen, 2017, 3859806}. Gestational age and PFOA branching characteristics influence transport across the placental. PFOA concentrations within the placenta increase during gestation from the first to third trimester {Mamsen, 2019, 5080595}. Linear PFOA is detected at higher frequency and at higher levels in maternal serum than branched isomers likely due to different binding affinities to serum proteins. However, branched PFOA is more efficiently transported into the placenta than linear PFOA {Cai, 2020, 6318671; Chen, 2017, 3859806}.

Several studies reported a strong positive correlation between maternal and cord serum levels {Kato, 2014, 2851230; Porpora, 2013, 2150057}. The ratio of PFOA in cord serum relative to maternal serum ranged from 0.55 to 1.33 (Table D-10) and generally increased with gestational age {Li, 2020, 6505874}. Factors such as exposure sources, parity, and other maternal demographics are postulated to influence variations in cord:maternal serum ratios {Kato, 2014, 2851230; Brochot, 2019, 5381552}. Cord:maternal serum ratios represent transplacental efficiencies (TTEs), which exhibit a U-shaped curve with PFAS chain length {Zhang, 2013, 3859792} and generally increased as the branching point moved closer to the carboxyl or sulfonate moiety {Zhao, 2017, 5085130}.

Lower levels of PFOA were measured in amniotic fluid compared to placenta and cord blood {Zhang, 2013, 3859792}. The mean concentration ratio between amniotic fluid and maternal blood was higher for PFOA (0.13) than for PFOS (0.0014). The mean concentration ratio between amniotic fluid and cord blood was higher for PFOA (0.023) than for PFOS (0.0065). 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.

PFOA 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. PFOA was detected in placenta, fetal liver, extremities, heart, intestines, lungs, connective tissues, spinal cord, and ribs and were highest in the placenta and lung. Different patterns of PFOA were observed in fetal tissues depending on fetal age {Mamsen, 2019, 5080595}. Fetal tissue:maternal serum ratios of PFASs were calculated by dividing the fetal tissue concentration by the maternal serum concentration. In general, fetal tissue:maternal ratios of PFOA in fetal tissue increased from first trimester to third trimester except for the liver and heart which showed the highest tissue:serum ratios in the second trimester compared with the third trimester.

New studies also confirm that distribution of PFOA 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 PFCA including length, lipophilicity, and branching. In the Mondal study {Mondal, 2014, 2850916}, the mean maternal serum PFOA concentrations were lower in breastfeeding mothers versus non-breastfeeding mothers. Conversely, breastfed infants had higher mean serum PFOA 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 PFOA levels in breastmilk were approximately 30-fold lower relative to maternal serum and the ratio between breastmilk and maternal serum PFOA was 0.038 ± 0.013 . The authors noted that the transfer rates from serum to breastmilk of PFAS were lower compared to other lipophilic persistent organic pollutants such as polychlorinated biphenyls.

Several studies have confirmed distribution from rat and mouse dams to fetuses and pups, as well as variable PFOA level across many fetal tissues {Han, 2003, 5081471; Hinderliter 2006, 3749132; Butenhoff, 2004, 1291063; Mylchreest, 2003, 9642031; Fenton, 2009, 194799; Macon, 2011, 1276151; White, 2011, 1276150; Blake, 2020, 6305864}. Interestingly, Fujii et al., {2020, 6512379} found that milk/plasma (M/P) concentration ratio for PFOA also exhibited a U-shaped curve with increasing chain length but did not correlate to lipophilicity of PFAS in FVB/NJcl mice. These findings suggest that the amount transferred from mothers to pup during lactation may also relate to chain length-dependent clearance.

3.2.1.2.5 Volume of Distribution in Humans and Animals

In humans, the V_d for PFOA has been assigned to values between 170 and 200 mL/kg (Table D-27). 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 mice and rats vary by species, age, sex, and dosing regimen. For example, Dzierlenga et al. {2019, 5916078} calculated the apparent volume of central and peripheral distribution in rats. A one-compartment model for males and a two-compartment model for females was used to characterize PFOA levels. In this study, both peripheral and central V_d values were calculated after oral dosing at all doses in both males and females. Peripheral V_d values were dramatically lower than central V_d values at all doses after oral administration and interestingly, also after IV administration. While peak tissue levels were reached readily in both males and females, tissue levels in males were steady over the course of several days whereas tissue levels in females dropped quickly (in the span of hours).

3.2.1.3 Metabolism

Consistent with other reports and reviews {U.S. EPA, 2016, 3603279; ATSDR, 2021, 9642134; Pizzuro et al., 2019, 5387175}, there is no evidence that PFOA 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 PFOA in humans, cynomolgus monkeys, and rats. Fewer studies measured elimination in mice, hamsters, and rabbits. 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 PFOA in humans and animals are provided in Section D.4.

3.2.1.4.1 Urinary and Fecal Excretion

Studies in animals provide evidence that urine is typically the primary route of excretion but that sex impacts excretion by both routes, and these sex differences appear to be species-specific. Limited evidence supports excretion through the fecal route in animals and humans and through hair in animals. Most studies indicate excretion by the fecal route is substantially lower than that observed by the urinary route. Excretion through the fecal route appears to be more prominent in males compared to females and in rodents compared to humans. Nevertheless, a comprehensive set of principles governing resorption by renal, hepatic, and enteric routes and how these impact excretion and retention of PFOA has not been established in either humans or animals.

Human studies examined PFOA excretion after oral exposure, primarily through the urinary route. The urinary excretion of PFOA in humans is impacted by the isomeric composition of the mixture present in blood and the sex and age of the individual. The half-lives of the branched-chain PFOA isomers are shorter than those for the linear molecule, an indication that renal resorption is less prevalent for the branched chains. {Zhang, 2014, 2851103; Fu, 2016, 3859819}.

Fujii et al. {2015, 2816710}, measured PFOA clearance in mice and humans. Male and female FVB/NJcl mice were administered PFOA by IV (0.31 $\mu\text{mol/kg}$) or gavage (3.13 $\mu\text{mol/kg}$) and serum concentration data was analyzed using a two-compartment model. Mouse urinary clearance was analyzed by dividing the total amount excreted in the urine

during a 24-h period with the area under the curve (AUC) of the serum concentration. Human data were analyzed from paired (bile-serum) archived samples from patients undergoing nasobiliary drainage, percutaneous transhepatic biliary drainage, or percutaneous transhepatic gallbladder drainage for 24 hours. Urine-serum pairs were collected from healthy donors. Urinary and biliary clearance was determined by dividing the cumulative urine or bile excretion in a 24-h period with the serum concentration. Fecal clearance was calculated using the estimated biliary resorption rate.

The authors estimated that human total clearances were 0.096 mL/kg/day and were 50-100 times smaller than those estimated in mice after oral gavage dosing. In humans, PFOA clearance rates via urinary, biliary, and fecal routes were estimated to be 0.044, 2.62, and 0.052 mL/kg/day, respectively. The reabsorption rate of bile excreting C8 was estimated to be 0.98 (derived by assigning a V_d of 200 mL/kg, a serum half-life of 3.8 years, and the presumption that C8 could only be excreted into the urine and feces via the bile).

In rats, urine PFOA concentrations differed with age, dose, and sex {Hinderliter, 2006, 3749132}. For all doses and ages, urinary excretion of PFOA was substantially higher in females than in males, and this difference increased with age. Several additional studies in rats found that females excreted much higher levels in urine compared to males and compared to feces {Kim, 2016, 3749289; Benskin, 2009, 1617974; Cui, 2010, 2919335}.

3.2.1.4.2 Renal and Enterohepatic Resorption

Several studies have been conducted to elucidate the cause of the sex difference in the elimination of PFOA by rats {Kudo, 2002, 2990271; Cheng, 2006, 6551310; Hinderliter, 2006, 3749132}. Many of the studies have focused on the role of transporters in the kidney tubules, especially the OATs and OATPs located in the proximal portion of the descending tubule {Nakagawa, 2007, 2919370; Nakagawa, 2009, 2919342; Yang, 2009, 2919328; Yang, 2010, 2919288}.

In vitro studies were supported by in vivo analysis of OATPs gene and protein expression in rat kidneys {Yang, 2009, 2919328}. Organic anion transporters polypeptide 1a1 (OATP1a1) is located on the apical side of proximal tubule cells and could be the mechanism for renal reabsorption of PFOA in rats. The level of messenger ribonucleic acid (mRNA) of OATP1a1 in male rat kidney is 5–20-fold higher than in female rat kidney and is regulated by sex hormones. Thus, higher expression of OATP1a1 in male rats would favor resorption of PFOA in the glomerular filtrate and reduce excretion.

Fewer studies have investigated enterohepatic resorption of PFOA. Gastrointestinal elimination of PFOA was reported in a case history of a single human male with high serum levels of perfluorinated chemicals that was treated with a bile acid sequestrant (cholestyramine [CSM]) {Genuis, 2010, 2583643}. Before treatment, PFOA was detected in urine (3.72 ng/mL) but not in stool (LOD = 0.5 ng/g) or sweat samples. After treatment with CSM for 1 week, his serum PFOA concentration lowered from 5.9 ng/g serum to 4.1 ng/g serum and stool PFOA levels increased to 0.96 ng/g. This observation suggests that PFOA is excreted in bile and that enterohepatic resorption via intestinal transporters limits the loss of PFOA via feces.

Studies in mice {Maher, 2008, 2919367; Cheng, 2008, 758807} suggest that increased expression of MRP3 and MRP4, coupled with decreased OATP levels leads to increased biliary excretion of

bile acids, bilirubin, and conjugated metabolites of toxic chemicals, including PFOA. Based on the results with the more extensive evaluation of perfluorodecanoic acid (PFDA) including mouse strains null for several receptors (PPAR α , CAR, PXR, and FXR), the authors concluded that the changes in receptor proteins were primarily linked to activation of PPAR α {Cheng, 2008, 758807}.

Zhao et al. {2017, 3856461} demonstrated that PFOA was a substrate for human OATP1B1, OATP1B3, and OATP2B1 transporters expressed in liver using in vitro studies of Chinese hamster ovary (CHO) and human embryonic kidney (HEK-293) cells transfected with transporter complementary DNA (cDNA). Under these conditions, the three OATPs expressed in human hepatocytes can transport the longer chain PFOA (C8) and perfluorononanoate (C9), but not the shorter chain perfluoroheptanoate (C7). Preliminary evidence suggests enterohepatic resorption could limit elimination of PFOA by the fecal route, including the recent observation that PFOA binds to NTCP, an uptake transporter in the gut {Ruggiero, 2021, 9641806}. The extent to which this pathway operates in vivo and whether enterohepatic resorption plays a substantial role in the retention of PFOA in humans and animals is still unknown.

3.2.1.4.3 Maternal Elimination through Lactation and Fetal Partitioning

PFOA 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 clearly eliminate PFOA through routes not available to males. The total daily elimination of PFOA in pregnant females was estimated to be 11.4 ng/day, lower than the 30.1 ng/day estimated for PFOS {Zhang, 2014, 2850251}. Mamsen et al. {2019, 5080595} estimated a placenta PFOA accumulation rate of 0.11% increase per day during gestation and observed that the magnitude of elimination may be influenced by the sex of the fetus. A study by Zhang et al. {2013, 3859792} observed that the mean levels in the cord blood, placenta, and amniotic fluid were 58%, 47%, and 1.3%, respectively, of those 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, 2851103} observed that the partitioning ratio of PFOA concentrations between urine and whole blood in pregnant women (0.0011) was lower than the ratios found in non-pregnant women (0.0028). The rate and extent of elimination through these routes are affected by parity {Lee, 2017, 3983576; Jusko, 2016, 3981718} 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, 2008, 1290895; Thomsen, 2010, 759807; Kang, 2016, 3859603}. Cariou et al., {2015, 3859840} measured PFOA in maternal serum, cord serum, and breast milk from females with planned cesarean births. The observed ratios of cord and maternal serum for PFOA was 0.78 in this study. However, the ratio between breast milk and maternal serum was 0.038, suggesting transfer from maternal blood to breast milk is lower than transfer from maternal blood to cord blood.

Studies in animals support elimination through pregnancy and lactation observed in humans. Fujii and colleagues {2020, 6512379} used the M/P concentration ratio as a measure of chemical transferability in FVB/NJcl mice. Maternal plasma PFOA concentrations were significantly higher than milk (M/P ratio was 0.32). The M/P ratios were similar for C8, C9, C12 and C13,

arguing against a direct relationship with lipophilicity. Potential roles for binding proteins in breast milk or transporters in breast tissue have not been investigated.

In summary, partitioning to the placenta, amniotic fluid, 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.

3.2.1.4.4 Other Routes of Elimination

Menstruation may be an important factor in the sex-specific differences observed in PFOA elimination. Zhang et al {2013, 3859849} estimated a menstrual serum clearance rate of 0.029 mL/day/kg. The link between menstruation and PFOA elimination is based on several observations. First, older females have longer PFOA elimination half-lives than young females and males {Zhang, 2013, 3859849}. 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. Second, several studies examined the 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 et al., 2017, 3981395} suggested that this association could be explained by reversed causality and more specifically, that PK bias could account for the observed association with epidemiological data. Additional studies may be needed to clarify the role of menstruation in PFOA elimination.

Gao et al. {2015, 2850134} found that hair is a potential route of PFAS elimination in rats. A dose-dependent increase in hair PFOA concentration was observed in all exposed animals. Interestingly, the hair PFOA concentrations for all treatment doses were significantly higher in males than in females. The sexual dimorphic difference in hair concentrations may be attributed to the sex differences observed in PFOA elimination rate and the transfer from serum to hair.

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 PFOA with estimates measured in years rather than months or weeks (see study details and summary tables in Section D.4.5). Because there is no evidence that PFOA is metabolized in mammals, half-life determinations are governed by excretion. The calculation of PFOA 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 and rodent females relative to males. In the single primate study discussed below, half-lives were shorter in males compared to females.

PFOA half-life values in humans ranged from 0.53 years for branched PFOA in young females {Zhang et al., 2013, 3859849} to 22 years in a study of primiparous women in Sweden {Glynn et al., 2012, 1578498} and varied by geographical region {Gomis, 2017, 3981280} (Table D-37). Age and sex difference in PFOA half-lives have not been rigorously evaluated, though estimates in males are generally longer than those in females {Fu, 2016, 3859819; Gomis, 2017, 3981280; Li, 2017, 4238434} and exhibit an age-related increase {Genuis et al., 2014, 2851045, Zhang et al., 2013, 3859849}. While most studies were conducted in adults and/or adolescents, one study

in newborns {Spliethoff et al., 2008, 2919368} calculated a half-life for PFOA of 4.4 years. Linear isoforms exhibit longer half-lives than branched isoforms {Zhang et al., 2013, 3859849}.

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 D-36). 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 a route of elimination {Zhang et al., 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 et al., 2020, 6781357; Worley et al., 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.

In animals, half-life values are reported in days rather than in years. Values in cynomolgus monkeys ranged from 13.6 to 41.7 days {Butenhoff et al., 2004b, 3749227} 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 0.03 days in females exposed to a high dose of 40 mg/kg {Dzierlenga et al., 2019, 5916078} to 13.4 days in males exposed to a relatively low dose of 0.4 mg/kg {Benskin et al., 2009, 1617974}. Rats exposed by the IV route exhibited shorter half-lives than rats administered the same dose by the oral gavage route {Kim et al., 2015, 2850129, Dzierlenga et al., 2019, 5916078}. Similar to humans and mice, half-life estimates were shorter in female rats compared to male rats. In contrast, female half-life values exceeded male values in cynomolgus monkeys suggesting species-specific factors impacting elimination across sexes. Similar to humans, PFOA isomers exhibited shorter half-lives compared to linear forms.

3.2.2 Pharmacokinetic Models

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 PFOA 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 require 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 PFOA ADME.

3.2.2.1 Classical Compartmental Analysis

The most common approach for the prediction of serum levels of PFOA 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 in the temporal gaps around, or between measured serum concentrations or exposures.

The most common use for these models is to predict serum concentrations from estimated exposures. Some examples of this include the work by Shin et al. (2011, 2572313) who evaluated the exposure predictions from an environmental fate and transport model by comparing the predicted serum PFOA concentrations to observed values and by Avanası et al. (2016, 3981510) who extended the work of Shin et al. (2011, 5082426) by applying a population model to investigate how variability and uncertainty in model parameters affect the prediction of serum concentration.

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 times 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 PFOA exposure that originates from drinking water.

Other applications are used to better understand the toxicokinetics of PFOA by combining estimated exposure values and serum values to estimate clearance and half-life in a population of interest. One example of this type of model application was presented by Gomis et al. (2016, 3749264) who used measurements of serum and exposure, in the form of air concentrations

during occupational exposure, to estimate an elimination half-life of PFOA. Those authors also were able to identify the relative contributions of direct occupational exposure to PFOA, indirect occupational exposure to PFOA precursors, and background, non-occupational PFOA exposure. Another example was presented by Worley et al. (2017, 3859800) who estimated the half-life of PFOA 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 (CL_R) (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 PFOA. Russell et al. (2015, 2851185) addressed this problem by estimating the amount of bias in elimination half-life that is introduced when the ongoing background exposure is not taken into account, with application to PFOA as an example.

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 PFOA, 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 younger ages because of growth during childhood and adolescence. This growth dilutes chemical in the body and results in lower levels than would be seen in its absence. Even though growth typically stops years prior to the age of 25, there is a period after growth ceases where PFOA 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, 3603279 (PFOA)}. 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 PFOA 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 PFOA levels and extrapolated that finding to clearance via menstruation.

In animals, three classical PK models for PFOA have been published since the original 2016 HESD for PFOA. In Dzierlenga et al. {2020, 5916078}, male Sprague-Dawley rats were dosed with PFOA via oral gavage at 6, 12, and 48 mg/kg, or intravenously at 6 mg/kg. Female Sprague-Dawley rats were dosed with PFOA via oral gavage at 40, 80, 320 mg/kg or intravenously at 40 mg/kg. Following the administration of PFOA, rats were sacrificed from five minutes to 50 days post-dosing for males and from five minutes to 12 days post-dosing in females. Differences in length of study for each sex represents the sex-dependent difference in half-lives where female rats eliminate PFOA more rapidly than males. For both sexes, measured plasma concentrations characterized the biphasic PK curve. From these exposure scenarios, Dzierlenga and coauthors 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 PFOA clearance. For each dosing scenario, a single set of PK parameters were fit making extrapolation to other dosing scenarios difficult. However, the authors demonstrate a significant difference between males and females in beta-phase half-life and overall clearance. This difference in half-life is critical for developmental PK for exposure to a pregnant dam.

Fujii et al. (2015, 2816710) conducted a PK analysis in mice by dosing male and female mice either intravenously with 0.313 $\mu\text{mol/kg}$ or through oral gavage with 3.13 $\mu\text{mol/kg}$. Following administration of PFOA, blood concentrations were collected through tail veins beginning immediately following dosing up to 24 hours post-dosing. Fujii and coworkers used these data to develop a two-compartment model to describe sex-dependent PK in mice. Unfortunately, the study design did not collect samples for long enough to characterize the beta-phase elimination of PFOA. This is reflected in a much larger predicted half-life for PFOA using the reported beta-phase elimination constant (627 days) compared to the half-life reported in Lou et al. (2009, 2919359) (15.6 – 21.7 days) where mouse plasma concentrations were measured up to 80 days post-dosing. In addition, the functional form fit for the oral gavage data in Fujii et al., 2015 reflects a one-compartment model with gavage dosing making it not possible to compare the predict half-lives between the two routes of exposure. While the reported data could be used for characterizing absorption and distribution of PFOA, it cannot be used for characterizing the elimination phase.

Finally, Gomis et al. (2016, 3749264) utilized the functional form of a two-compartment model with oral gavage to predict internal dosimetry of PFOA in rats using PK data from Perkins et al. (2004, 1291118)}. 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 PFOA AND PFOS concentrations in breast milk {Abdallah, 2020, 6316215}. Verner et al. (2016, 3299692) presented another developmental model to predict PFOA 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 developmental model was developed by Goeden et al. (2019, 5080506) to evaluate the relationship between drinking water concentrations and infant serum levels during breastfeeding, due to gestational and lactational transfer of PFOA that had accumulated within the mother. A distinguishing feature of the Goeden et al. (2019, 5080506) model is that it incorporates an adjustment for the increased intracellular water in infants and young children compared to adults, under the assumption that PFAS distribution into tissues,

quantified by the V_d , will increase proportionally to body water. This difference may explain why the ratio of PFOA in cord blood vs. maternal blood at childbirth tends to be less than one. Monroy et al. (2008, 2349575) reported median cord blood PFOA concentration to be 87% of maternal serum), while the median ratio of fetal tissue to placenta PFOA concentration was found to be generally greater than one {Mamsen, 2019, 5080595}. One oversight of this model is that the rate equations are in terms of concentration, but the equations do not account for the decrease in concentration due to increasing body weight (growth dilution). This is a significant factor for infants who grow quickly.

Other unique analyses that extended the one-compartment framework were publications by Shan et al. (2016, 3360127), who estimated the exposure to specific isomers of PFOA using measurements in food, tap water, and dust to estimate the isomeric profiles of the substances in human serum and Convertino et al. (2018, 5080342) who used a 2-compartment PK-pharmacodynamic model to describe changes in serum concentration during a dose-escalation, phase one clinical trial with PFOA and how those serum changes are correlated with changes in serum total cholesterol (TC) and free thyroxine (FT4).

Toxicokinetic models that can accommodate longer half-life values than would be predicted based on standard absorption, distribution, metabolism, and excretion 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}. The underlying assumption for all 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 et al., 2006, 818501} was created using the post-dosing plasma data from the Butenhoff et al. (2004, 3749227) study in cynomolgus monkeys. In this study, groups of six monkeys (three per sex per group) were dosed for 26 weeks with 0, 3, 10, and 20 mg/kg PFOA (and also a high dose of 30 mg/kg PFOA for only the first 12 days) and followed for more than 160 days after dosing. Metabolism cages were used for overnight urine collection. Since urine specimens could account for only overnight PFOA excretion, the total volume and total PFOA were extrapolated to 24-hour values based on the excretion rate (volume per hour) for the volume collected and the hours of collection.

The Andersen et al. (2006, 818501) model was based on the hypothesis that saturable resorption capacity in the kidney would possibly account for the unique half-life properties of PFOA across species and genders. 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 {Andersen, 2006, 818501}.

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 with transfer to the central compartment can occur. Transfer from the filtrate compartment to the central compartment decreases the rate of excretion. The resorption in the model was saturable, meaning that there was proportionally less resorption and greater excretion at high serum PFOA concentrations than at low concentrations. Here, renal excretion decreased below the glomerular filtration rate (GFR) due to the renal resorption. Additionally, excretion was also reduced by

implementing a constant proportion of PFOA that was bound to protein in plasma and was not available for renal filtration.

The model was parameterized using the body weight and urine output of 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 optimized to fit the data for plasma and urine at lower concentrations and then applied for the 20 mg/kg/day dose, which was assumed to represent a concentration at which renal resorption was saturated. Based on the data for the dose of 20 mg/kg/day, the model was able to predict the decline in plasma levels after the cessation of dosing. The predictions were adequate for one of the three modeled monkeys; for the other two monkeys, the model predicted higher serum concentrations than were observed. That discrepancy between model prediction and observations could have occurred because the model did not allow for efflux of PFOA into the glomerular filtrate through transporters on the basolateral surface of the tubular cells. The authors observed that three monkeys had faster CL_R of PFOA than the other three monkeys.

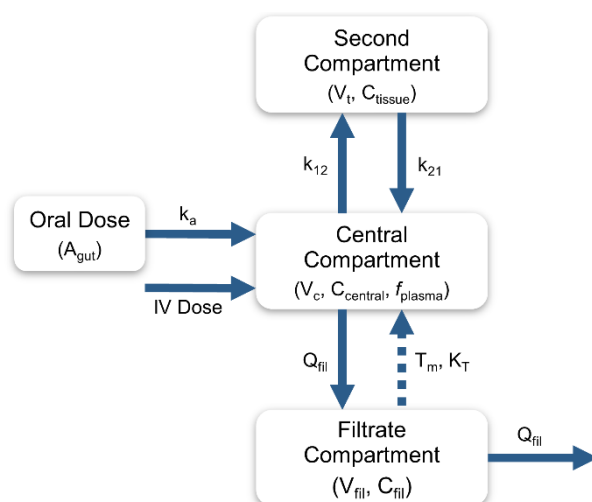


Figure 6. Schematic for a Physiologically Motivated Renal Resorption PK Model

Adapted from {Wambaugh, 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 PFOA {U.S. EPA, 2016, 3603279}. The model was applied to data from studies conducted in monkeys, rats, and mice that demonstrated an assortment of systemic, developmental, reproductive, and immunological effects. A saturable renal resorption term was used. This concept has played a fundamental role in the design of all of the published PFOA 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, but the rate of transfer from serum to tissue was also estimated from the data.

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. This sex assumption was applied to male and female rats to determine sex-specific parameters because of established sex-specific toxicokinetic differences. Conversely, monkeys and mice were only grouped by species and strain. Body weight, the number of doses, and magnitude of the doses were the only parameters varied for different studies. 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* PFOA PK studies. The monkey PK data were derived from Butenhoff et al. (2004, 3749227), and the data for the rats (M/F) were from Kemper et al. (2003, 6302380). Two strains of female mice were analyzed separately, with CD1 information derived from Lou et al. (2009, 2919359) and C57Bl/6 information derived from DeWitt et al. (2008, 1290826). The data were analyzed within a Bayesian framework using Markov Chain Monte Carlo sampler implemented as an R package developed by EPA to allow predictions across species, strains, and genders and to 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 is 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 PFOA 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 2-compartment model), an absorption compartment, and a renal filtrate compartment with saturable renal resorption {Tan, 2008, 2919374}. The work of Tan et al. (2008, 2919374) was a development of the earlier work of Andersen et al. (2006, 818501) previously discussed. The PBPK model of Loccisano and colleagues then extended this “biologically motivated” model by

the addition of discrete tissue compartments, rather than a single compartment representing all tissues.

A series of follow-up studies applied the Loccisano and coauthors' model structure, with extensions, to address how PK variation in human 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 PFOA 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 PFOA 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 PFOA levels, and Dzierlenga et al. (2020, 6315786) and Dzierlenga et al. (2020, 6833691) who applied a model of thyroid disease {Dzierlenga, 2019, 7947729} to describe changes in PFOA AND PFOS CL_R 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 a region in Spain. The use of human tissue data is relatively rare due to the challenges in sourcing human tissue 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 to blood PCs 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 human 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 PFOA 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 PFOA is the estimation of the chemical-dependent parameters such as those involved in protein binding and CL_R . One way to achieve this goal is to perform in vitro experiments 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 PFOA. Cheng et al. (2017, 3981304) went farther in their use of in vitro data and used measurements of PFOA interactions with binding proteins, as well the measured rates of several transporters to parameterize a rat PBPK model.

There are no new animal PBPK models for PFOA published since the original 2016 EPA assessment {U.S. EPA, 2016, 3603279}. See the 2016 HESD {U.S. EPA, 2016, 3603279} for a more in-depth review of PFOA PBPK models.

3.2.3 Half-Life Data

Many half-life values have been reported for the clearance of PFOA in humans, as reviewed in Section D.4.5. The slow excretion of PFOA 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 PFOA 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 PFOA 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 depend on the ratio of the prior and ongoing exposures. That is, for a given ongoing exposure, a high prior exposure will be less prone to bias than a lower prior exposure. 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, because of non-linearities in PK that may occur due to the saturation of PFAS-protein interactions. This will likely take the form of an under-estimation of the half-life that is relevant to lower levels, that are more representative of the general population, due to saturation of renal resorption and increased urinary clearance in the study population.

We chose to select a reported half-life value from an exposure to a study population that is demographically representative of the general population, with a clear decrease in exposure at a known time, with a high number of participants and a long follow-up time. Based on these criteria, a half-life of 2.7 years was determined for PFOA as reported in Li et al. (2017, 9641333; 2018, 4238434). This value comes from a large population (n=455) who originally had contaminated drinking water for which the study documents the decrease in exposure levels after the installation of filtration with an average final serum sample taken 3.9 years after the beginning of water filtration. Li et al. (2018, 4238434) also reported a similar half-life of 2.7 years for PFOA in a separate community with a similar study design. In that study, serial blood samples were collected from participants after the beginning of drinking water filtration after a long period of exposure to drinking water contaminated with PFOA. This second study involved 106 participants with a median number of 6 samples per person but with only a two-year observation period. The good agreement between the second study and the previous, larger study in diverse populations support the use of this value as a good estimate of the PFOA elimination half-life.

A summary of PFOA 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 of PFOA, 170 ml/kg, was sourced from Thompson et al. {2010, 2919278} who used a one-compartment PK model. This calculation involves several assumptions: that the participants' serum concentrations are at steady-state, that their exposure can be estimated from the drinking water concentration in their community, that there is 91% bioavailability for exposure from drinking water, and that the half-life of PFAS is 2.3 years, which comes from the report of Bartell et al. (2010, 379025). EPA considered updating this parameter to 200 ml/kg, which is the value that would be calculated using the EPA chosen half-life value of 2.7 years. However, the value of 2.3 years was calculated under very similar conditions as the other data in the Thompson et al. (2010, 2919278) population and 2.3 years

may better reflect the clearance rate in that specific population at that time. This calculation was performed in a population with PFOA contamination. V_d is a parameter that is relatively easily obtained from an analysis of PK data from controlled experimental studies, 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 for PFOA from oral dosing in monkeys was 140 ml/kg {Andersen, 2006, 818501}.

Another group has approached the calculation of V_d by taking the average of reported animal and human values and reported values of 200 ml/kg for PFOA {Gomis, 2017, 3981280}. This calculation included the V_d value from Thompson et al. (2010, 2919278) and did not include additional values derived from human data. The resulting average value shows that the value from Thompson et al. (2010, 2919278) is reasonable, and we selected the Thompson et al. (2010, 2919278) result based on the fact that it is the only value derived from human data that EPA considers to be reliable for risk estimation in the general population. Dourson et al. (2021) also reported a human V_d value from a clinical trial undertaken with PFOA. Their reported value of 91 ml/kg for PFOA was much lower than other reported values (across mammalian species) and may reflect an earlier initial distribution step rather than the distribution observed after chronic exposure. A longer, chronic exposure may result in a greater distribution to the tissues relative to the plasma, and this process may be slowed by the extensive binding to plasma proteins. Additionally, the exposure doses seen in the clinical trial are much higher than typically seen in the general population, which could result in a different distribution profile.

A summary of PFOA V_d values is presented in Section D.2.5 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

This section describes studies of PFOA 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.

3.3.1.1.2 Study Evaluation Considerations

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., GFR and blood volume changes over the course of pregnancy) because these factors may be related to both PFOA levels and the developmental effects examined here. More confidence was placed in the epidemiologic studies that adjusted for GFR 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 PFOA in humans noted in section 3.2.3, samples collected during all three trimesters, before birth or 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 (BWT) 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, BWT 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 PFOA 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 BWT, small for gestational age (SGA), ponderal index [i.e., BWT grams)/birth length (cm³) × 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 BWT and BWT-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 BWT such as mean BWT (or variations of this endpoint such as standardized BWT z-scores), as well as binary measures such as SGA (e.g., lowest decile of BWT stratified by gestational age and other covariates) and low BWT (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 PFOA 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 (PTB, 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 PFOA exposure. While they could impact precision and study sensitivity, they were not considered a major concern for risk of bias.

3.3.1.1.2.1 Study Inclusion

Sixty-one developmental epidemiological studies of PFOA that were not included in the 2016 HESD report have been identified based on a literature search from October 2020. Although every study is included in the endpoint-specific study quality evaluation heat maps for comprehensiveness, five studies identified in the literature search were excluded from the mean BWT synthesis due to study population overlap with other included studies (i.e., were considered duplicative). For example, the Bjerregaard-Olesen et al. (2019, 3045435) study from the Aarhus birth cohort overlaps with Bach et al. (2016, 3981534). 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, 2015, 2850268; Kobayashi, 2017, 3981430; Minatoya, 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 as Kashino et al. (2020, 6311632). For those studies with the same endpoints analyzed across different subsets from the same cohort, such as mean BWT, the analysis with the largest sample size was used in forest plots and tables (e.g., {Kashino, 2020, 631163} for the Hokkaido birth cohort study). 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, 2017, 4238465}. 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 additional studies with overlapping cohorts were all included in the synthesis, as they 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 the authors provided additional mean BWT data. The mean BWT results for singleton and twin births from Bell et al. (2018, 5041287) are included in forest plots here, while the postnatal growth trajectory data in the same UPSTATE KIDS cohort by Yeung et al. (2019, 5080619) are also included as they target different developmental endpoints.

Following exclusion of the five duplicative studies, 56 studies were available for the synthesis. Five additional studies {Alkhalawi, 2016, 3859818; Jin, 2020, 6315720; Lee, 2013, 3859850; Lee, 2016, 3981528; Maekawa, 2017, 4238291} were considered uninformative due to critical deficiencies in some risk of bias domains (e.g., confounding) or multiple domain deficiencies and are not further examined here. Thus, 51 studies were included across various developmental endpoints for further examination and synthesis.

Thirty-eight of the 51 studies examined PFOA in relation to fetal growth restriction measured by the following endpoints: SGA, low BWT, head circumference, as well as mean and standardized BWT and birth length measures. Seventeen studies examined gestation duration, three examined fetal loss, and two examined birth defects.

3.3.1.1.3 Growth Restriction: Fetal Growth

3.3.1.1.3.1 Birth Weight

Among the fetal growth restriction studies, 35 total studies examined either mean BWT, standardized BWT z-score measures, or both. Twenty-five of the 35 studies examined mean BWT only {Bell, 2018, 5041287; Buck Louis, 2018, 5080619; Callan, 2016, 3858524; Cao, 2018, 5080197; Chu, 2020, 6315711; de Cock, 2016, 3045435; Gao, 2019, 5387135; Govarts, 2016, 3230364; Hjermitslev, 2020, 5880849; Kashino, 2020, 6311632; Kwon, 2016, 3858531; Lauritzen, 2017, 3981410; Lenters, 2016, 5617416; Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Marks, 2019, 5081319; Robledo, 2015, 2851197; Shi, 2017, 3827535; Starling, 2017, 3858473; Valvi, 2017, 3983872; Wang, 2016, 3858502; Woods, 2017, 4183148; Workman, 2019, 5387046; Wu, 2012, 2919186; Xu, 2019, 5381338}, three examined standardized BWT z-score measures only {Chen, 2017, 3981292; Shoaff, 2018, 4619944; Xiao, 2019, 5918609}, and seven examined both mean BWT and standardized BWT z-score measures in relation to PFOA exposures {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Gyllenhammar, 2018, 4238300; Meng, 2018, 4829851; Sagiv, 2018, 4238410; Wang, 2019, 5080598; Wikström, 2019, 6311677} (Figure 11, Figure 12).

Twenty-two of the 32 mean BWT studies shown on Figure 11 and Figure 12 were prospective birth cohort studies and the remaining ten 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; Wu, 2012, 2919186; Xu, 2019, 5381338}. Overall, eight of these PFOA 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, 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}. Nineteen 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 {Buck Louis, 2018, 5016992; Lauritzen, 2017, 3981410}, three {Callan, 2016, 3858524; Chu, 2020, 6315711; Kashino, 2020, 6311632; Valvi, 2017, 3983872; Wang, 2016, 3858502; Wu, 2012, 2919186}, or across multiple trimesters ({Hjermitslev, 2019, 5880849; Lenters, 2016, 5617416; Marks, 2019, 5081319; Starling, 2017, 3858473; Wikström, 2019, 6311677; Woods, 2017, 4183148}). The study by {Meng, 2018, 4829851} pooled exposure data from two study populations, one which measured PFOA in umbilical cord blood and one which measured PFOA 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 provided by study authors were used.

Fourteen of the 32 included mean BWT studies were rated *high* in overall study confidence {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Bell, 2018, 5041287; Buck Louis, 2018, 5016992; 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; Wang, 2016, 3858502; Wikström, 2019, 6311677}, while 10 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; Woods et al., 2017, 4183148} and eight were classified as *low* {Callan, 2016, 3858524; Cao, 2018, 5080197; Gao, 2019, 5387135; Marks, 2019, 5081319; Shi, 2017, 3827535; Workman, 2019, 5387046; Wu, 2012, 2919186; Xu, 2019, 5381338} (Figure 11, Figure 12).

Of the 24 *high* or *medium* confidence studies detailed in this synthesis, two had *deficient* study sensitivity {Bell, 2018, 5041287; de Cock, 2016, 3045435}. Seven studies {Lauritzen, 2017, 3981410; Lenters, 2016, 5617416; Robledo, 2015, 2851197; Starling, 2017, 3858473; Wang, 2016, 3858502; Wikström, 2019, 6311677; Woods et al., 2017, 4183148} were considered to have *good* study sensitivity, and fifteen studies {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Buck Louis, 2018, 5016992; Chu, 2020, 6315711; Govarts, 2016, 3230364; Gyllenhammar, 2018, 4238300; Hjermitslev, 2019, 5880849; Kashino, 2020, 6311632; Kwon, 2016, 3858531; Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Meng, 2018, 4829851; Sagiv, 2018, 4238410; Valvi, 2017, 3983872; Wang, 2019, 5080598} were considered *adequate* (Figure 11, Figure 12). The median exposure values across all studies ranged from 0.86 ng/mL {Callan, 2016, 3858524} to 16.95 ng/mL {Wu, 2012, 2919186}.

3.3.1.1.3.1.1 Mean Birth Weight Study Results: Overall Population Studies

Twenty-seven of the 32 included studies with mean BWT data examined data in the overall population, while five were sex-specific studies only {Ashley-Martin, 2017, 3981371; Lind, 2017, 3858512; Marks, 2019, 5081319; Robledo, 2015, 2851197; Wang, 2016, 3858502}. Twenty-one of the twenty-seven studies reported some mean BWT deficits in the overall population, albeit these were not always statistically significant. Two of these mean BWT studies in the overall population reported null associations {Bach, 2016, 3981534, Buck Louis, 2018, 5016992; Gao, 2019, 5387135}, while three reported increased mean BWT deficits {de Cock,

2016, 3045435; Shi, 2017, 3827535; Xu, 2019, 5381338}. Among the 21 studies showing some adverse associations in the overall population, there was a wide distribution of deficits ranging from 11 to 267 grams across both categorical and continuous exposure estimates with results based on a per unit (continuous measure) when studies presented both. Sixteen of these 21 studies reported deficits from 26 to 127 grams. Few definitive patterns were observed among studies based on sample timing and overall study confidence, although the three largest deficits were among two studies with umbilical cord or post-birth maternal blood samples (1 *medium* and 2 *low* confidence studies). Among the seven studies presenting results based on categorical data, two studies {Meng, 2018, 4829851; Starling, 2017, 3858473} showed monotonic exposure-response relationships (Figure 7, Figure 8).

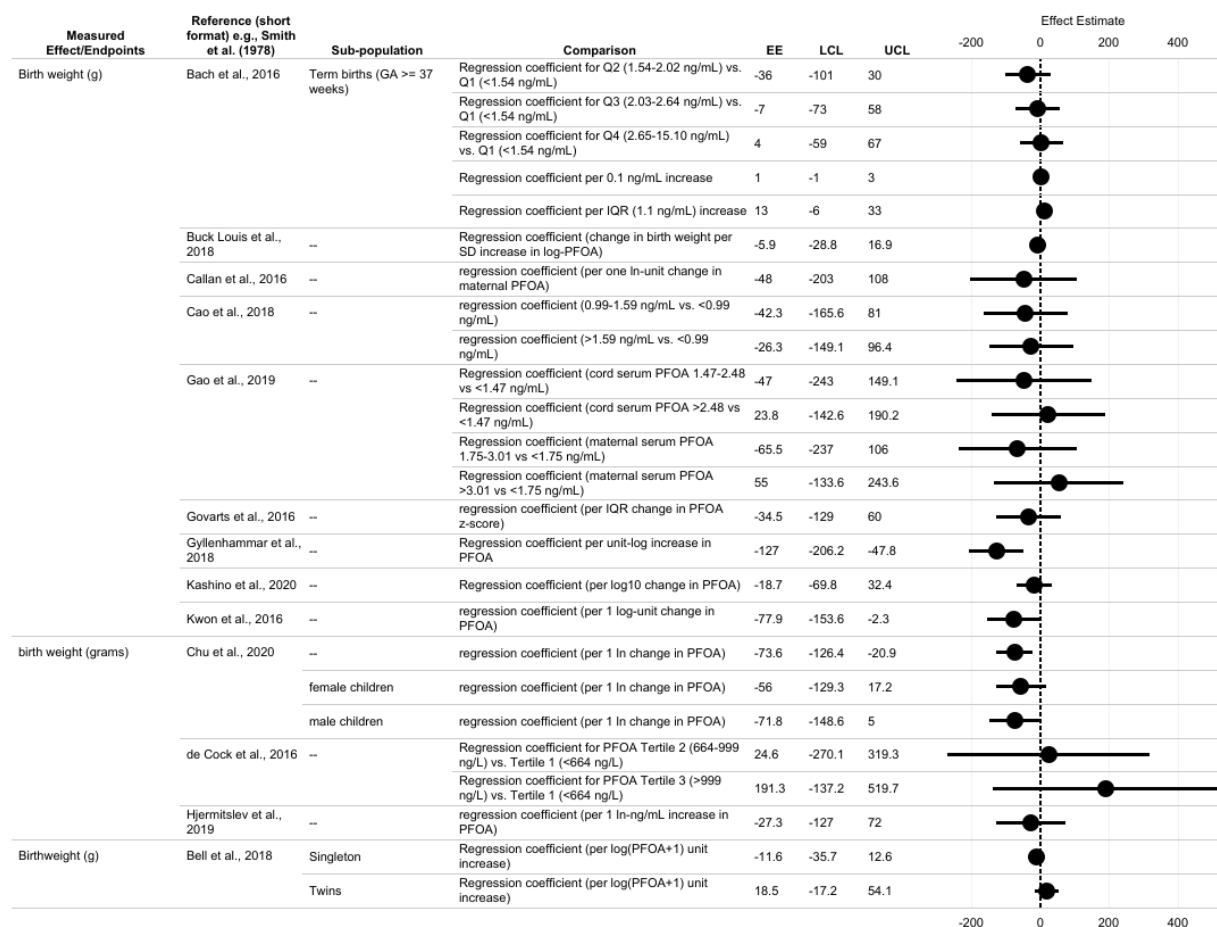


Figure 7. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA

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

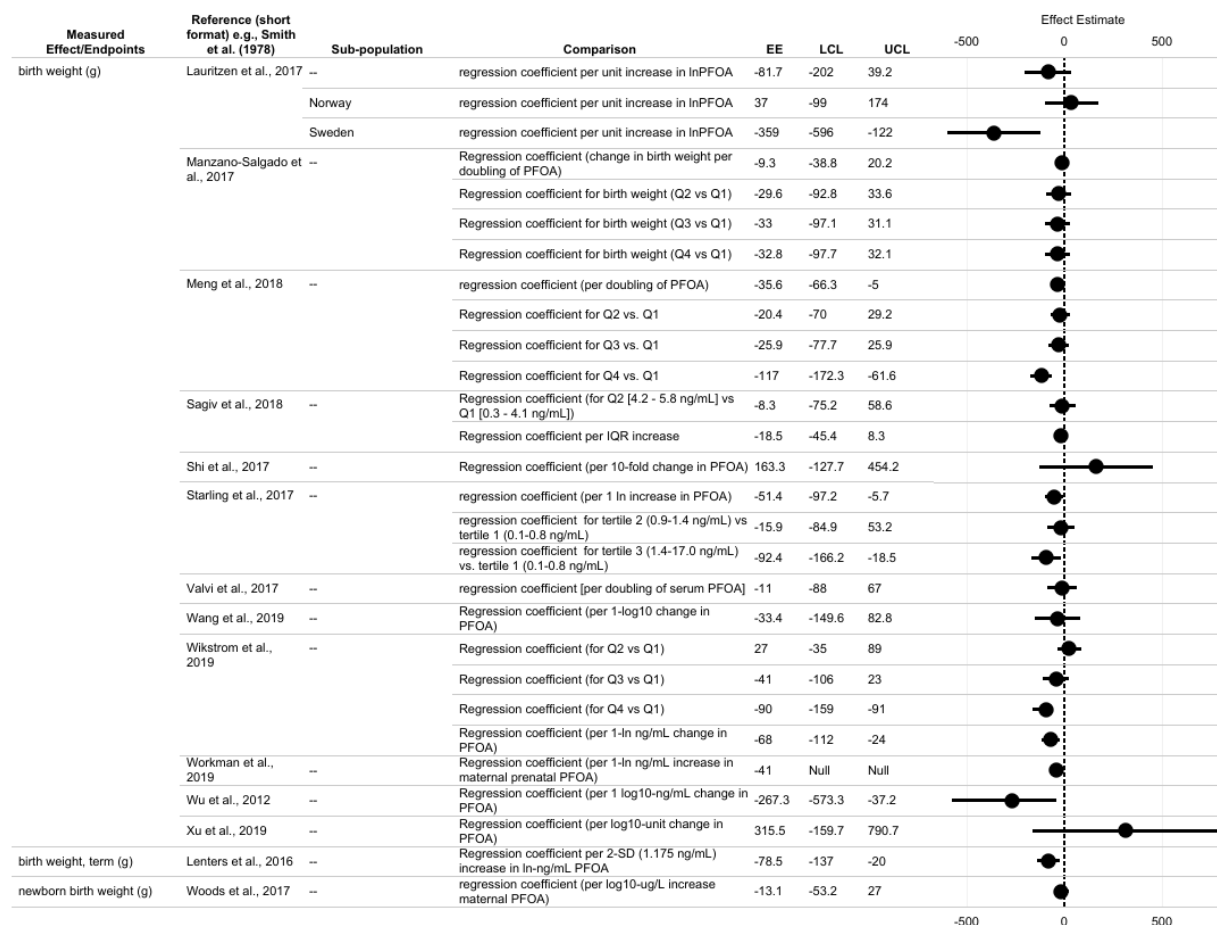


Figure 8. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA (Continued)

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

3.3.1.1.3.1.2 Mean Birth Weight Study Results: Sex Specific Studies

Twenty-one epidemiological studies also examined sex-specific results, with four studies {Bach, 2016, 3981534; de Cock, 2016, 3045435; Lind, 2017, 3858512; Shi, 2017, 3827535} not showing any evidence of adverse associations. The *low* confidence study by Marks et al. (2019, 5081319) which only had data on male neonates reported large deficits in the upper two PFOA tertiles (-53 and -46 grams, respectively) with no exposure-response relationship. Among these 20 studies, examining relationships in both male and female neonates, sixteen studies reported some deficits in either or both sexes. Three studies reported mean BWT deficits only in boys {Kashino, 2020, 6311632; Manzano-Salgado, 2017, 4238465; Valvi, 2017, 3983872}, while four studies reported larger deficits in girls {Hjermitslev, 2019, 5880849; Robledo, 2015, 2851197; Wang, 2016, 3858502; Yeung, 2019, 5080619}. Among the nine studies showing deficits in both sexes, two studies reported sex-specific deficits comparable in magnitude among boys and girls {Lenters, 2016, 5617416; Li, 2017, 3981358}. Four studies {Ashley-Martin, 2017, 3981371; Cao, 2018, 5080197; Wang, 2019, 5080598; Wikström, 2019, 6311677} showed larger deficits among girls and three showed larger deficits among boys {Chu, 2020, 6315711; Lauritzen, 2017, 3981410; Meng, 2018, 4829851}.

No consistent patterns in magnitude of deficits were observed with the sex-specific studies. Although other studies based on different exposure measures were more variable, some consistency was seen amongst four studies {Ashley-Martin, 2017, 3981371; Wang, 2016, 3858502; Wang, 2019, 5080598; Wikström, 2019, 6311677} in girls (range: –80 to –90 g) based on continuous (i.e., per each ln or log10 increases) PFOA exposures. The magnitude of deficits in boys across seven studies {Ashley-Martin, 2017, 3981371; Kashino, 2020, 6311632; Lenters, 2016, 5617416; Manzano-Salgado, 2017, 4238465; Meng, 2018, 4829851; Wang, 2019, 5080598; Wikström, 2019, 6311677} were fairly consistent per each continuous unit PFOA change (range: –21 to –49 g), although four studies {Chu, 2020, 6315711; Lauritzen, 2017, 3981410; Li, 2017, 3981358; Valvi, 2017, 3983872} reported deficits larger in magnitude (Figure 9, Figure 10).

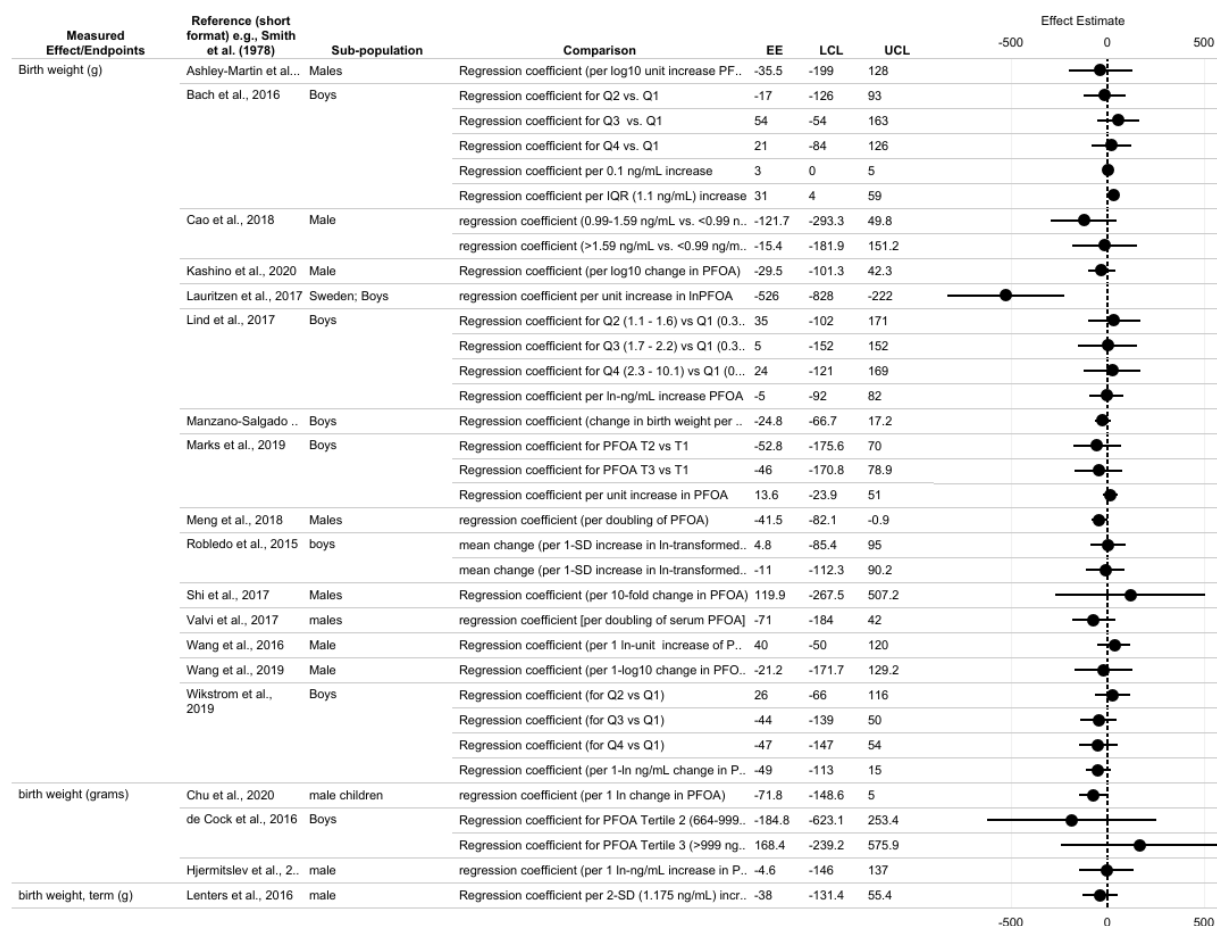


Figure 9. Mean Birth Weight in Males Following Exposure to PFOA

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

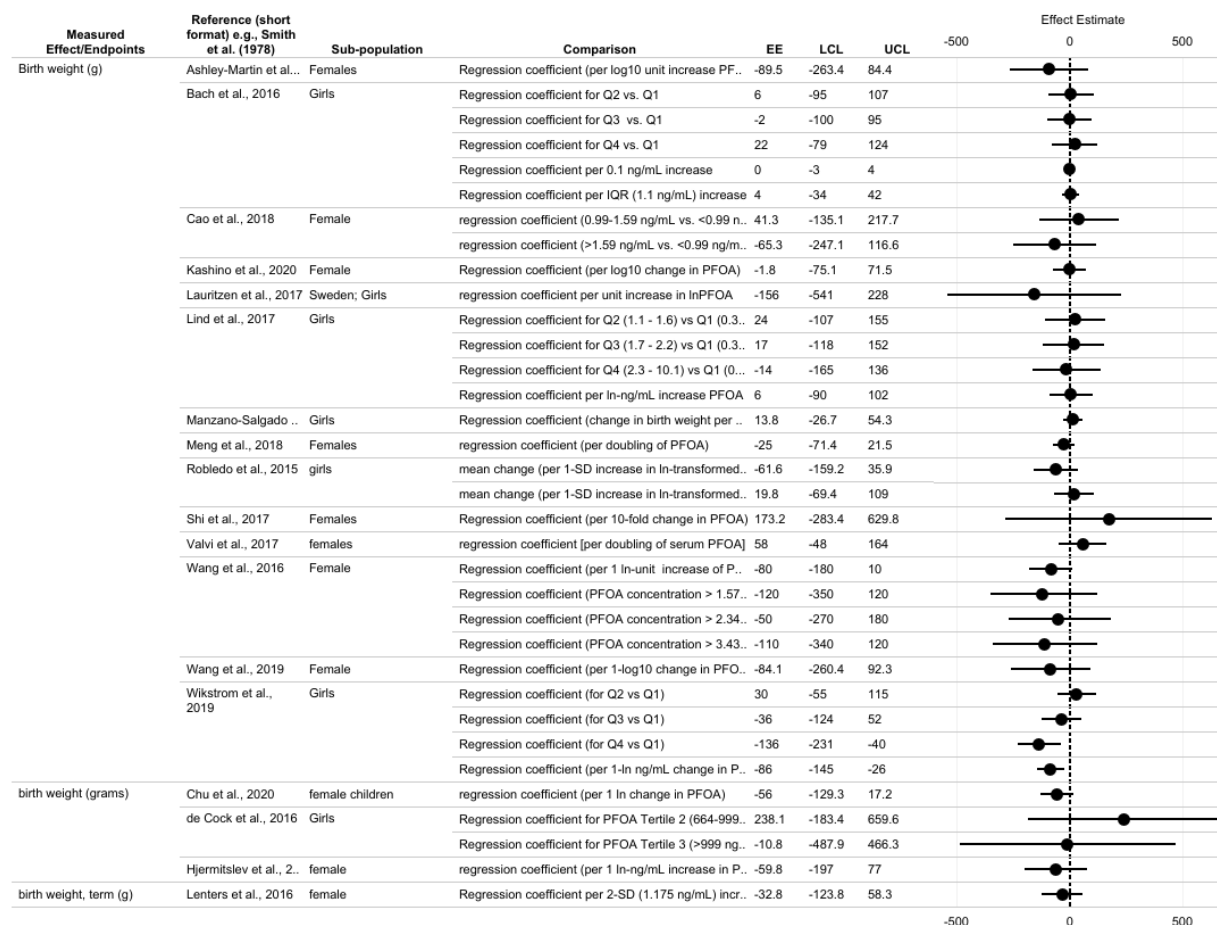


Figure 10. Mean Birth Weight in Females Following Exposure to PFOA

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

3.3.1.1.3.1.3 Birth Weight z-scores

Ten studies examined for BWT z-scores in relation to PFOA {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 11, Figure 12). No associations were reported between BWT-z scores and PFOA exposures in six studies {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Gyllenhammar, 2018, 4238300; Meng, 2018, 4829851; Sagiv, 2018, 4238410; Wang, 2019, 5080598}.

Among the four studies that showed adverse associations between PFOA exposures and BWT z-scores, three of these were *high* {Shoaff, 2018, 4619944; Wikström, 2019, 6311677; Xiao, 2019, 5918609} confidence and one was *medium*. The *medium* confidence study by Chen et al. (2017, 3981292) reported an adverse association in males only (-0.15; 95% CI: -0.30, -0.006). Shoaff et al. (2018, 4619944) reported an association similar in magnitude for their overall population (-0.15; 95% CI: -0.40, 0.01). Compared to quartile 1, Wikström et al. (2019, 6311677) reported adverse associations in quartile 4 in the overall population (-0.20; 95% CI: -0.36, -0.05); these results seemed to be driven by associations detected in female neonates (-0.30; 95% CI: -0.51,

–0.09). Among the four studies showing some deficits, the largest associations were detected in Xiao et al. (2019, 5918609) for the overall population (–0.39; 95% CI: –0.79, –0.01), male neonates (–0.29; 95% CI: –0.55, –0.01), and female neonates (–0.20; 95% CI: –0.57, 0.16). None of the four different studies with categorical data showed any strong evidence of exposure-response relationships but one study {Wikström, 2019, 6311677} showed increasing risk among quartiles 3 and 4 among girls and the overall population (Figure 13, Figure 14, Figure 15).

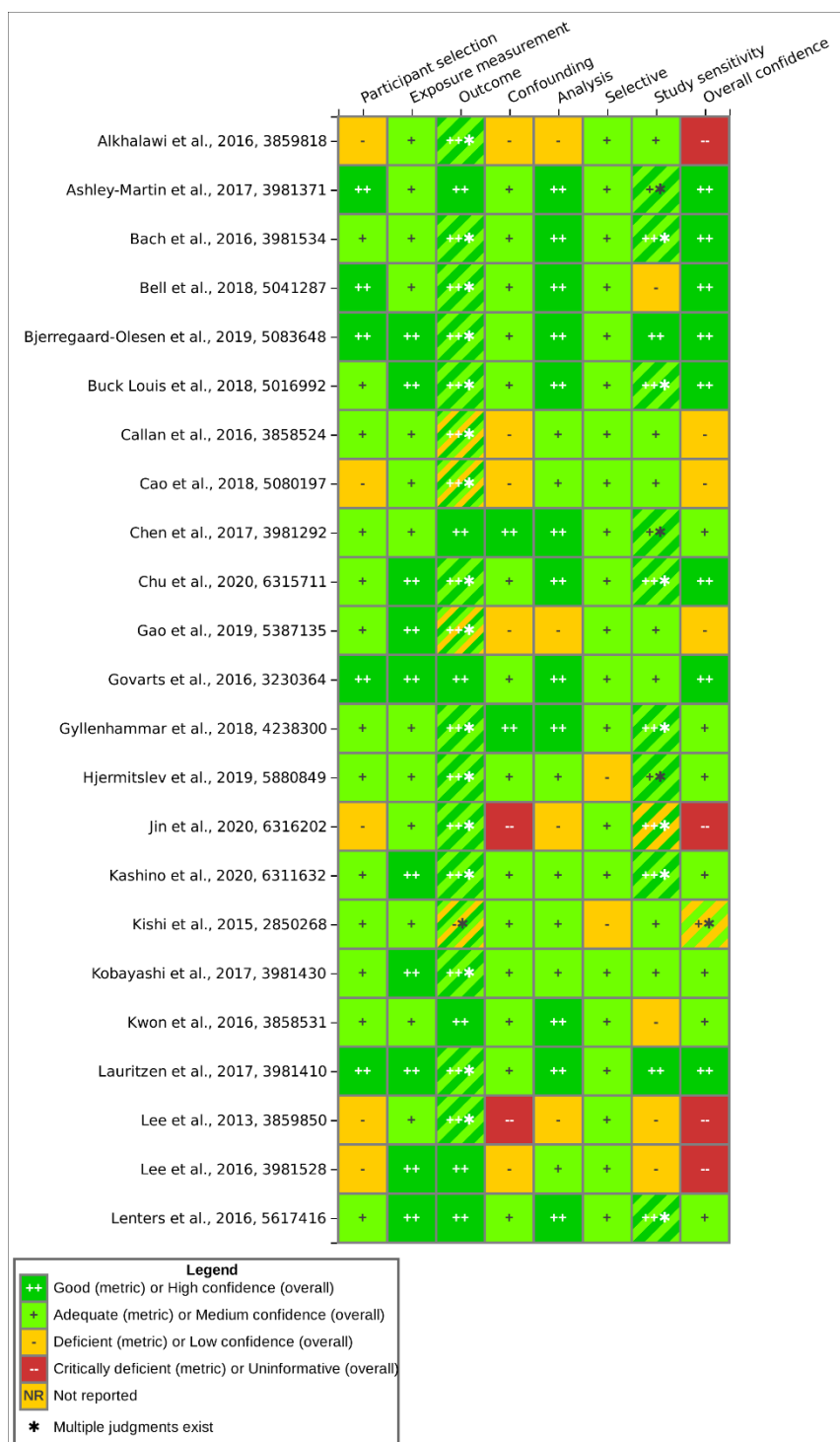


Figure 11. Summary of Study Evaluation for Epidemiology Studies of PFOA and Birth Weight Effects

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

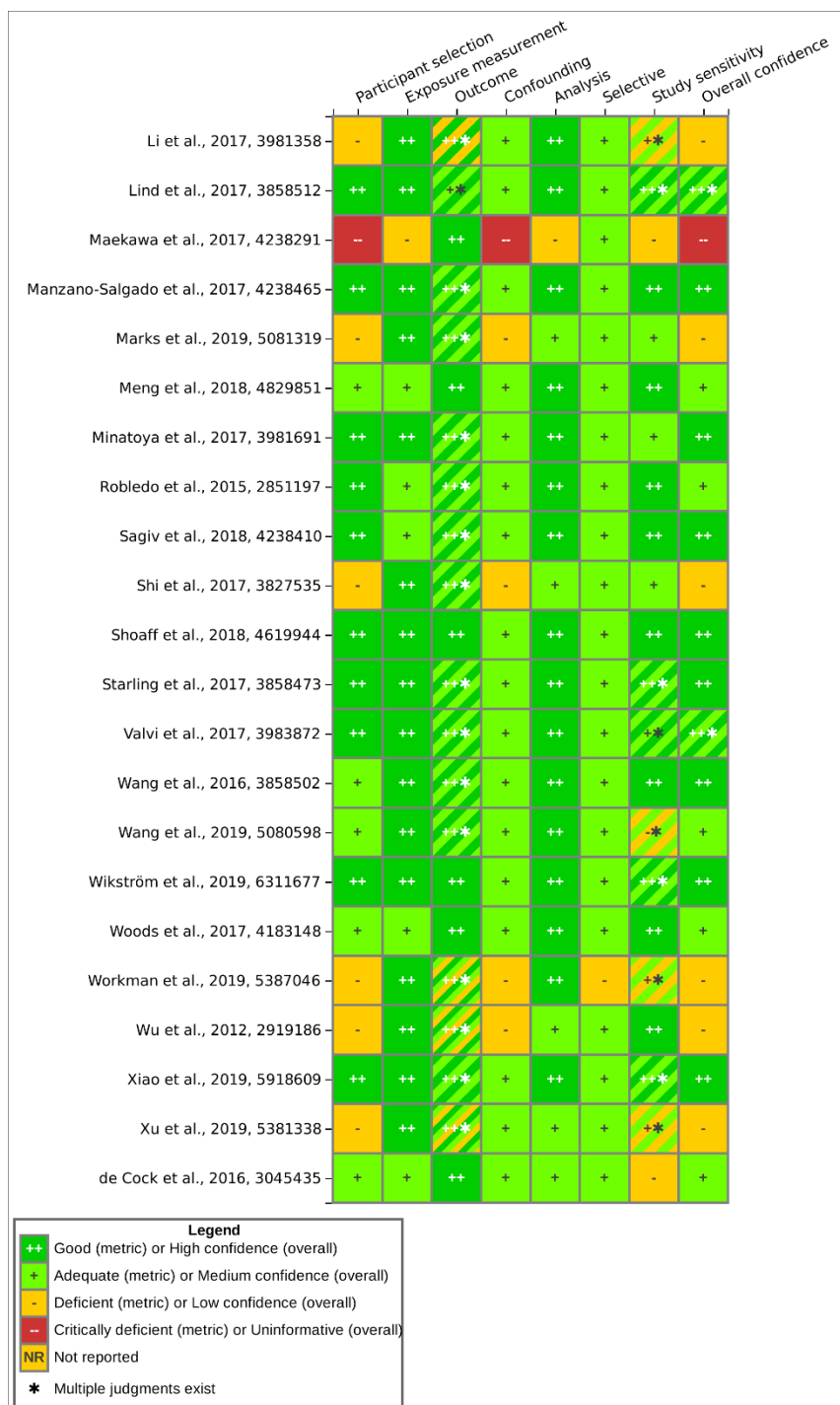


Figure 12. Summary of Study Evaluation for Epidemiology Studies of PFOA and Birth Weight Effects (Continued)

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

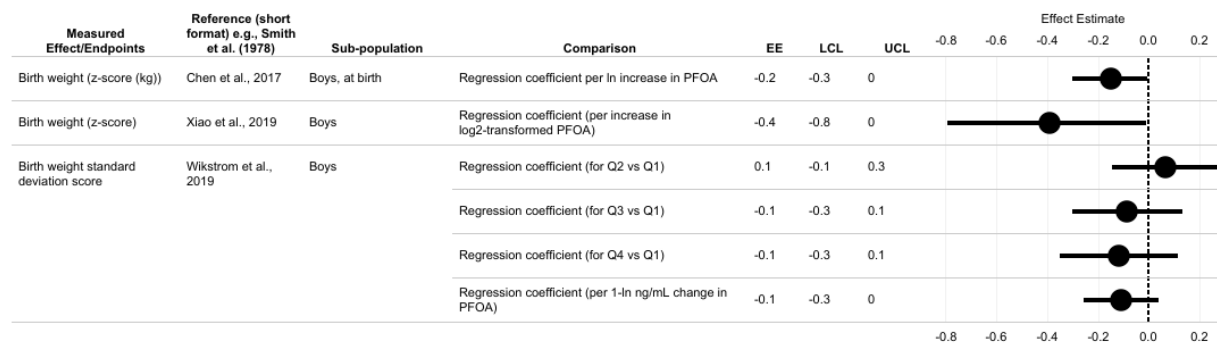


Figure 13. Birth Weight Z-scores in Males Following Exposure to PFOA

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

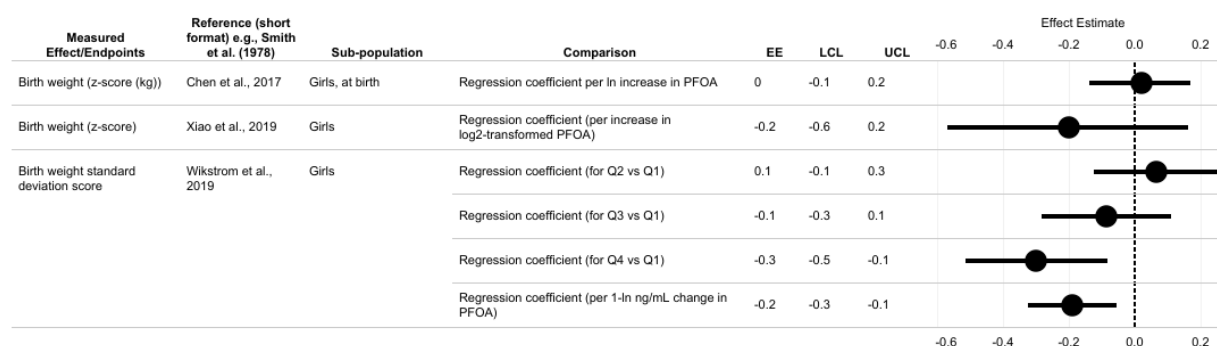


Figure 14. Birth Weight Z-scores in Females Following Exposure to PFOA

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

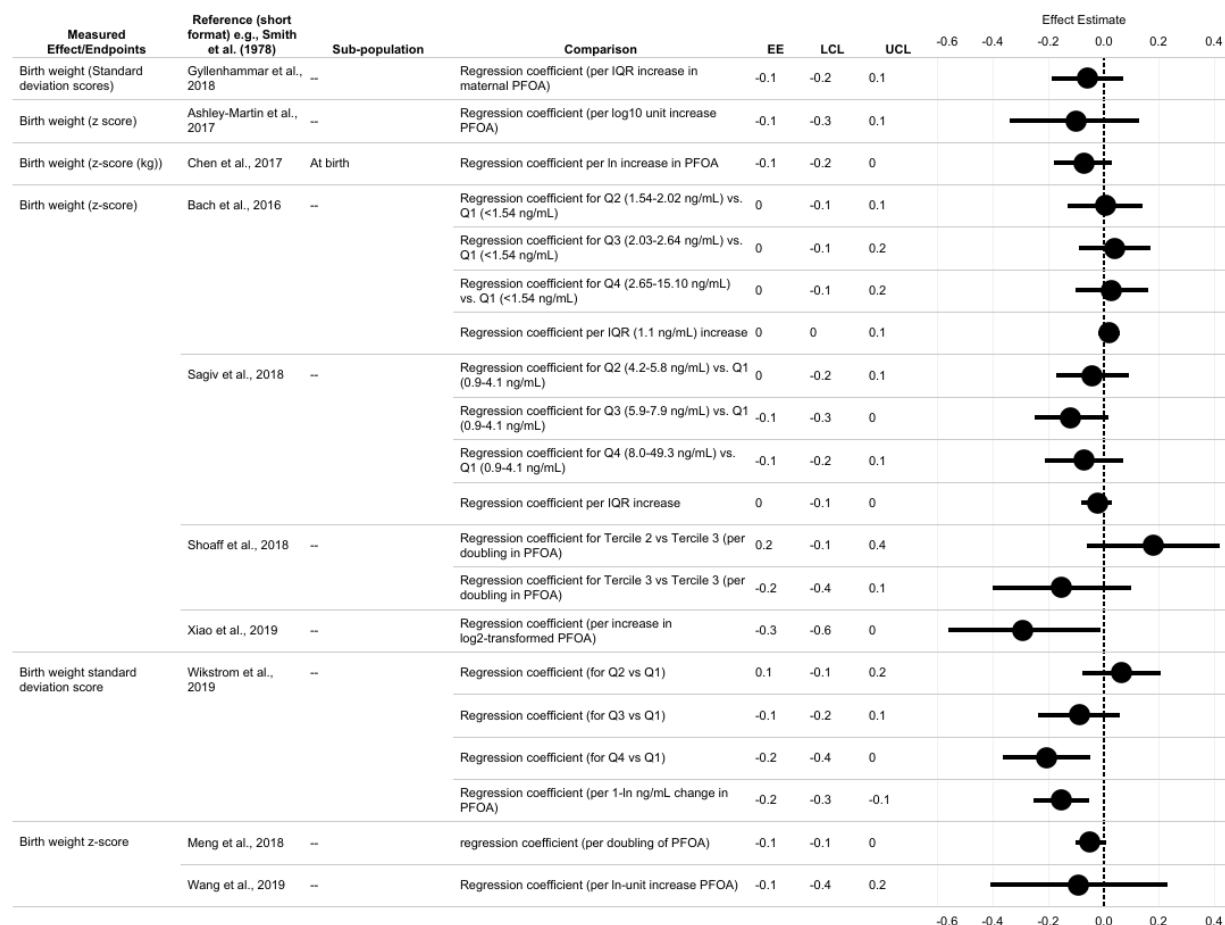


Figure 15. Overall Birth Weight Z-scores from Epidemiology Studies Following Exposure to PFOA

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

3.3.1.1.3.2 Small for Gestational Age/Low Birth Weight

Ten epidemiological studies examined associations between PFOA exposure and different dichotomous fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints), low birth weight (LBW), or both (i.e., {Manzano-Salgado, 2017, 4238465}). Five studies were *high* confidence {Chu, 2020, 6315711; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Wang, 2016, 3858502; Wikström, 2019, 6311677}, three were *medium* confidence {Govarts, 2018, 6311677, Hjermitsev, 2019, 5880849; Meng, 2018, 4829851} and two were *low* confidence studies {Souza, 2020, 6833697; Xu, 2019, 5381338}. Five of these studies had *good* study sensitivity {Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Meng, 2018, 4829851; Wang et al. 2016, 3858502; Wikström, 2019, 6311677}, while two were considered *adequate* {Chu, 2020, 6315711; Hjermitsev, 2019, 5880849} and three were *deficient* {Govarts, 2018, 6311677; Souza, 2020, 6833697; Xu, 2019, 5381338}.

Five of the seven SGA studies {Govarts, 2018, 6311677; Lauritzen, 2017, 3981410; Souza, 2020, 6833697; Wang, 2016, 3858502; Wikström, 2019, 6311677} showed some adverse associations, while two studies were entirely null {Manzano-Salgado, 2017, 4238465; Xu, 2019,

5381338} (Figure 16). Although they were not always statistically significant, the relative risks reported in the four studies examining the overall population based on either categorical or continuous exposures (per each unit increase) were fairly consistent in magnitude (odds ratio (OR) range: 1.44 to 2.81). Govarts et al. (2018, 6311677) reported an increased risk (OR = 1.64; 95% CI: 0.97, 2.76) per each PFOA IQR increase. Lauritzen et al. (2017, 3981410) showed a slight increased risk in the overall population (OR = 1.21; 95% CI: 0.69, 2.11) per each ln-unit PFOA increase, but this was driven by associations only in participants from Sweden (OR = 5.25; 95% CI: 1.68, 16.4). The high confidence study by Wang et al. (2016, 3858502) showed and increased risk (OR = 1.48; 95% CI: 0.63, 3.48 per each log₂ unit increase) for SGA among girls only. One {Souza, 2020, 6833697} of the three studies examining exposure quartiles detected an exposure-response relationship in the overall population (OR range: 1.26–2.81). Another study by Wikström et al. (2019, 6311677) did not detect an exposure-response relationship but reported increased risk in the upper two quartiles (OR range: 1.64–2.33) for female neonates only.

Two of four LBW studies {Manzano-Salgado, 2017, 4238465; Meng, 2018, 4829851} showed some associations within the overall population, and/or in boys or girls (Figure 16). Meng et al. (2018, 4829851) reported non-significant increased ORs (range: 1.2–1.5) across all quartiles but saw no evidence of an exposure-response relationship. One study showed some suggestion of an increased risk (OR = 1.67; 95% CI: 0.72, 3.86) for term LBW but was detected among boys only {Manzano-Salgado, 2017, 4238465}.

Overall, seven of the ten different studies examining either SGA or LBW or both showed some increased risks with increasing PFOA exposures. The magnitude of the associations was typically from 1.2 to 2.8 with limited evidence of exposure-response relationships among the categorical studies. Although the number of studies was small, few discernible patterns across study characteristics or confidence were evident across the SGA or LBW findings. Collectively, the majority of SGA and LBW studies were supportive of an increased risk with increasing PFOA exposures.

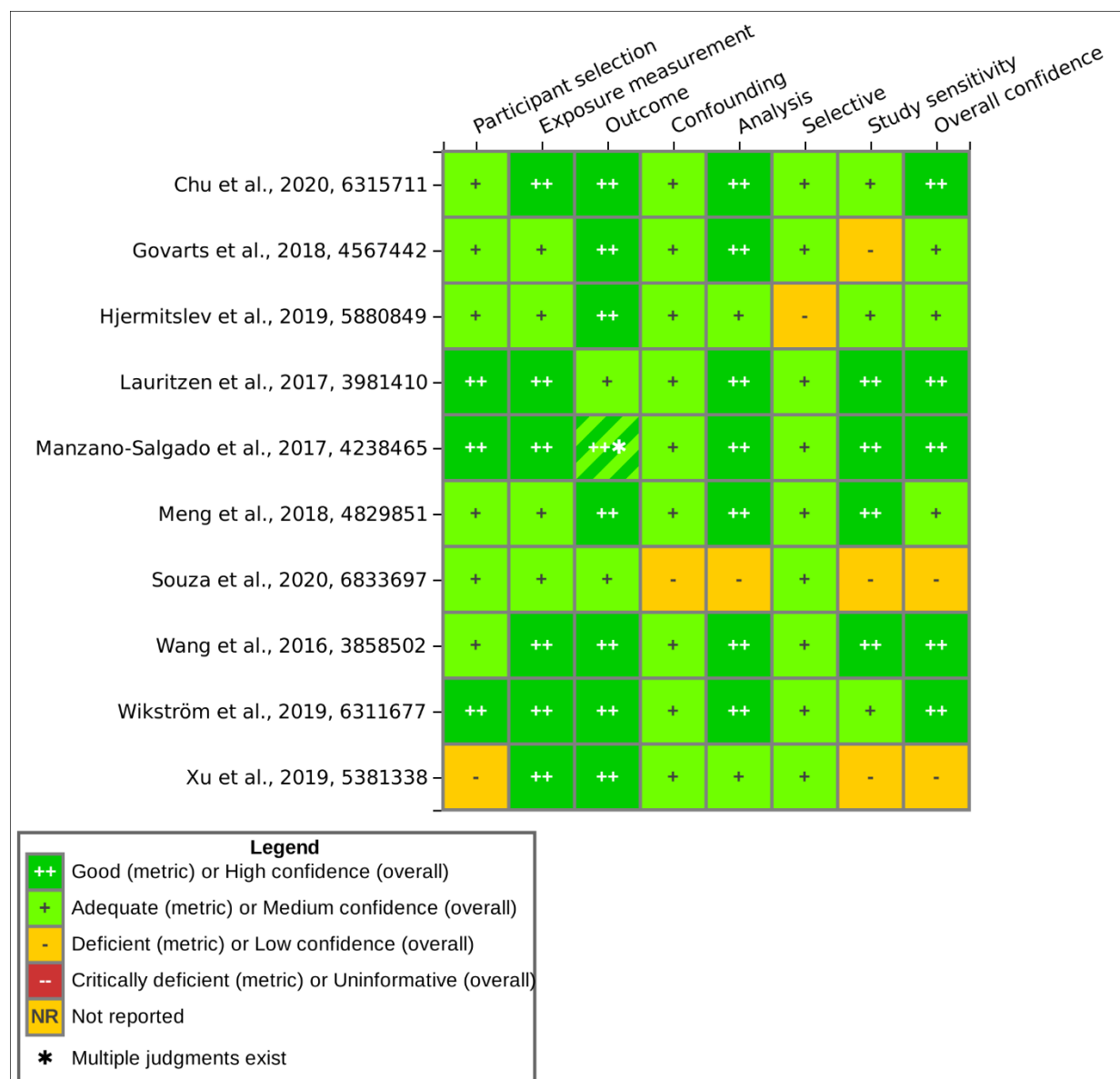


Figure 16. Summary of Study Evaluation for Epidemiology Studies of PFOA and Small for Gestational Age and Low Birth Weight Effects^a

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

^aManzano-Salgado, 2017, 4238465: High confidence for SGA; medium confidence for LBW

3.3.1.1.3.3 Birth Length

As shown in Figure 17 and Figure 18, twenty-nine birth length studies were considered as part of the study quality evaluation. Twenty-three non-overlapping informative studies examined birth length in relation to PFOA with four studies examining standardized birth length measures {Chen, 2017, 3981292; Gyllenhammar, 2018, 4238300; Shoaff, 2018, 4619944; Xiao, 2019, 5918609}, and two evaluating both measures {Wang, 2019, 5080598; Workman, 2019, 5387046}. Sixteen studies examined mean birth length differences in the overall study population (Figure 19, Figure 22). Eleven studies examined sex-specific data with three studies

{Marks, 2019, 5081319; Robledo, 2015, 2851197; Wang, 2016, 3858502} reporting only sex-specific results (Figure 20, Figure 21).

Nine of the 23 different studies were high confidence {Bach, 2016, 3981534; Bell, 2018, 5041287; Buck Louis et al, 2018; Lauritzen et al. 2017, 3981410; Manzano-Salgado, 2017, 4238465; Shoaff, 2018, 4619944; Valvi, 2017, 3983872; Wang, 2016, 3858502; Xiao, 2019, 5918609}, six were medium {Chen, 2017, 3981292; Gyllenhammar, 2018, 4238300; Hjermitsev, 2019, 5880849; Kashino, 2020, 6311632; Robledo, 2015, 2851197; Wang, 2019, 5080598} and eight were low confidence studies {Callan, 2016, 3858524; Cao, 2018, 5080197; Gao, 2019, 5387135; Marks, 2019, 5081319; Shi, 2017, 3827535; Workman, 2019, 5387046; Wu, 2012, 2919186; Xu, 2019, 5381338}. Six PFOA studies had good study sensitivity, ten had adequate sensitivity and four were considered deficient.

Ten of the overall 23 birth length studies showed some adverse associations including two of the five studies that reported standardized birth length data showed adverse associations. The high confidence study by Xiao et al. (2019, 5918609) reported a reduced birth length z-score (-0.14 ; 95% CI: $-0.40, 0.13$) in the overall population per each log₂ increase in PFOA that appeared to be driven by male neonates (-0.27 ; 95% CI: $-0.65, 0.10$). The other study high confidence study by Shoaff et al. (2018, 4619944) of standardized birth length measures showed a deficit only for tertile 3 (-0.32 ; 95% CI: $-0.72, 0.07$) compared to tertile 1.

Eight of the nineteen studies that examined mean birth length in relation to PFOA showed adverse associations including two studies reporting only sex-specific results. In the medium confidence study by Robledo et al. (2015, 2851197) slight deficits in birth length were detected for both male and female neonates per each 1 standard deviation (SD) PFOA increase. In contrast, the high confidence study by Wang et al. (2016, 3858502) only showed deficits among females for only PFOA quartiles 1 (-0.39 cm; 95% CI: $-1.80, 1.02$) and 3 (-0.60 cm; 95% CI: $-1.98, 0.77$). The high confidence study by Lauritzen et al. (2017, 3981410) showed a small deficit in the overall population (-0.49 cm; 95% CI: $-0.99, 0.02$), but detected the strongest association when restricted to the Swedish population (-1.2 cm; 95% CI: $-2.1, -0.3$) and especially Swedish boys (-1.6 cm; 95% CI: $-2.9, -0.4$). Four {Cao, 2018, 5080197; Marks, 2019, 5081319; Workman, 2019, 5387046; Wu, 2012, 2919186} of these studies showing adverse associations were low confidence and the magnitude of deficits was quite variable (range: -0.16 to -1.91 cm). For example, the low confidence study by Wu et al. (2012, 2919186) showed the largest deficit (-1.91 cm; 95% CI: $-3.31, -0.52$). The low confidence study by Cao et al. (2018, 5080197) showed consistent results across their overall population (-0.45 cm; 95% CI: $-0.79, -0.10$), male (-0.36 cm; 95% CI: $-0.80, 0.09$), and female neonates (-0.58 cm; 95% CI: $-1.12, -0.04$) with evidence of exposure-response relationships in all three of these groups. This was the only study (out of 6) with categorical data that demonstrated an exposure-response relationship.

Ten of 23 studies in total showed some adverse associations including five high confidence studies that all had good study sensitivity. Four of the eight low confidence studies reported adverse associations which were quite variable in magnitude and precision. There was limited evidence of exposure-response relationships in the categorical data, but several of the results here were large in magnitude. Few other patterns were detected across these ten studies, although only one examined PFOA exposures before trimester 2. Comparable sex-specific results were seen for

both male and female neonates. Overall, there is moderate evidence for associations between PFOA and birth length.

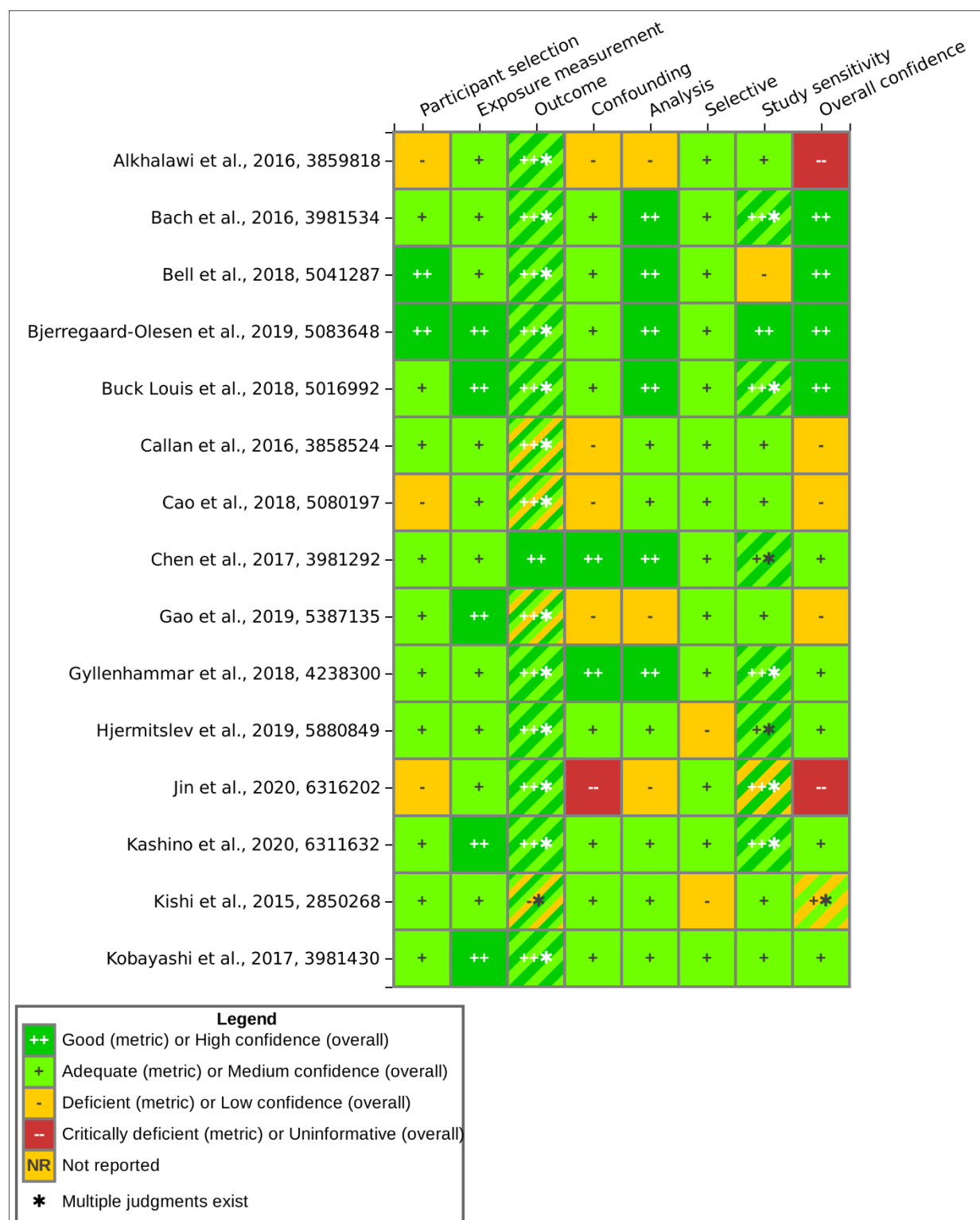


Figure 17. Summary of Study Evaluation for Epidemiology Studies of PFOA and Birth Length Effects

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

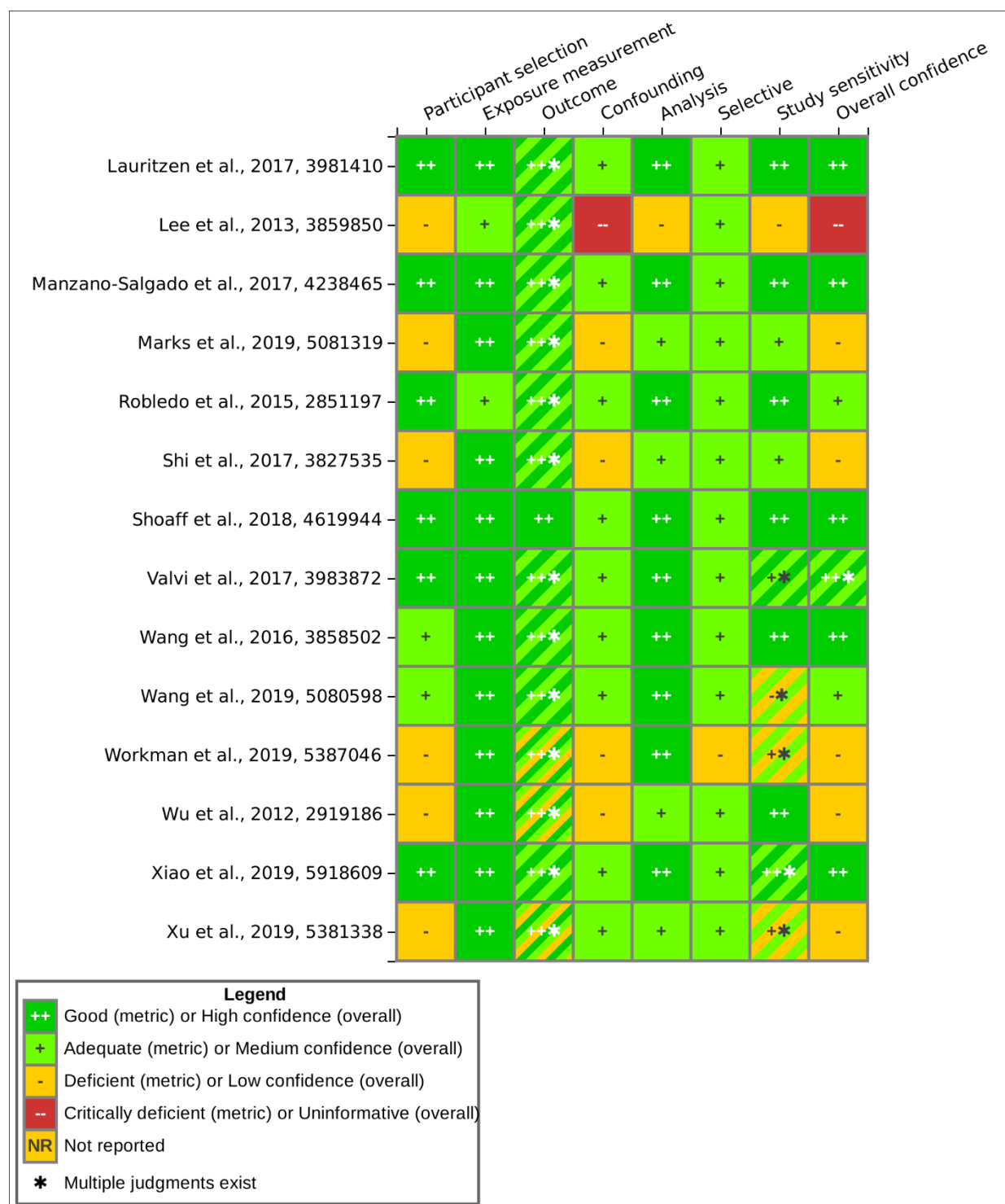


Figure 18. Summary of Study Evaluation for Epidemiology Studies of PFOA and Birth Length Effects (Continued)

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

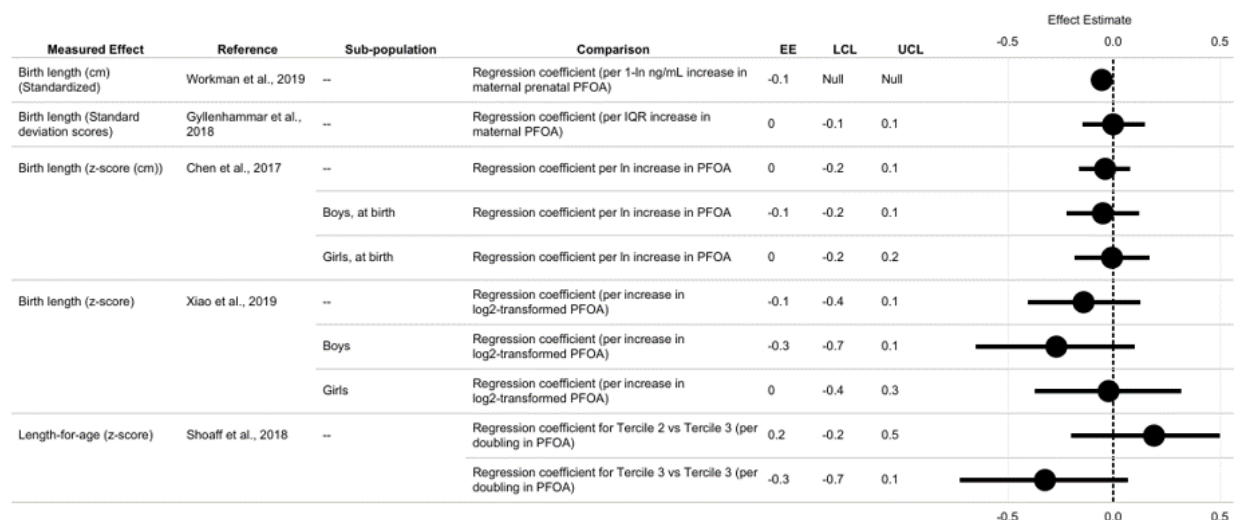


Figure 19. Overall Birth Length Z-scores from Epidemiology Studies Following Exposure to PFOA

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

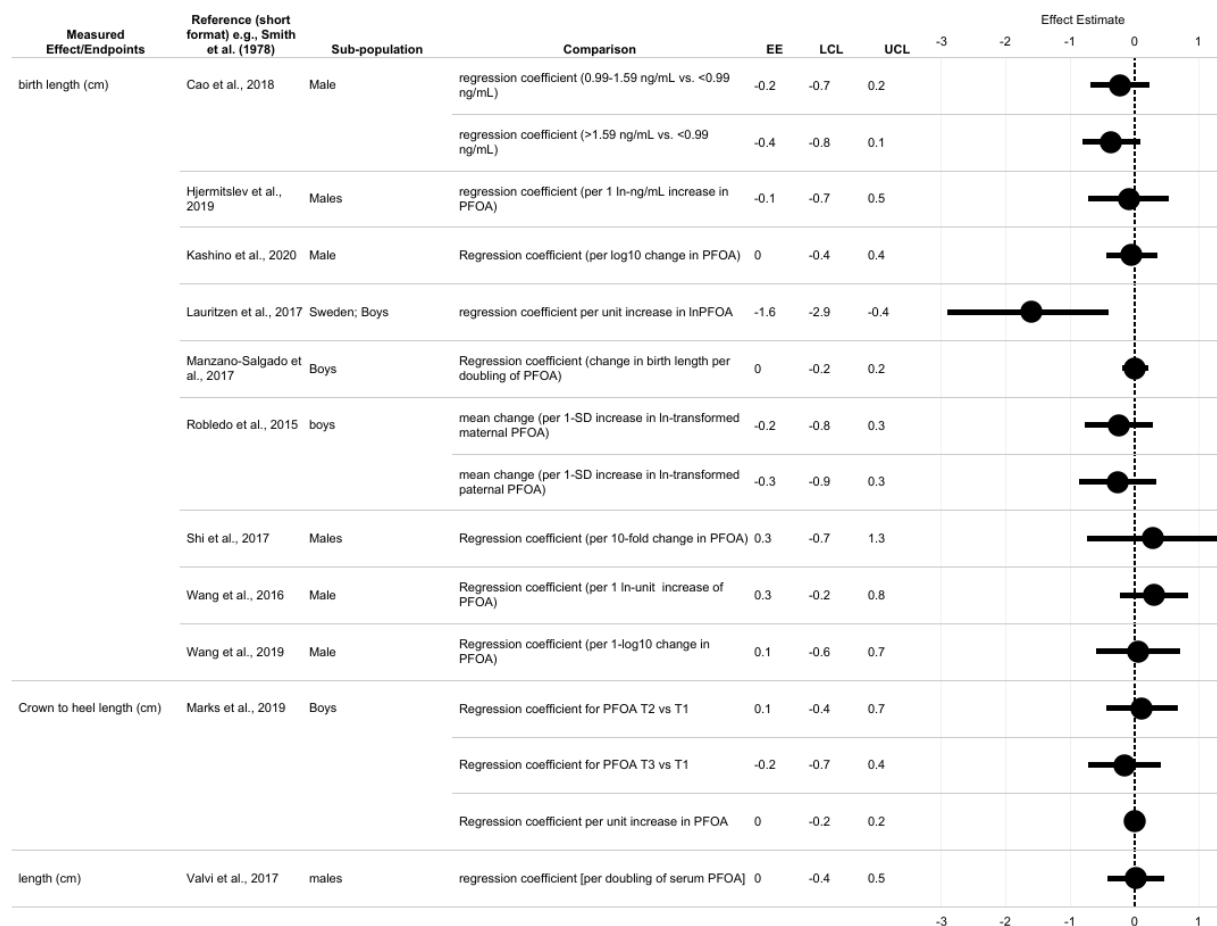


Figure 20. Mean Birth Length in Males Following Exposure to PFOA

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

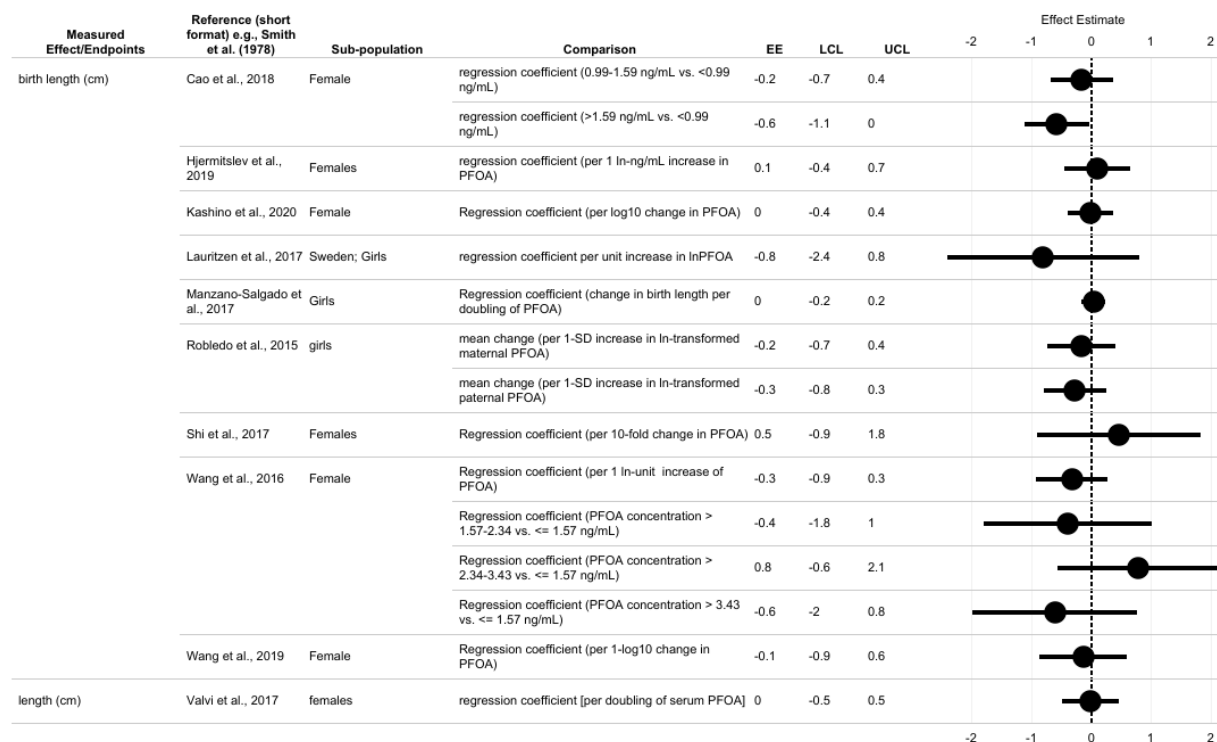


Figure 21. Mean Birth Length in Females Following Exposure to PFOA

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

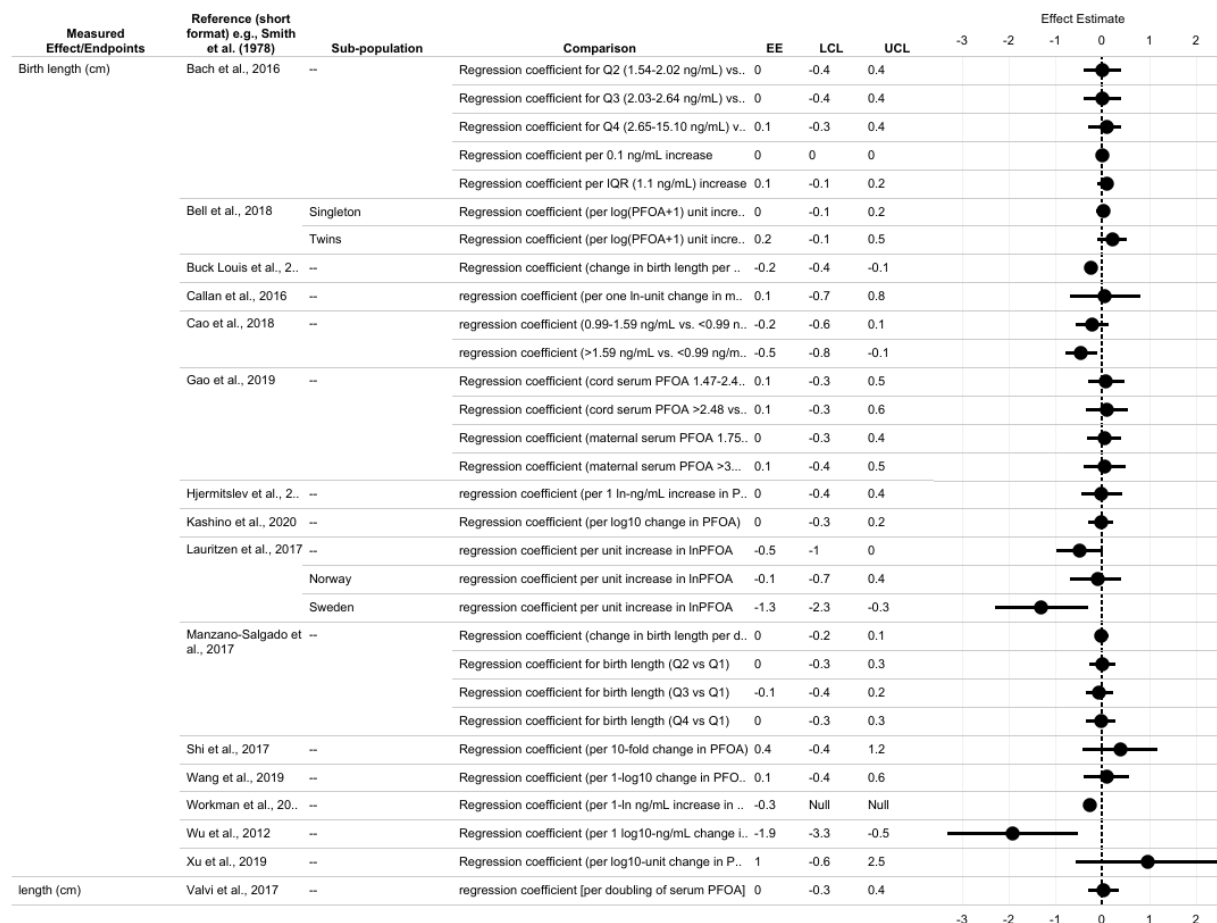


Figure 22. Overall Mean Birth Length from Epidemiology Studies Following Exposure to PFOA

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

3.3.1.1.3.4 Head Circumference at Birth

As shown in Figure 23, 18 unique informative studies measured head circumference at birth. Four of the 18 studies were low confidence {Callan, 2016, 3858524; Marks, 2019, 5081319; Workman, 2019, 5387046; Xu, 2019, 5381338}, while studies six were medium {Gyllenhammar, 2018, 4238300; Hjermitslev, 2019, 5880849; Kashino, 2020, 6311632; Lind, 2017, 3858512; Robledo, 2015, 2851197; Wang, 2019, 5080598} and eight were high confidence {Bach, 2016, 3981534; Bell, 2018, 5041287; Buck Louis, 2018, 5016992; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Valvi, 2017, 3983872; Wang, 2016, 3858502; Xiao, 2019, 5918609} (Figure 23). Four studies were deficient in study sensitivity {Bell, 2018, 5041287; Wang, 2019, 5080598; Workman, 2019, 5387046; Xu, 2019, 5381338}, while seven each had good {Gyllenhammar, 2018, 4238300; Lauritzen, 2017, 3981410; Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Robledo, 2015, 2851197; Valvi, 2017, 3983872; Wang, 2016, 3858502} and adequate {Bach, 2016, 3981534; Buck Louis, 2018, 5016992; Callan, 2016, 3858524; Hjermitslev, 2019, 5880849; Kashino, 2020, 6311632; Marks, 2019, 5081319; Xiao, 2019, 5918609} study sensitivity. Fourteen of the 18 studies examined PFOA in relation to mean head circumference differences including 10 studies with sex-specific

data (Figure 24, Figure 25) and 12 studies with results in the overall population (Figure 26, Figure 27). Four of the mean head circumference studies {Lind, 2017, 3858512; Marks, 2019, 5081319; Robledo, 2015, 2851197; Wang, 2016, 3858502} only reported sex-specific data. Two additional studies {Gyllenhammar, 2018, 4238300; Xiao, 2019, 5918609} examining unitless standardized measures are not included on the forest plots.

Seven of the 18 studies reported some adverse associations between PFOA exposures and head circumference in the overall population, in either or both male and female neonates or across different racial strata. The high confidence study by Buck Louis et al. (2018, 5016992), reported non-significant deficits (−0.14 cm; 95% CI: −0.29, 0.02) among black neonates but no associations amongst the overall population. Four of the mean head circumference studies only reported sex-specific data {Lind, 2017, 3858512; Marks, 2019, 5081319; Robledo, 2015, 2851197; Wang, 2016, 3858502} including male neonate data only in the low confidence study by Marks et al. (2019, 5081319). 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) reported a reduced head circumference z-score (−0.17; 95% CI: −0.48, 0.15) in the overall population per each log2 increase in PFOA that appeared to be driven by female neonates (−0.30; 95% CI: −0.74, 0.13).

Among the 12 studies that examined mean head circumference at birth in the overall population, four of them reported adverse associations. Five of the ten studies which examined sex-specific results showed adverse associations including three each in female neonates and three in male neonates. A deficit similar in magnitude was also reported among male neonates and the overall population in the medium confidence study by Wang et al. (2019, 5080598), with larger deficits noted in female neonates (−0.57 cm; 95% CI: −1.07, −0.08). The medium confidence study by Hjermitsev et al. (2019, 5880849) showed a non-significant reduction in head circumference for the overall population (−0.14 cm; 95% CI: −0.42, 0.14 per each ng/ml PFOA increase) which seemed to be driven by results in females (−0.25 cm; 95% CI: −0.65, 0.14). The high confidence study by Manzano-Salgado et al. (2017, 4238465) reported a non-significant decrease only in quartile 4 (−0.16 cm; 95% CI: −0.38, 0.06) compared to quartile 1 and deficit among male neonates only (−0.13 cm; 95% CI: −0.27, 0.0) per each log2 PFOA increase. In the medium confidence study by Robledo et al. (2015, 2851197) opposite results were seen for male (0.18 cm; 95% CI: −0.25, 0.60) and female neonates (−0.18 cm; 95% CI: −0.59, 0.23) per each 1 SD PFOA increase. The low confidence study by Callan et al. (2016, 3858524) reported a −0.40 cm (95% CI: −0.96, 0.16) difference per each 1 ln unit PFOA change. The high confidence study by Lauritzen et al. (2017, 3981410) reported a similar deficit but only in their Swedish population (−0.4 cm; 95% CI: −1.0, 0.1) per each 1 ln unit PFOA change.

Seven of the 18 epidemiological studies reported some adverse associations between PFOA exposures and head circumference including five of twelve studies examining the overall population and five of ten sex-specific studies. Three studies each reported deficits in male and female neonates. Few patterns by study characteristics or overall confidence levels were evident across the eleven studies showing null associations with head circumference, but nine of the eleven were either high or medium confidence. Overall, the evidence for head circumference was considered moderate.

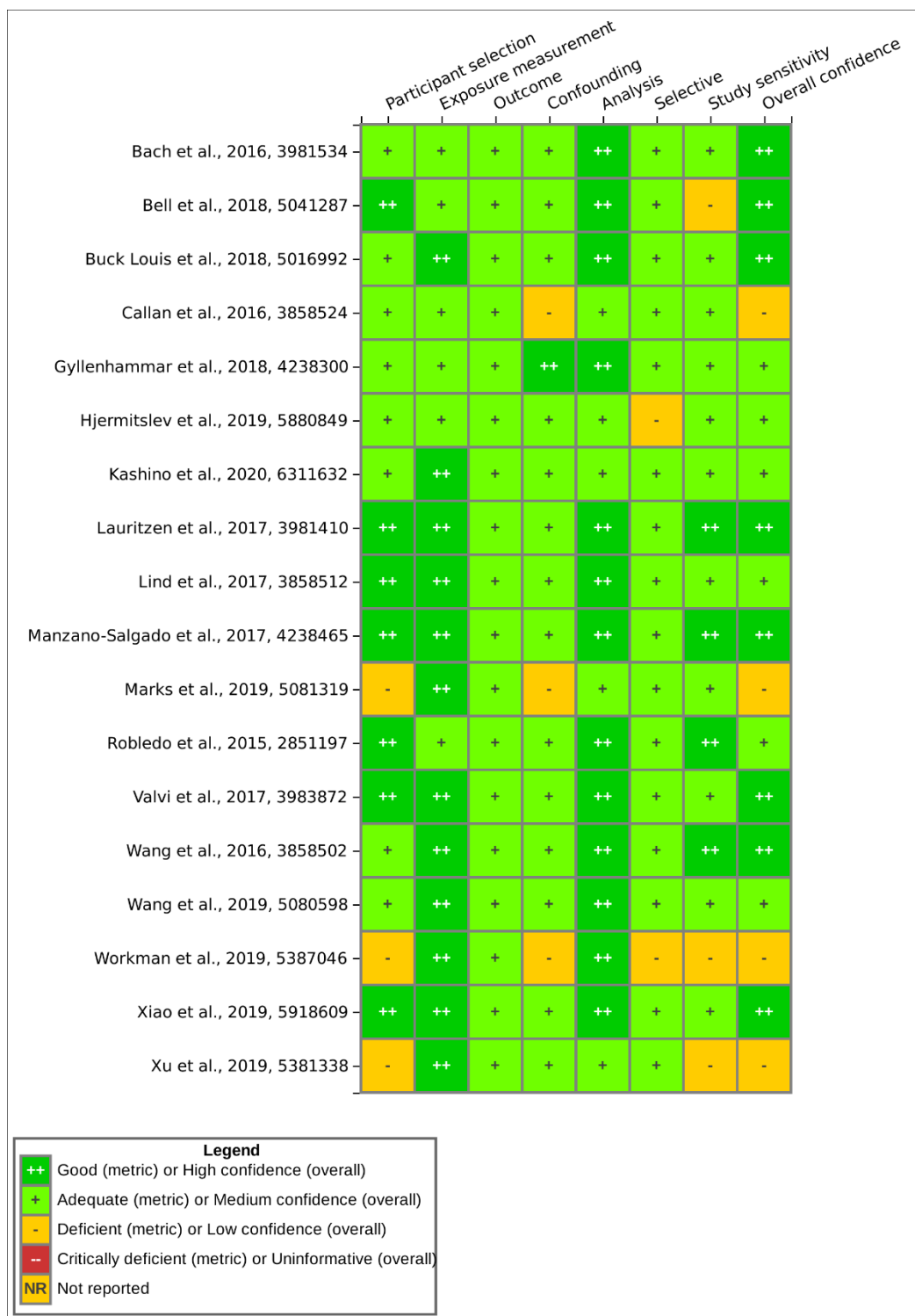


Figure 23. Summary of Study Evaluation for Epidemiology Studies of PFOA and Birth Head Circumference Effects

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

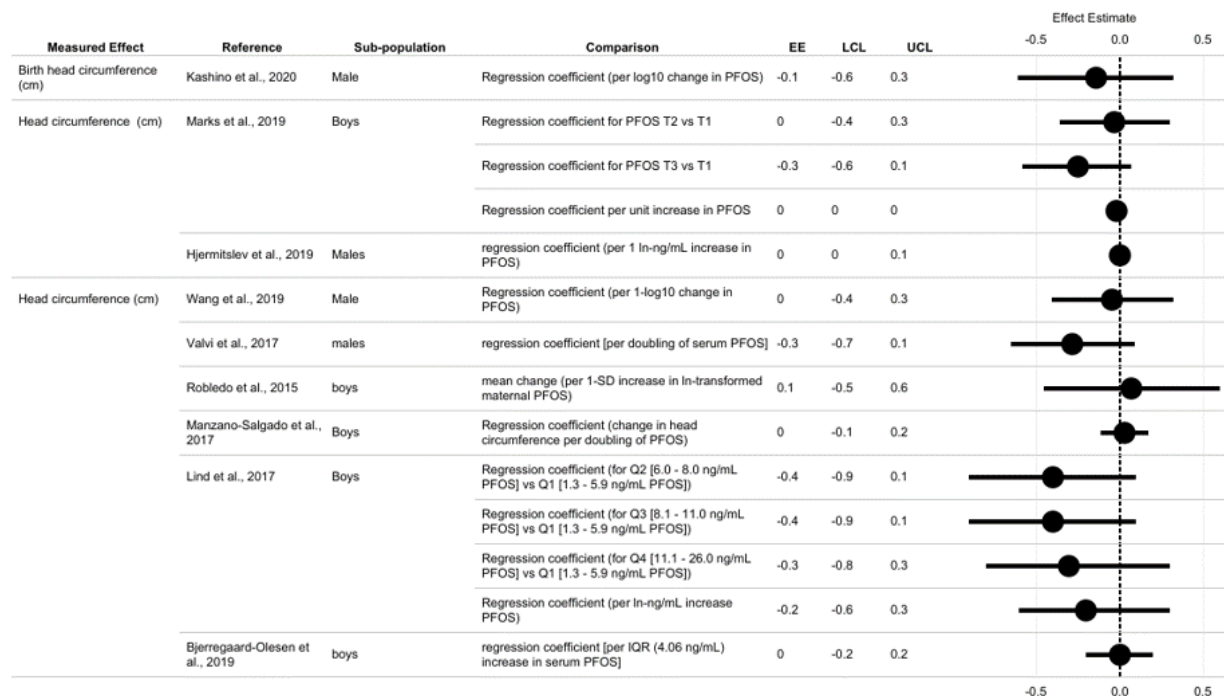


Figure 24 Head Circumference at Birth in Males Following Exposure to PFOA

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

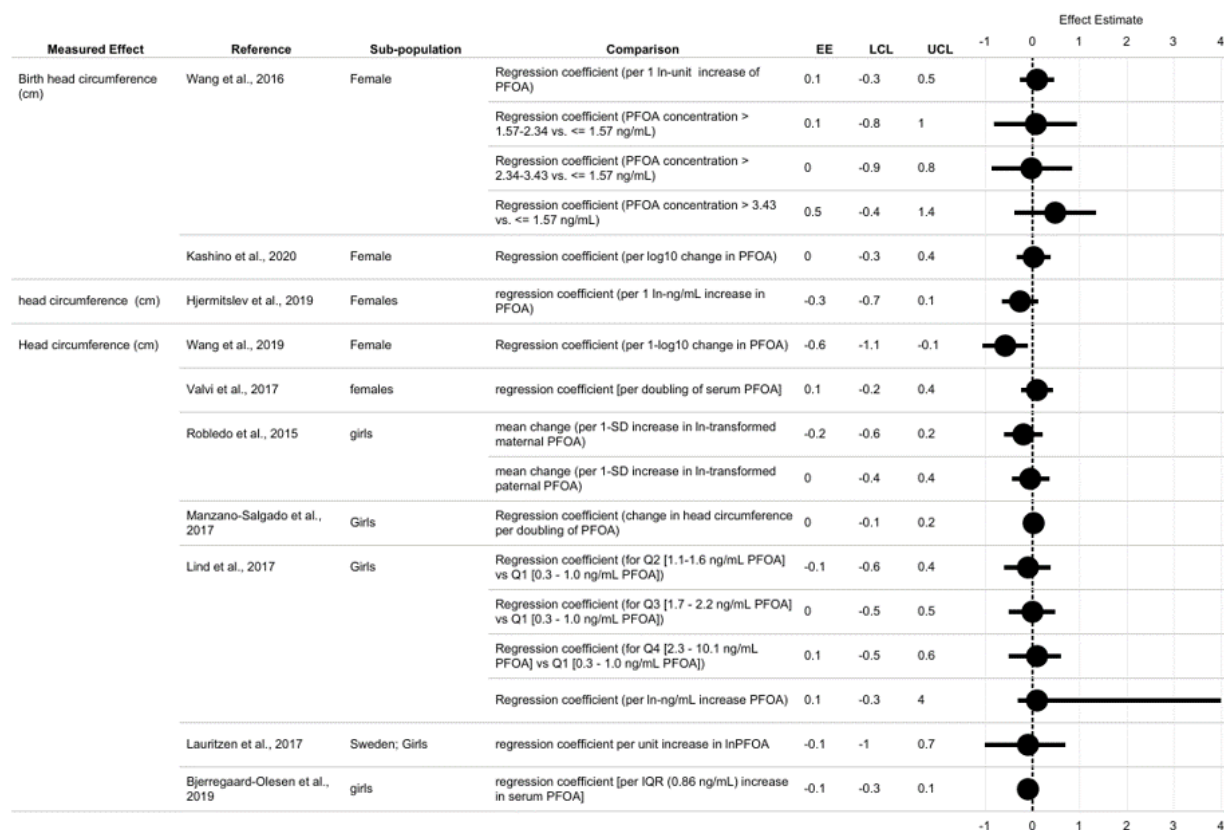


Figure 25. Head Circumference in Females Following Exposure to PFOA

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

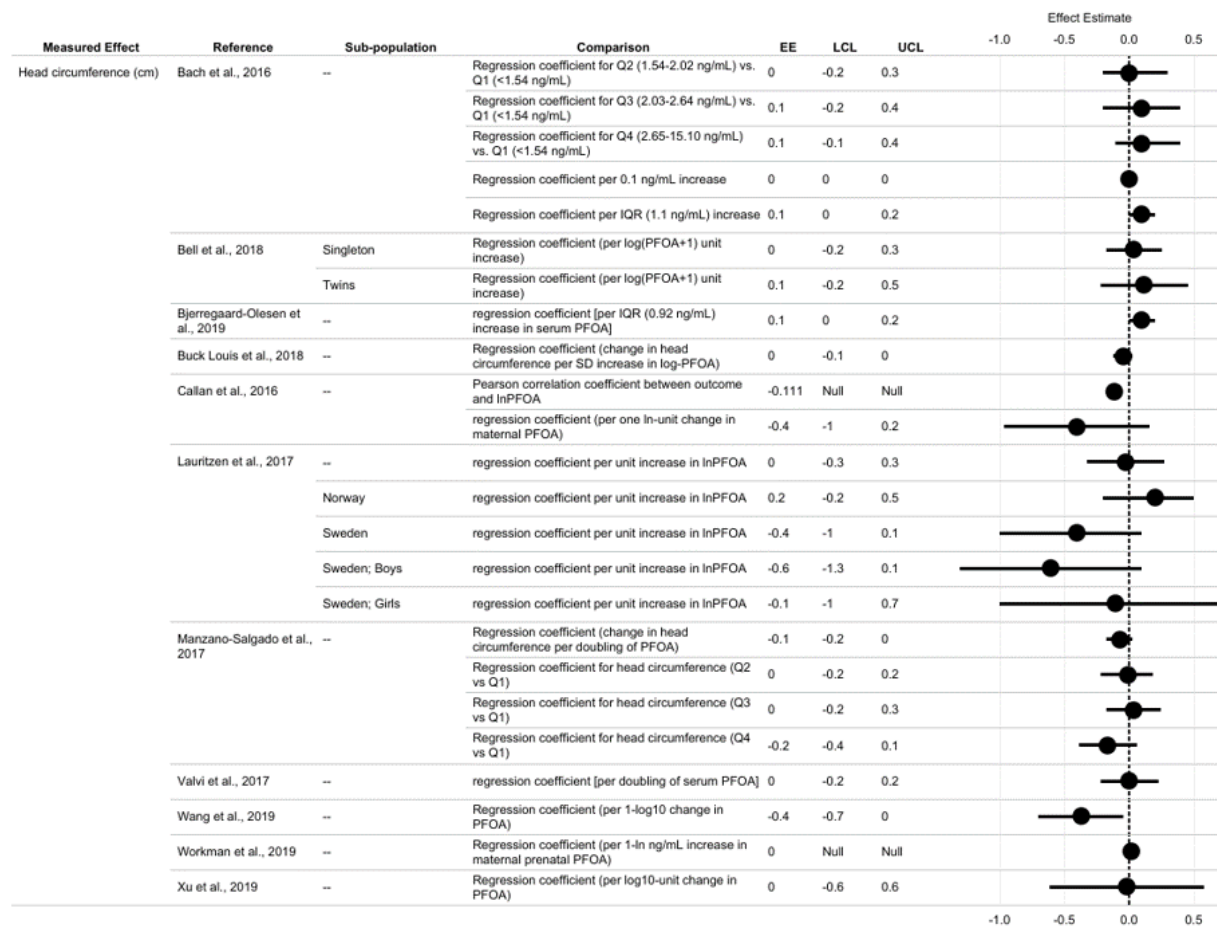


Figure 26. Overall Head Circumference at Birth from Epidemiology Studies Following Exposure to PFOA

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

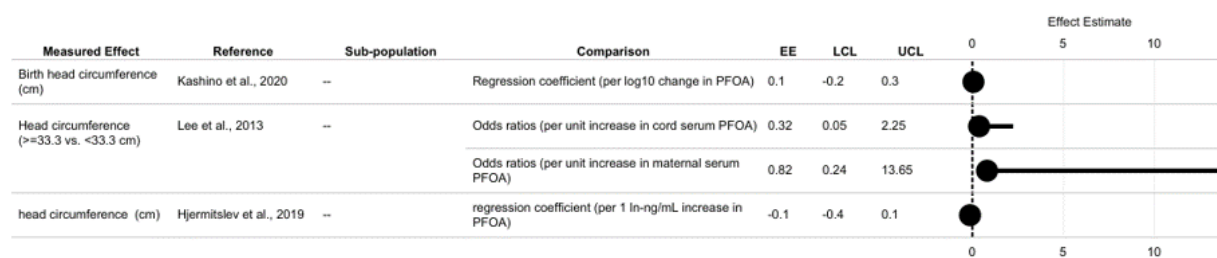


Figure 27. Overall Head Circumference at Birth from Epidemiology Studies Following Exposure to PFOA (Continued)

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

3.3.1.1.4 Postnatal Growth

Twelve studies examined PFOA exposure in relation to post-natal growth measures {Cao, 2018, 5080197; Chen, 2017, 3981292; de Cock, 2014, 2713590; Gyllenhammar, 2018, 4238300; Jensen, 2020; Lee, 2018, 4238394; Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944;

Starling, 2019, 5412449; Tanner, 2020, 6322293; Wang, 2016, 3858502; Yeung, 2019, 5080619}(Figure 28). Seven post-natal growth studies were high confidence, three were medium confidence and two were low confidence. The synthesis here is focused on postnatal growth mean and standardized weight, height, head circumference and body mass index (BMI)/adiposity measures. Rapid growth during infancy is also included as it was examined in five studies {Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944; Starling et al. 2019; Tanner et al. 2020; Yeung, 2019, 5080619).

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 PFOA exposures among singletons followed for three years. An exposure-response relationship was evident with decreasing BMI z-scores across PFOA quartiles, but 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 PFOA tertile 3 compared to tertile 1 with exposure-response relationships detected for infant BMI and weight for length z-scores. Small deficits that were not statistically significant were observed in tertile 3 for height measured as length for age z-score (-0.18 ; 95%CI: $-0.45, 0.09$). The high confidence study by Manzano-Salgado et al. (2017, 4238509) reported null associations for their overall population and female neonates measured at 6 months but reported an increased weight gain z-score for males (0.13 ; 95%CI: $0.01, 0.26$) per each log2 PFOA increases. They also reported null associations for rapid growth measured from birth until 6 months.

The low confidence study by Lee et al. (2018, 4238394) reported that for each PFOA ln unit increase statistically significant associations were detected for mean height differences at age 2 years (-0.91 cm; 95%CI: $-1.36, -0.47$) as well as height change from birth to 2 years (-0.86 cm; 95%CI: $-1.52, -0.20$). Small differences were seen for mean weight differences at age 2 years (-0.21 cm; 95%CI: $-0.43, 0.02$) but not for weight change from birth to 2 years. The medium confidence study by Chen et al. (2017, 3981292) study did not report associations between each per ln unit PFOA exposure increase and height z-score measures up to 24 months of age. They did report slight deficits in the overall population which appeared to be driven by male neonates for both infant weight z-scores (-0.15 ; 95%CI: $-0.30, -0.01$) and BMI z-scores (-0.20 ; 95%CI: $-0.36, -0.04$). The medium confidence study by deCock et al. (2014, 2713590) did not report effect estimates for postnatal infant height (p-value = 0.045), weight (p-value = 0.35) and BMI (p-value = 0.81) up to 11 months of age and reported a statistically significant association for infant height only. In the medium confidence study by Gyllenhammar et al. (2018, 4238300), no associations were detected for infant height deficits among participants followed from 3 months to 60 months of age per each interquartile range (IQR) PFOA change. Although Gyllenhammar et al. (2018, 4238300) did not report statistically significant standardized BWT deficits per each IQR PFOA change, they did show slight weight deficits (approximately -0.2) at 3 months that gradually decreased over time (to approximately -0.1) at 60 months of age. No associations were found in the overall population from the high confidence study by Starling et al. (2019, 5412449) for postnatal measures at 5 months of age, but an exposure-response relationship of increased adiposity was seen among male neonates with increasing PFOA tertiles (2.81 ; 95% CI: $0.79, 4.84$ for tertile 3). Similarly, no associations were found in the overall population for weight-for-

age or weight-for-length z-scores and PFOA exposures, but both measures were increased among male neonates. They also reported small increased ORs (range: 1.25 to 1.43) for rapid growth in the overall population based on either weight for age z-or weight for length-based z-scores. Compared to the umbilical cord PFOA tertile 1 referent, the low confidence study by Cao et al. (2018, 5080197) reported slight increases (1.37 cm; 95% CI: -0.5, 3.28) in postnatal length (i.e., height) amongst infants (median age of 19.7 months) while large postnatal weight deficits were reported for tertile 2 (-429.2 g; 95% CI: -858.4, -0.12) and tertile 3 (-114.9 g; 95% CI: -562.0, 332.1). These height increases were predominately due to female infants, while the weight deficits were driven by males. Few differences were observed in the overall population for postnatal head circumference with slight non-significant deficits seen amongst females only. In their high confidence study, Wang et al. (2016, 3858502) reported statistically significant childhood weight (-0.14; 95% CI: -0.39, 0.11) and height (-0.15; 95% CI: -0.38, 0.08) z-scores for female neonates when averaged over the first 11 years and per 1-ln-unit PFOA increase. Results were null for male neonates for childhood average weight (0.03; 95% CI: -0.11, 0.18) and height (0.01; 95% CI: -0.24, 0.25) z-scores. However, when they examined the first 2 years only, statistically significant deficits in both height and weight z-scores were only seen for male neonates. These weight deficits dissipated in males later during childhood, while the height deficits detected at age 2 years continued through age 11. In contrast, the height deficits in female children that were detected at birth were no longer evident in older kids until later ages (i.e., 11 years). The weight deficits in female children detected at birth did not persist. Lastly, Jensen et al. (2020, 6833719) reported null associations between adiposity and per each 1-unit increase in PFOA measured at 3 and 18 months.

Eight of the 11 studies examining different infant weight measures showed some evidence of adverse associations with PFOA exposures. Two {Manzano-Salgado, 2017, 4238509; Starling et al., 2019} of the three studies that did not report adverse associations in either the overall population and females, but did detect increased infant weight measures among males. Four of the eight studies reported adverse associations between PFOA exposures and infant height. There was mixed evidence for six studies of infant BMI or adiposity measures, with two studies each reporting null, adverse or inverse associations (i.e., lower adiposity with increasing PFOA). Although the postnatal database is not entirely consistent, the preponderance of data suggests that PFOA may be associated with many post-natal growth measures up to two years of age.

3.3.1.1.4.1 Rapid Weight Gain

Five studies examined rapid infant growth, with all five considered high confidence. Mixed evidence was reported with these studies, with one of two studies showing increased odds or rapid weight gain with increasing PFOA {Starling et al., 2019, 5412449}. The most detailed evaluation by Tanner et al. (2020, 6322293) also showed some adverse associations including higher prenatal PFOA concentrations related to a longer duration of time needed to complete 90% of the infant growth spurt (Δ tertile 1: 0.06; 95%CI: 0.01, 0.11). Higher prenatal PFOA concentrations were also significantly related to delayed infant peak growth velocity (δ 1: 0.58; 95%CI: 0.17, 0.99) and a higher post-spurt weight plateau (α 1: 0.81; 95%CI: 0.21, 1.41). Overall, the evidence of impacts of PFOA on rapid growth is inconsistent across five high confidence studies and was considered limited.

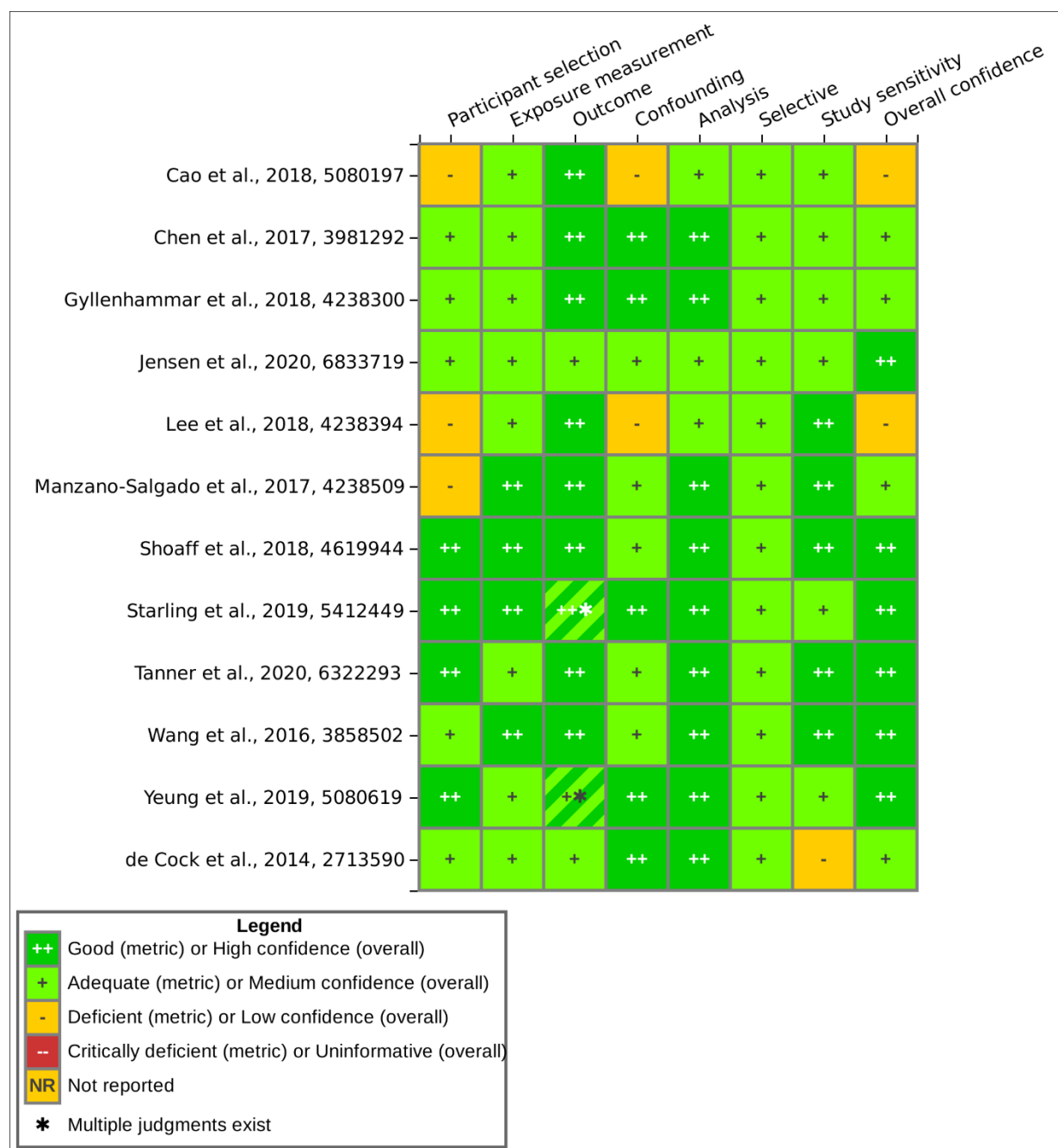


Figure 28. Summary of Study Evaluation for Epidemiology Studies of PFOA and Postnatal Growth

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

3.3.1.1.5 Gestational Duration

Nineteen different studies examined gestational duration measures (i.e., PTB or gestational age measures).

3.3.1.1.5.1 Gestational Age

Eighteen studies examined gestational age in relation to PFOA exposures including one uninformative study {Lee, 2013, 3859850} and one overlapping cohort {Li, 2017, 3981358} (Figure 29). Sixteen non-overlapping and informative studies examined mean gestational age (in weeks) in relation to PFOA exposures. Eight of these 16 studies were high confidence {Bach, 2016, 3981534; Bell, 2018, 5041287; Buck Louis, 2018, 5016992; Chu, 2020, 6315711; Huo, 2020, 6505752; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Sagiv, 2018, 4238410}, four were medium {Gyllenhammar, 2018, 4238300; Hjermitslev, 2019, 5880849; Lind, 2017, 3858512; Meng, 2018, 4829851} and four were low confidence {Gao, 2019, 5387135; Workman, 2019, 5387046; Wu, 2012, 2919186; Xu, 2019, 5381338}. Eight of the studies had good study sensitivity, five were adequate and three were deficient.

Six of the 16 studies showed some evidence of adverse impacts on gestational age either in the overall population or either sex (Figure 30, Figure 31, Figure 32, Figure 33). Among these six studies, four were high confidence, and one each was medium and low confidence. The low confidence study by Wu et al. (2012, 2919186) study reported an extremely large difference (–2.2 weeks; 95% CI: –4.0, –0.6) in gestational age per each log₁₀ unit PFOA change. The high confidence study by Huo et al. (2020, 6505752) reported largely null results but did report a large non-significant deficit for tertile 2 (–0.69; 95% CI: –1.75, 0.37) versus tertile 1. The medium confidence study by Meng et al. (2018, 4829851) also reported statistically significant gestational age deficits (range: –0.17 to –0.24 weeks) across all quartiles but no evidence of an exposure-response relationship. The high confidence study by Lauritzen et al. (2017, 3981410) reported a slight decrease in the overall population (–0.2 weeks; 95% CI: –0.34, 0.14). They also showed larger deficits in their Swedish population (–0.3 weeks; 95% CI: –0.9, 0.3) which was predominately driven by results among male neonates (–0.4 weeks; 95% CI: –1.2, 0.5). The high confidence study by Chu et al. (2020, 6315711) showed larger deficits in the overall population (–0.21 weeks; 95% CI: –0.44, 0.02) which was driven by female neonates (–0.83 weeks; 95% CI: –0.53, –0.23). The high confidence study by Lind et al. (2017, 3858512) examined only sex-specific data and reported larger deficits in female (–0.21 cm; 95% CI: –0.61, 0.19 per each ln unit PFOA increase) than male neonates (–0.10 cm; 95% CI: –0.41, 0.21).

Six of the 16 studies showed some evidence of adverse impacts on gestational age. Five of the six studies were either medium or high confidence studies. Few patterns emerged based on study confidence or other study characteristics. For example, only two of the studies reporting adverse associations were sampled late in pregnancy (i.e., third trimester).

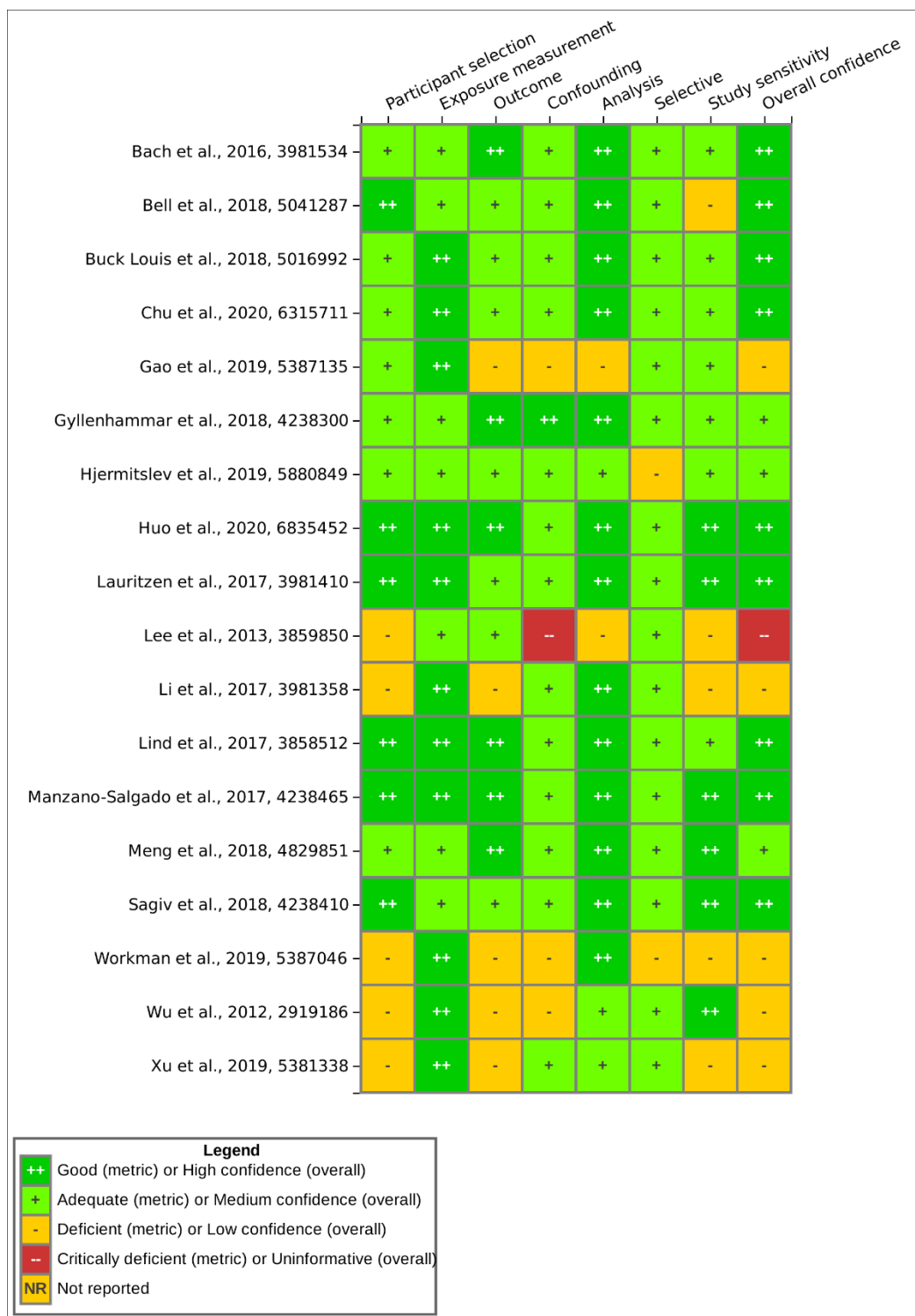


Figure 29. Summary of Study Evaluation for Epidemiology Studies of PFOA and Gestational Age Effects

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

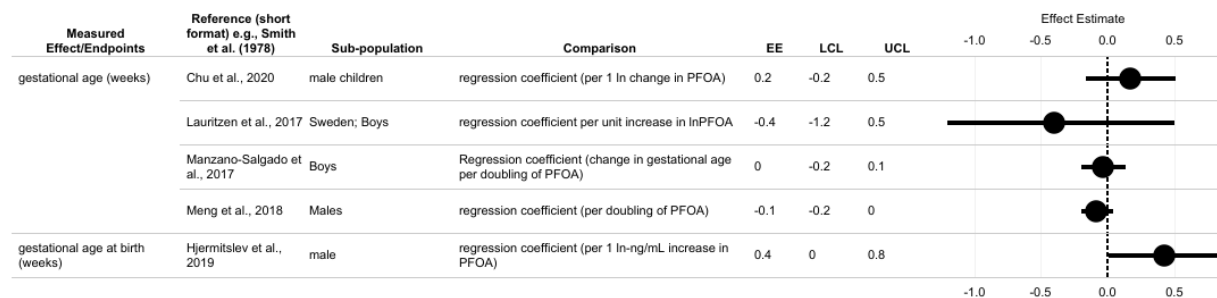


Figure 30. Gestational Age in Males Following Exposure to PFOA

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

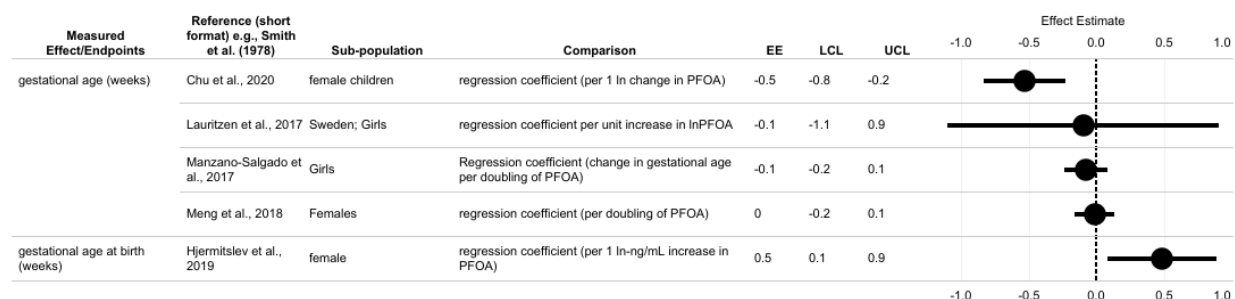


Figure 31. Gestational Age in Females Following Exposure to PFOA

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

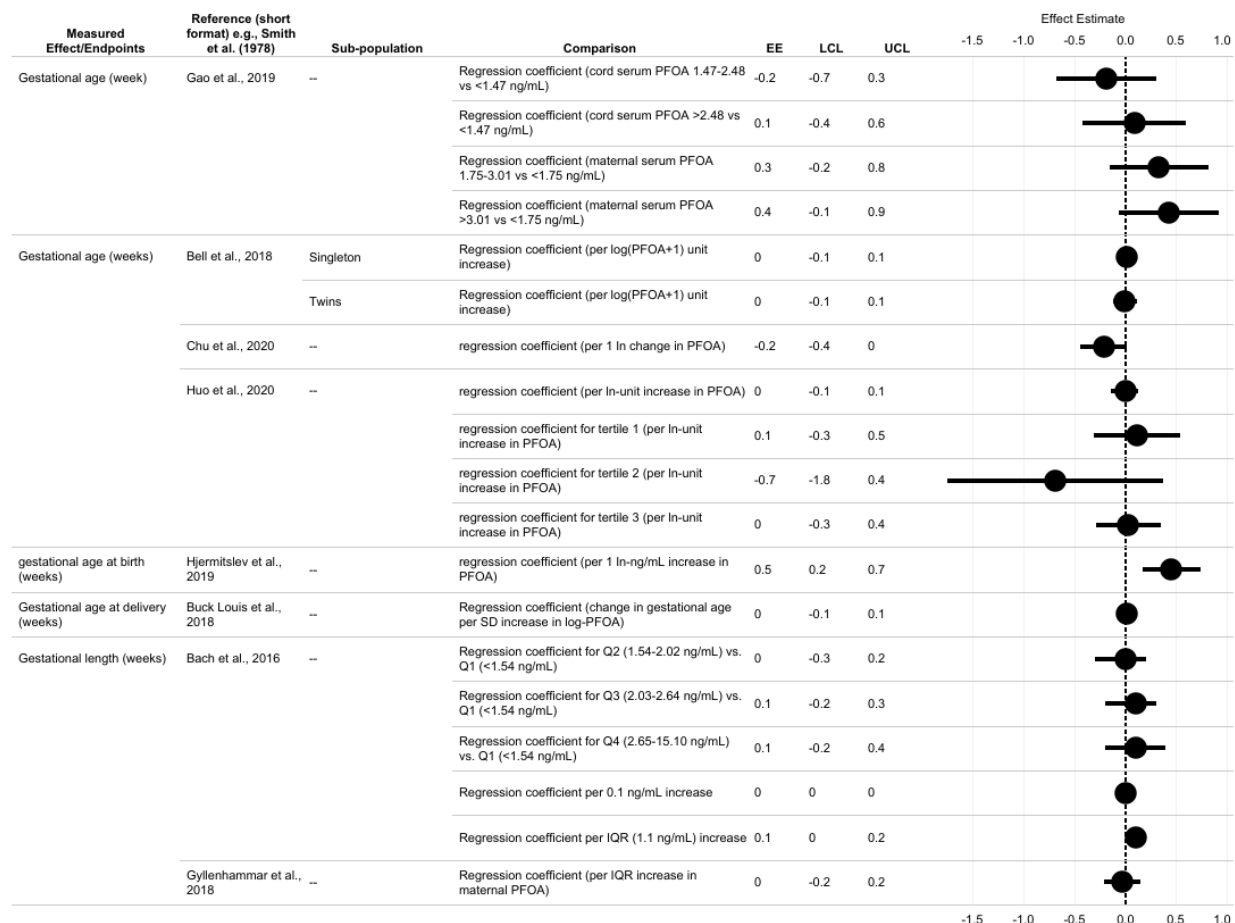


Figure 32. Overall Gestational Age from Epidemiology Studies Following Exposure to PFOA

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

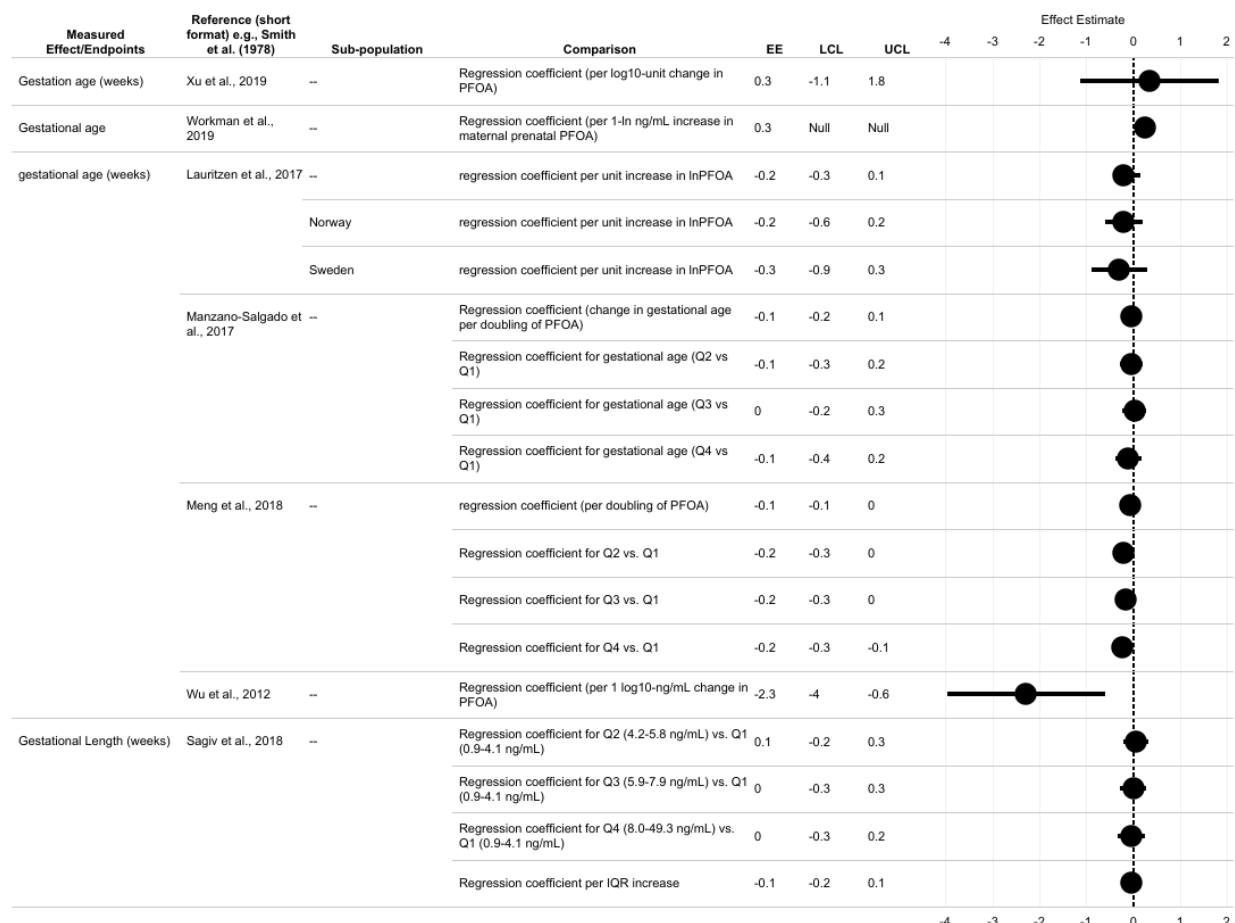


Figure 33. Overall Gestational Age from Epidemiology Studies Following Exposure to PFOA (Continued)

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

3.3.1.1.5.2 Preterm Birth

Eight studies examined the relationship between PFOA and PTB (Figure 34); 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, and the remaining 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}. The *medium* confidence study by Meng et al. (2018, 4829851) pooled exposure data from two study populations, one which measured PFOA in umbilical cord blood and one which measured PFOA in maternal blood samples collected in trimesters 1 and 2. The *high* confidence study by Huo et al. (2020, 6835452) was considered to have *good* study sensitivity, while the other seven studies were rated *adequate* in this domain.

The median exposure values across all studies ranged from 0.79 ng/mL {Liu, 2020, 6833609} to 11.85 ng/mL {Huo, 2020, 6835452}.

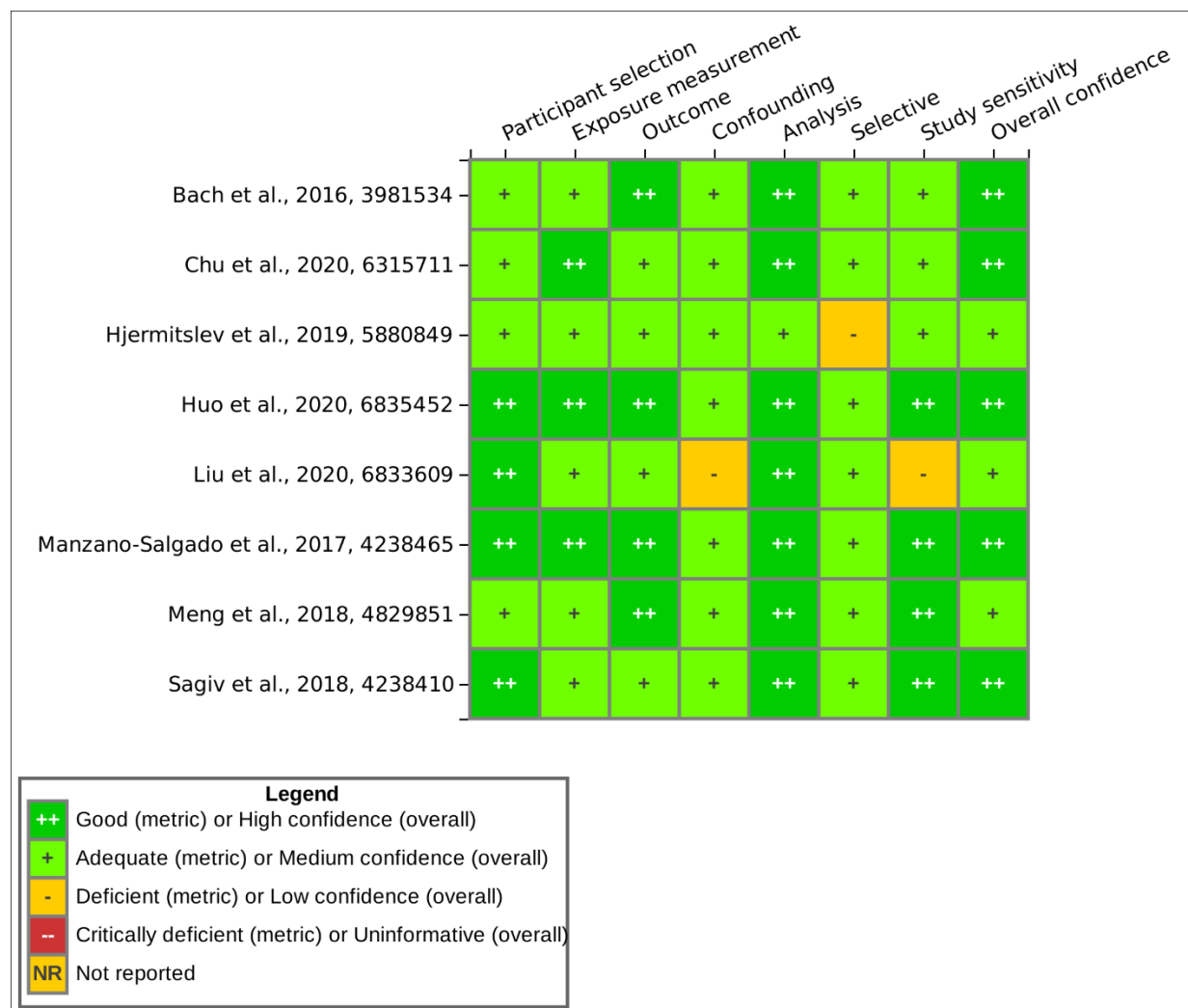


Figure 34. Summary of Study Evaluation for Epidemiology Studies of PFOA and Preterm Birth Effects

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

Limited adverse associations were reported with only three of the eight studies consistently showing increased risk of PTB with PFOA exposures. The Meng et al. (2018, 4829851) study reported consistently elevated non-monotonic ORs for PTB in the upper three PFOA quartiles (OR range: 1.7–3.2), but little evidence was seen when examined per each doubling of PFOA exposures (OR=1.1; 95%CI: 0.8, 1.5). Although they were not statistically significant, Chu et al. (2020, 6315711) reported increased ORs of similar magnitude per 1 ln ng/mL unit increase (OR=1.49; 95%CI: 0.94, 2.36) or when quartile 3 (OR=1.60; 95% CI: 0.60, 4.23) and quartile 4 (OR=1.84; 95%CI: 0.72, 4.71) exposures were compared to the referent. Associations between PFOA and (overall) PTB near or just below the null value were consistently detected in the Huo et al. (2020, 6835452) study. Few patterns emerged across PTB subtypes, although there was an

increase in clinically indicated PTBs per each ln-unit increase in PFOA (OR=1.71; 95%CI: 0.80, 3.67). The high confidence study by Sagiv et al. (2018, 4238410) showed increased non-significant risks (OR range: 1.1–1.2) for PTB across all PFOA quartiles. Null or inverse associations were reported by Bach et al. (2016, 3981534), Hjermitsev et al. (2019, 5880849), Liu et al. (2020, 6833609), and Manzano-Salgado et al. (2017, 4238465). None of the six studies showed strong evidence of exposure-response relationships (Figure 35, Figure 36, Figure 37).

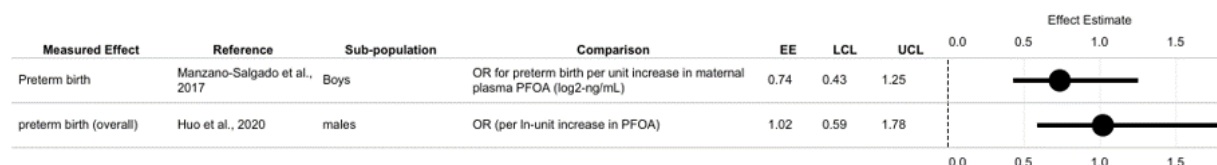


Figure 35. Preterm Birth in Males Following Exposure to PFOA

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

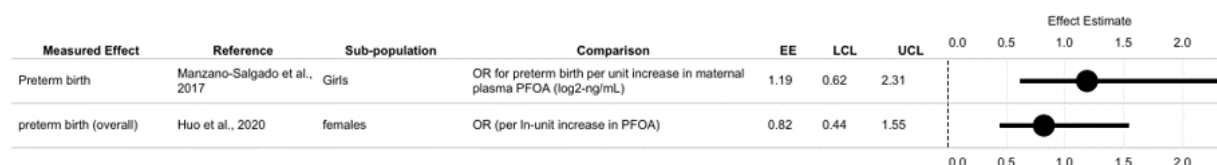


Figure 36. Preterm Birth in Females Following Exposure to PFOA

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

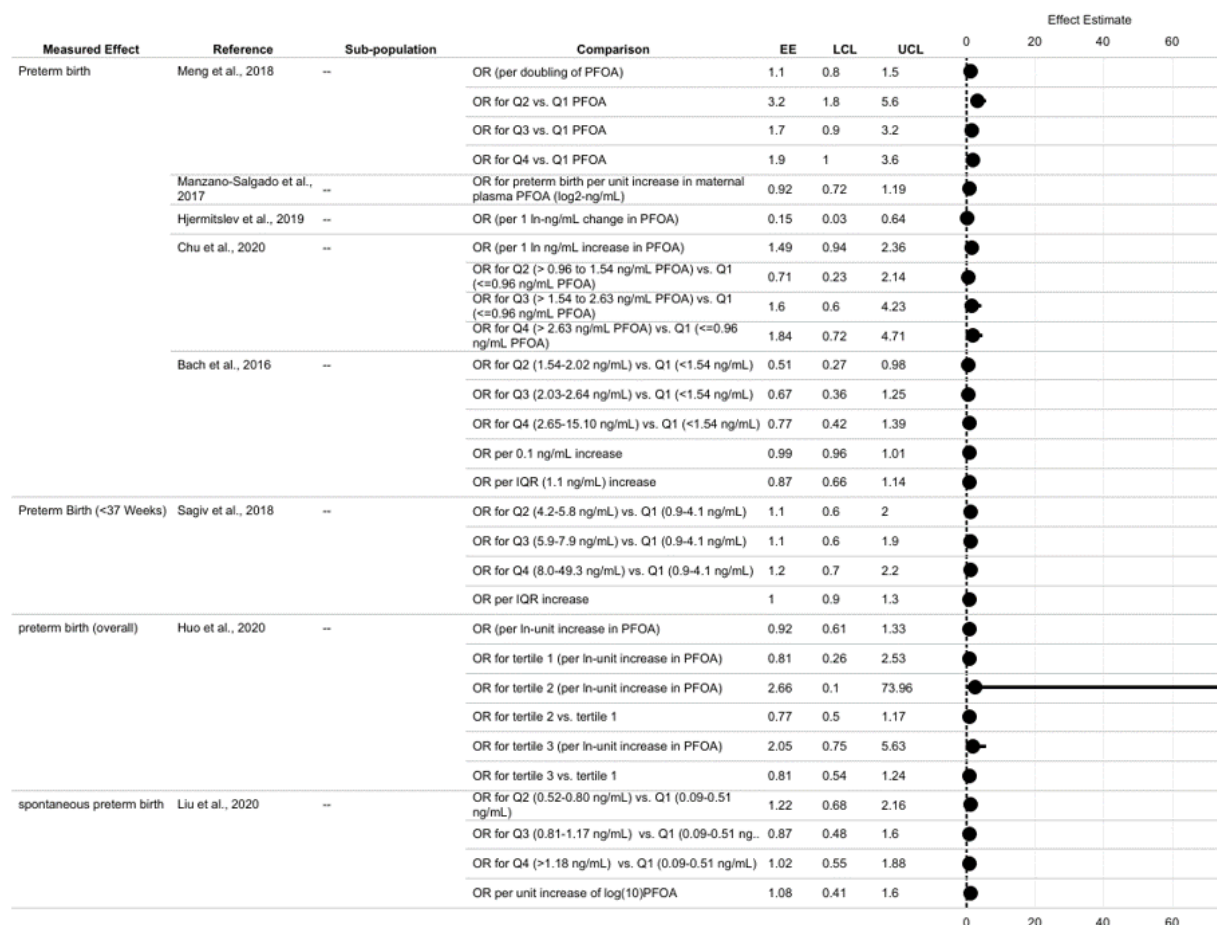


Figure 37. Overall Preterm Birth from Epidemiology Studies Following Exposure to PFOA

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

3.3.1.1.5.3 Gestational Duration Summary

Overall, there is *limited* evidence of an impact of PFOA exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) as most of the studies did not show any adverse associations. Only three of the eight studies consistently showed increased risk of PTB with PFOA exposure. Six of the 16 studies showed some evidence of adverse impacts of PFOA on gestational age with five of these being *medium* or *high* confidence studies. There was also little evidence of evidence of exposure-response relationships for both endpoints. Few patterns were evident as explanatory factors for heterogeneous results based on our qualitative analysis.

3.3.1.1.6 Fetal Loss

Three (2 *medium* and 1 *low* confidence) studies examined PFOA exposure and fetal loss with *limited* evidence as only one study showing increased risks of miscarriage (Figure 38). The *medium* confidence study by Liew et al. (2020, 6387285) detected a 40% increased risk of miscarriage (OR = 1.4; 95% CI: 1.0, 1.9) with some suggestion of an exposure-response relationship across quartiles three (1.4; 95% CI: 0.8, 2.6) and four (2.2; 95% CI: 1.2, 3.9). 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 PFOA exposures. No associations with miscarriages were reported for PFOA tertiles in the *low* confidence study by Jensen et al. (2015, 2850253), but an inverse association was suggested (OR range: 0.64; 95%CI: 0.36, 1.18) per each ln-unit increase in PFOA exposure. The overall evidence for fetal loss was *limited* given the increased relative risk estimates in only one of three studies, although there was a suggestion of an exposure-response relationship detected.

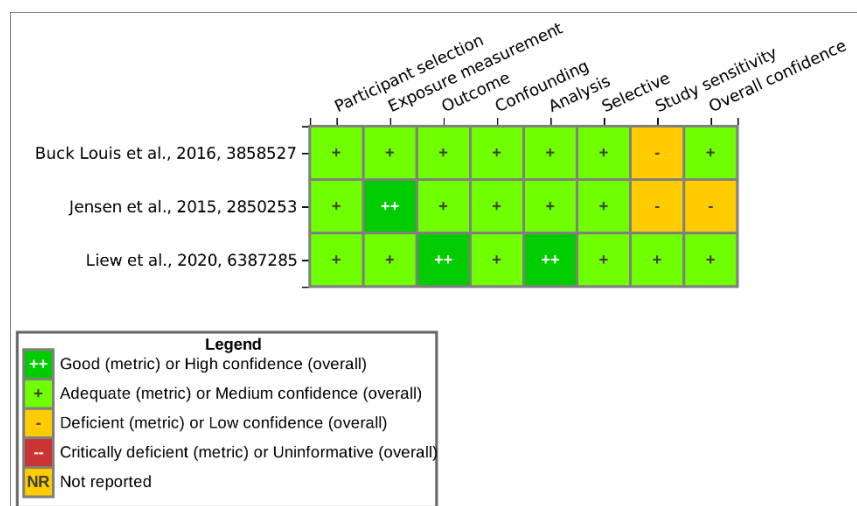


Figure 38. Summary of Study Evaluation for Epidemiology Studies of PFOA and Fetal Loss

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

3.3.1.1.7 Birth Defects

Two birth defect studies (Figure 39) examined PFOA exposure including one *low* confidence study {Cao, 2018, 5080197} of a non-specific grouping of all birth defects that reported limited evidence of an association (OR = 1.24; 95% CI: 0.57, 2.61). The *medium* confidence study by Vesterholm Jensen et al. (2014, 2850926) reported no adverse associations for cryptorchidism (OR = 0.83; 95% CI: 0.44, 1.58) per each ln-unit increase in PFOA exposures. Overall, there was *very limited* evidence of associations between PFOA and birth defects based on the available epidemiological studies.

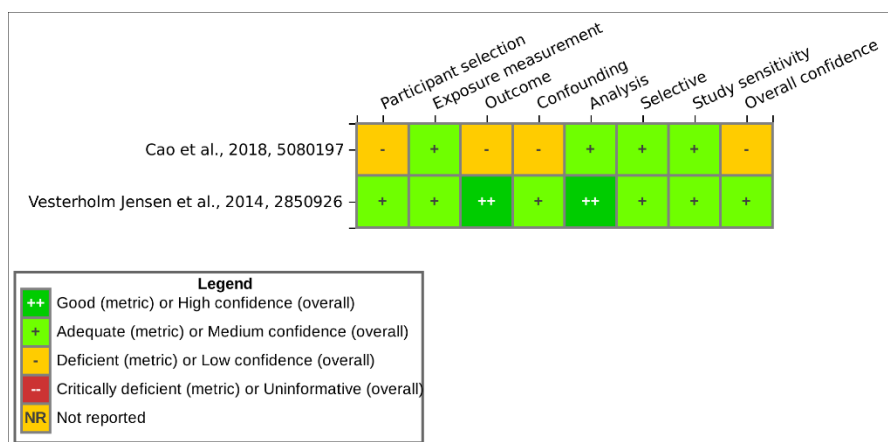


Figure 39. Summary of Study Evaluation for Epidemiology Studies of PFOA and Birth Defects

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

3.3.1.2 Animal Evidence

There are 9 studies from the most recent literature search conducted in 2020 and 4 key studies from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and developmental effects. Study quality evaluations for these 13 studies are shown in Figure 40.

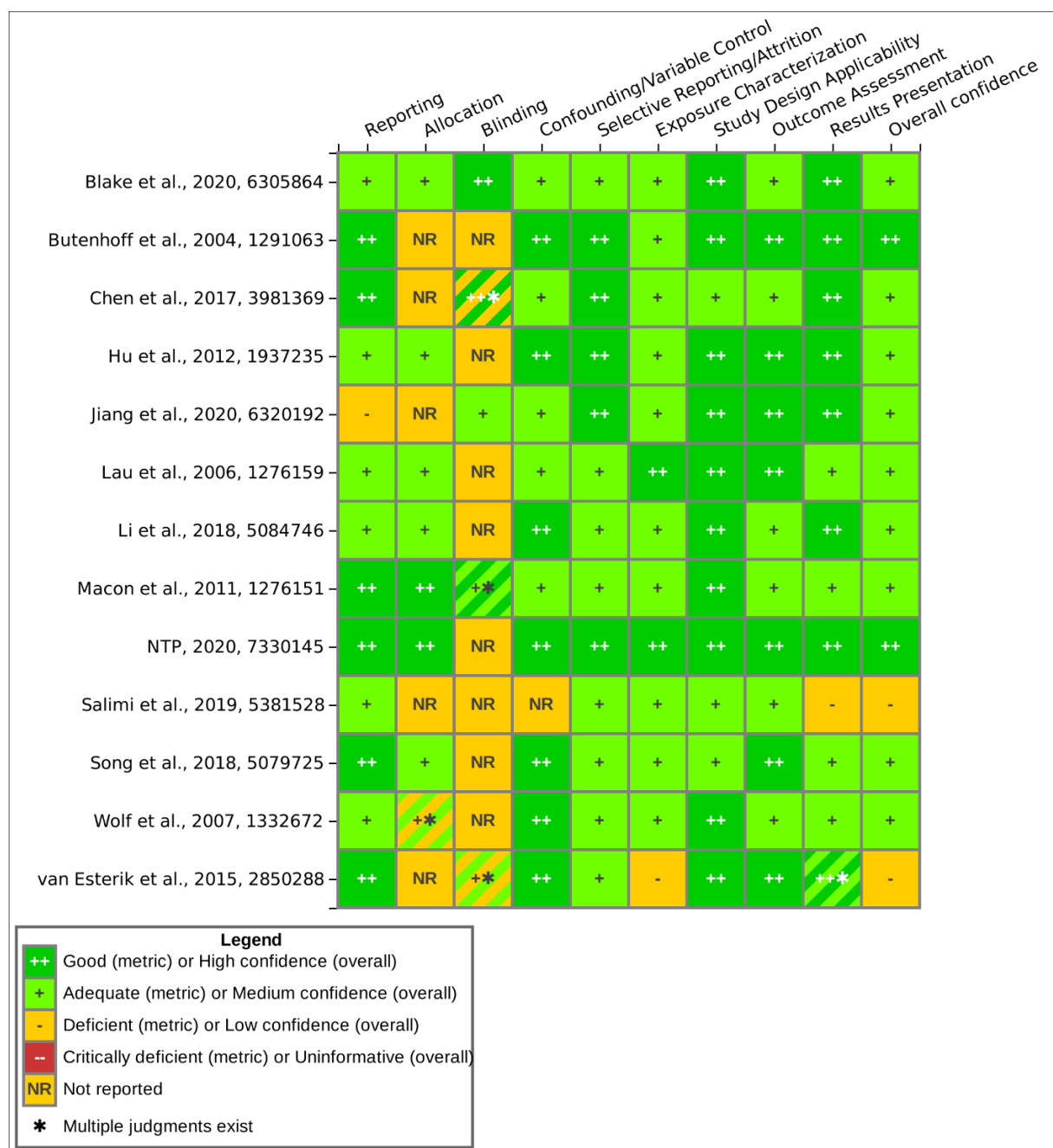


Figure 40. Summary of Study Evaluation for Toxicology Studies of PFOA and Developmental Effects

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

Evidence suggests that PFOA exposure can adversely affect development. Oral studies in mice and rats report effects in offspring including decreased survival, decreased body weights, structural abnormalities (e.g., reduced skeletal ossification), delayed eye opening, altered puberty (delayed vaginal opening in females and accelerated preputial separation in males), and altered mammary gland development. Doses that elicited responses were generally lower in mice than in

rats. Additionally, three studies of gestational PFOA exposure to mice reported effects on placental weight and histopathological changes in placental tissue, suggesting that the placenta may be a target of PFOA. In some cases, adverse developmental effects of PFOA exposure that relate to other health outcomes may be discussed in the corresponding health outcome section (e.g., neurodevelopmental effects are discussed in Section 3.3.8.2).

3.3.1.2.1 Maternal Effects

Exposure to PFOA resulted in significant decreases in maternal body weight and/or weight gain at ≥ 10 mg/kg/day in multiple strains of pregnant mice {Li, 2018, 5084746; Lau, 2006, 1276159; Yahia, 2010, 1332451} and at ≥ 30 mg/kg/day in pregnant Sprague Dawley rats {Butenhoff, 2004, 1291063; Hinderliter, 2005, 1332671}. The effect followed a dose-related trend in some studies. PFOA exposure was also associated with significantly delayed parturition at ≥ 3 mg/kg/day in CD-1 mice {Lau, 2006, 1276159} and at 10 mg/kg/day in ICR mice {Yahia, 2010, 1332451}.

3.3.1.2.1.1 Mice

Li et al. (2018, 5084746) reported marked, dose-related decreases in maternal body weight gain at ≥ 10 mg/kg/day in pregnant Kunming mice exposed from gestation day 1 to 17 (GD1 to GD17; no statistical tests performed). Dose-related decreases in body weight gain were also seen in pregnant CD-1 mice exposed to 10, 20, or 40 mg/kg/day (significant at 20 and 40 mg/kg/day) by Lau et al. (2006, 1276159); significantly delayed time to parturition was also seen at 3, 10, and 20 mg/kg/day in this study (all litters at 40 mg/kg/day were resorbed). Yahia et al. (2010, 1332451) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day from GD0 to GD17 (sacrificed on GD18) or GD0 to GD18 (allowed to give birth), and at 10 mg/kg/day, observed significant decreases in body weight gain from GD12 onward in dams allowed to give birth as well as significantly decreased terminal body weight in dams sacrificed on GD18. In the same study, a significant decrease in food intake during early gestation was also reported for the dams allowed to give birth, but data were not shown. Delayed parturition was also observed at 10 mg/kg/day (data not shown). Pregnant CD-1 mice exposed to 25 mg/kg/day from GD11 to GD16 exhibited significantly decreased body weight from GD13 to GD16 {Suh, 2011, 1402560}. In contrast to the above-described findings, two studies in pregnant CD-1 mice reported significantly increased maternal body weight gain after exposure to 5 mg/kg/day {Blake, 2020, 6305864} or 3 or 5 mg/kg/day {Wolf, 2007, 1332672} from GD1–17.

3.3.1.2.1.2 Rats

A two-generation oral gavage reproductive toxicity study in Sprague-Dawley rats reported no effect on parental generation (P₀) maternal body weight or food consumption, but significantly decreased body weight in first-generation (F₁) parental females at 30 mg/kg/day during pre-cohabitation, gestation (GD0–GD14), and lactation day 5 to 15 (LD5–LD15). Decreased absolute food consumption was reported, but data were not shown; relative feed consumption was unaffected {Butenhoff, 2004, 1291063}. In pregnant Sprague-Dawley rats dosed with 30 mg/kg/day from GD4 to LD21, body weight gain was decreased during gestation and body weight was 4% lower than controls during lactation (statistical significance not indicated) {Hinderliter, 2005, 1332671}.

In a two-year chronic toxicity/carcinogenicity assay conducted by NTP (NTP, 2020, 7330145), female Sprague Dawley (Hsd:Sprague Dawley® SD®) rat dams were exposed to 0, 150, or 300

parts per million (ppm) PFOA in feed during the perinatal period. In study 1, F₁ male rats were administered 0, 150, or 300 ppm PFOA and F₁ female rats were administered 0, 300, or 1,000 ppm PFOA in feed during the postweaning period. For study 2, lower postweaning exposure levels (0, 20, 40, or 80 ppm) were utilized for males due to unexpected toxicity in male offspring using the original exposure regime. Exposure for all F₁ generations in both studies occurred for 107 weeks or until the 16-week interim necropsy. The perinatal and postweaning exposure regimes for females and males for both studies are presented in Table 6. **Error! Reference source not found..** Dose groups for this study are referred to as “[perinatal exposure level]/[postweaning exposure level]” (e.g. 300/100).

Table 6. Study Design for Perinatal and Postweaning Exposure Levels for F₁ Male and Female Rats for the NTP, 2020 (7330145) Study

Perinatal Dose	Postweaning Dose						
	0 ppm	20 ppm	40 ppm	80 ppm	150 ppm	300 ppm	1,000 ppm
Study 1 Females							
0 ppm	X	–	–	–	–	X	X
150 ppm	–	–	–	–	–	X	
300 ppm	–	–	–	–	–	–	X
Study 1 Males							
0 ppm	X	–	–	–	X	X	–
150 ppm	–	–	–	–	X		–
300 ppm	–	–	–	–	–	X	–
Study 2 Males							
0 ppm	X	X	X	X	–	–	–
300 ppm	X	X	X	X	–	–	–

X = exposure level used; F₁ = first generation.

In pregnant Sprague-Dawley rats exposed to 150 or 300 ppm via diet (equivalent to approximately 11 and 22 mg/kg/day during gestation and 22 and 45 mg/kg/day from LD1 to LD14), no consistent effects were observed on body weight or body weight gain during gestation or lactation (Figure 41). Food consumption was marginally but significantly decreased (up to 4%) at one or both dose levels at various intervals. In a repeat of this study that tested a single dose level of 300 ppm (approximately 21.8 mg/kg/day during gestation and 48.3 mg/kg/day from LD1 to LD14), no effects were observed on maternal body weight or body weight gain during gestation; from LD1 to LD14, there was a marginal but significant decrease (2–3%) in maternal body weight and body weight gain and a significant decrease (5%) in food consumption {NTP, 2020, 7330145}.

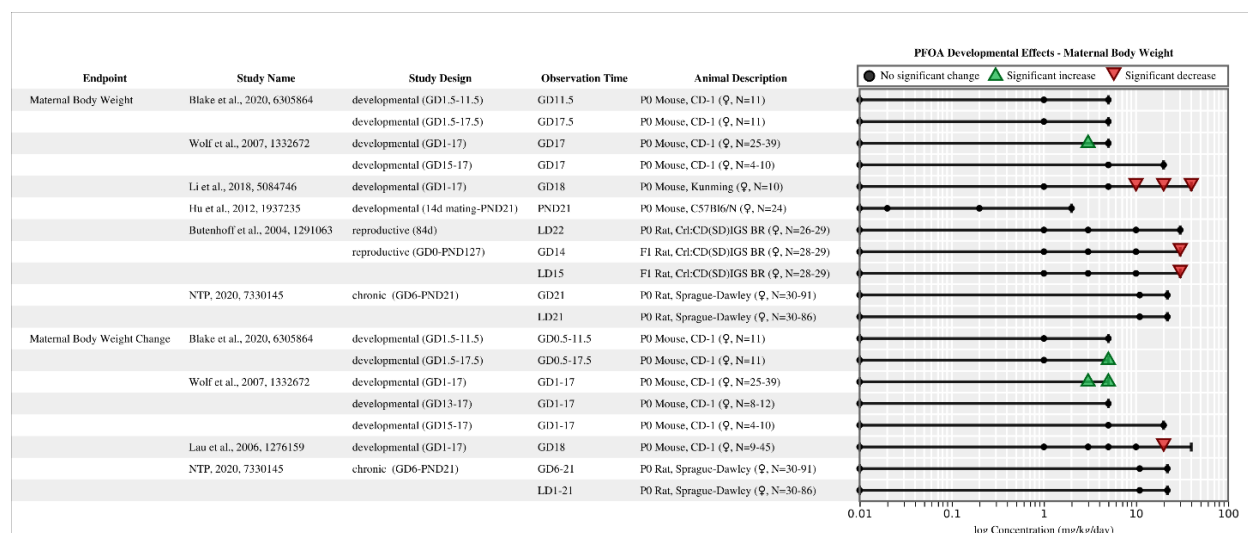


Figure 41. Maternal Body Weight in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA 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 = lactation day; P0 = parental generation; F1 = first generation.

3.3.1.2.2 Placenta Effects

Two oral gavage studies in CD-1 mice reported significant decreases in embryo to placenta weight ratio at 5 mg/kg/day {Blake et al., 2020, 6305864} or ≥ 2 mg/kg/day {Suh et al., 2011, 1402560} and treatment-related histopathological lesions at 5 mg/kg/day {Blake et al., 2020, 6305864} or ≥ 10 mg/kg/day {Suh et al., 2011, 1402560}. A third study in Kunming mice reported decreased placenta to body weight ratio at ≥ 5 mg/kg/day and histopathological changes in placental tissue at ≥ 2.5 mg/kg/day {Jiang et al., 2020, 6320192} (Figure 42).

Blake et al. (2020, 6305864) administered 0, 1, or 5 mg/kg/day to pregnant CD-1 mice from GD1.5 through sacrifice on GD11.5 or GD17.5, Suh et al. (2011, 1402560) administered 0, 2, 10, or 25 mg/kg/day to CD-1 mice from GD11 through sacrifice on GD16, and Jiang et al., {2020, 6320192} administered 0, 2.5, 5, or 10 mg/kg/day to Kunming mice from GD1 through sacrifice on GD13. The embryo to placental weight ratio was significantly decreased at 5 mg/kg/day in Blake et al. {2020, 6305864} and at ≥ 2 mg/kg/day in Suh et al. (2011, 1402560). Blake et al. (2020, 6305864) observed significantly increased placental weight at 5 mg/kg/day at GD17.5 and no changes in the numbers of viable fetuses or resorptions, whereas Suh et al. (2011, 1402560) observed significantly decreased placental weight and increased numbers of resorptions and dead fetuses at ≥ 2 mg/kg/day. Jiang et al. (2020, 6320192) observed significantly decreased relative placental weight at ≥ 5 mg/kg/day (decreases were also seen at lower dose levels, but they did not reach statistical significance). Histopathological changes in placental tissue were also observed at ≥ 2.5 mg/kg/day (increased area of spongiorotrophoblast, decreased blood sinusoidal area in labyrinth), ≥ 5 mg/kg/day (increased interstitial edema of spongiorotrophoblast), or 10 mg/kg/day (decreased labyrinth area, increased ratio of spongiorotrophoblast to labyrinth area). Jiang et al. (2020, 6320192) found no effect on fetus to maternal body weight ratio. Viable fetus weight was significantly decreased in Blake et al. (2020, 6305864) at 5 mg/kg/day and in Suh et al. (2011, 1402560) at ≥ 10 mg/kg/day and corresponded with treatment-related lesions in the

placenta. The incidence of GD17.5 placentas within normal limits was significantly lower in mice exposed to 5 mg/kg/day {Blake, 2020, 6305864}, and the lesions observed in placentas from that group included labyrinth atrophy (3/40 placentas), labyrinth congestion (23/40), and early fibrin clot (1/40). In dams treated with 1 mg/kg/day, labyrinth necrosis was observed in 1/32 placentas and placental nodules were observed in 2/32 placentas. Histopathologic examination by Suh et al. (2011, 1402560) showed normal placental tissue in 0 and 2 mg/kg/day groups and dose-dependent necrotic changes in placentas from the 10 and 25 mg/kg/day groups (incidences of specific lesions and statistical significance not reported).

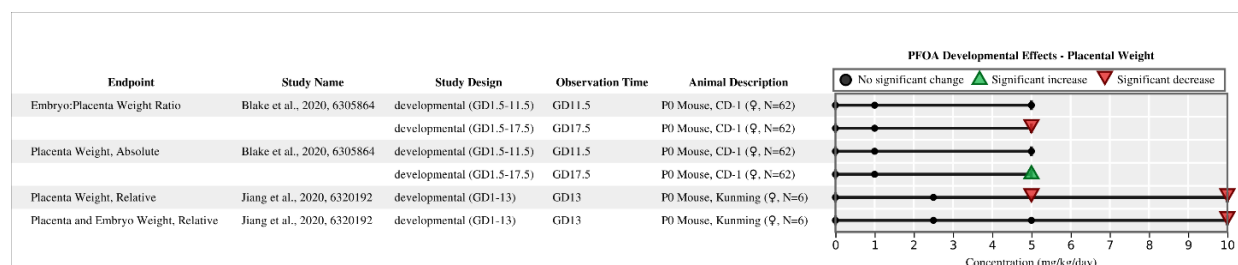


Figure 42. Placental Weights in Mice Following Exposure to PFOA

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

3.3.1.2.3 Offspring Mortality

Studies of oral PFOA exposure to mice report significant increases in resorptions and dead fetuses at dose levels as low as 2 mg/kg/day in prenatal evaluations {Li, 2018, 5084746; Suh, 2011, 1402560; Lau, 2006, 1276159}. Stillbirths, pup mortality, and total litter loss were observed in several strains of mice at ≥ 5 mg/kg/day {Lau, 2006, 1276159; Song, 2018, 5079725; White, 2011, 1276150; Wolf, 2007, 1332672; Yahia, 2010, 1332451}; increased litter loss was seen as low as 0.6 mg/kg/day in one study in 129S1/SvImJ mice {Abbott, 2007, 1335452}. Comparatively, rat pup mortality (pre- and post-weaning) was reported at a higher dose of 30 mg/kg/day {Butenhoff, 2004, 1291063}. Maternal effects observed in some of these studies were not sufficient to explain effects observed in the offspring, as some studies reported effects on offspring survival at dose levels that did not produce maternal effects.

3.3.1.2.3.1 Mice, Prenatal Evaluations

In two studies of gestational PFOA exposure to pregnant Kunming mice, Li et al. (2018, 5084746) reported significantly decreased GD18 fetal survival at 10 and 20 mg/kg/day and total fetal resorption at 40 mg/kg/day (fetal survival was also decreased at 5 mg/kg/day, but the effect did not reach statistical significance), and Chen et al. (2017, 3981369) reported a significant increase in the number of resorbed fetuses at GD13, but not GD7, after exposure to 10 mg/kg/day beginning on GD1 (there were no effects on the number of implantation sites). Suh et al. (2011, 1402560) exposed pregnant CD-1 mice to 0, 2, 10, or 25 mg/kg/day from GD11 to GD16 (dams were sacrificed on GD16) and observed a significant increase in the number of resorptions and dead fetuses at all dose levels; post-implantation loss was 3.87, 8.83, 30.98, and 55.41% at 0, 2, 10, and 25 mg/kg/day, respectively. In pregnant CD-1 mice exposed from GD1 to GD17, Lau et al. (2006, 1276159) reported significant increases in the number of full-litter resorptions at ≥ 5 mg/kg/day, with complete loss of all pregnancies at the high dose of 40 mg/kg/day (no effect was observed on the number of implantation sites in litters that were fully

resorbed). At 20 mg/kg/day, a significant increase in the percentage of prenatal loss per live litter was observed. White et al. (2011, 1276150) reported significantly fewer implants in F₁-generation CD-1 mouse dams that had been exposed to 5 mg/kg/day.

3.3.1.2.3.2 Mice, Postnatal Evaluations

Wolf et al. (2007, 1332672) reported a significant increase in total litter loss following oral exposure of pregnant CD-1 mice to 5 mg/kg/day (no effect on the number of implantation sites). In offspring exposed to 5 mg/kg/day *in utero* and throughout lactation, significantly decreased pup survival was observed from postnatal day 4 to 22 (PND4 to PND22; this effect was not seen in cross-fostered offspring exposed during gestation only or during lactation only). In a separate study, these authors exposed pregnant CD-1 mice to 5 mg/kg/day for different lengths of time (GD7–GD17, GD10–GD17, GD13–GD17, or GD15–GD17) and to 20 mg/kg/day from GD15–17. Control mice received deionized water from GD7 to GD17. Although gestational PFOA exposure from GD1 to GD6 was not required to elicit adverse developmental responses in pups, the severity of postnatal responses, including decreased pup weight during lactation and delayed eye opening, increased with earlier and longer exposure durations (i.e., GD7–GD17 exposure resulted in more severe decreases in pup body weight when compared to pups exposed from GD15 to GD17). The authors could not attribute the observed adverse effects to a sensitive window of development as the pups exposed for longer durations had higher serum PFOA levels than pups exposed for shorter durations. Notably, significantly decreased offspring survival was observed in pups exposed to 20 mg/kg/day with the shortest exposure duration from GD15 to GD17 {Wolf, 2007, 1332672}.

Lau et al. (2006, 1276159) reported significant increases in the incidence of stillbirths and pup mortality at 5, 10, and 20 mg/kg/day in CD-1 mice exposed from GD1 to GD18 and allowed to deliver naturally (complete loss of all pregnancies was observed at the high dose of 40 mg/kg/day); there were no effects on the number of implantation sites). At 10 and 20 mg/kg/day, most of the pups died on PND1. After exposure of pregnant Kunming mice to 1, 2.5, or 5 mg/kg/day from GD1 to GD17, Song et al. (2018, 5079725) reported a significant decrease in the number of surviving pups/litter on PND7, 14, and 21 at 5 mg/kg/day (a dose-related trend was observed, but statistical significance was achieved only at the high dose). Yahia et al. (2010, 1332451) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day from GD0 to GD18, and the dams were allowed to give birth naturally. Approximately 58% of pups born to high-dose dams were stillborn, and the remaining pups died within 6 hours of birth. Mean PND4 survival rate was 98, 100, 84.4, and 0% at 0, 1, 5, and 10 mg/kg/day, respectively (significantly decreased at 5 and 10 mg/kg/day). In the same study, some of the pregnant mice were exposed to the same dose levels from GD0 to GD17 and sacrificed on GD18, and the number of live GD18 fetuses from these dams was not significantly affected at any dose level. White et al. (2011, 1276150) conducted a multi-generational study and dosed pregnant CD-1 mice with 0, 1, or 5 mg/kg/day from GD1 to GD17. Exposure to 5 mg/kg/day significantly increased prenatal loss, significantly decreased the number of live pups born, and significantly reduced postnatal survival. In adult female F₁ animals, no effects were observed on the prenatal loss or postnatal pup survival of the second generation (F₂) offspring. Abbott et al. (2007, 1335452) exposed pregnant 129S1/SvImJ wild-type and PPAR α -null mice from GD1 to GD17 to dose levels ranging from 0.1 to 20 mg/kg/day and allowed the mice to deliver naturally. Wild-type dams exposed to ≥ 0.6 mg/kg/day and PPAR α -null dams exposed to ≥ 5 mg/kg/day had significantly increased litter loss compared to their respective controls. At ≥ 5 mg/kg/day in wild-type dams and 20 mg/kg/day in PPAR α -

null dams, 100% litter loss occurred. Pup survival from birth to weaning was significantly decreased in wild-type litters exposed to ≥ 0.6 mg/kg/day (no effect seen in PPAR α -null litters). Survival was significantly decreased for wild-type and heterozygous pups born to wild-type dams dosed with 1 mg/kg/day and for heterozygous pups born to PPAR α -null dams dosed with 3 mg/kg/day.

3.3.1.2.3.3 Rats, Postnatal Evaluations

The NTP two-year carcinogenicity studies in Sprague-Dawley rats found no effects on offspring survival {NTP, 2020, 7330145}, but Butenhoff et al. (2004, 1291063) reported an increase in the total number of dead F₁ rat pups during lactation (26/388 deaths at 30 mg/kg/day and 10/397 in the control group; statistically significant only on LD6–LD8) and a significant increase in F₁ female pup deaths at 30 mg/kg/day on post-weaning days 2–8. F₂ generation pup survival was unaffected. In pregnant Sprague-Dawley rats dosed with 0, 3, 10, or 30 mg/kg/day from GD4 to LD21, one dam at 3 mg/kg/day and two dams at 30 mg/kg/day delivered small litters (3–6 pups/litter compared to 12–19 pups/litter in the control group); however, statistical significance was not indicated, and given the small sample size (5 dams/group), the biological significance of this finding is unclear {Hinderliter, 2005, 1332671} (Figure 43).

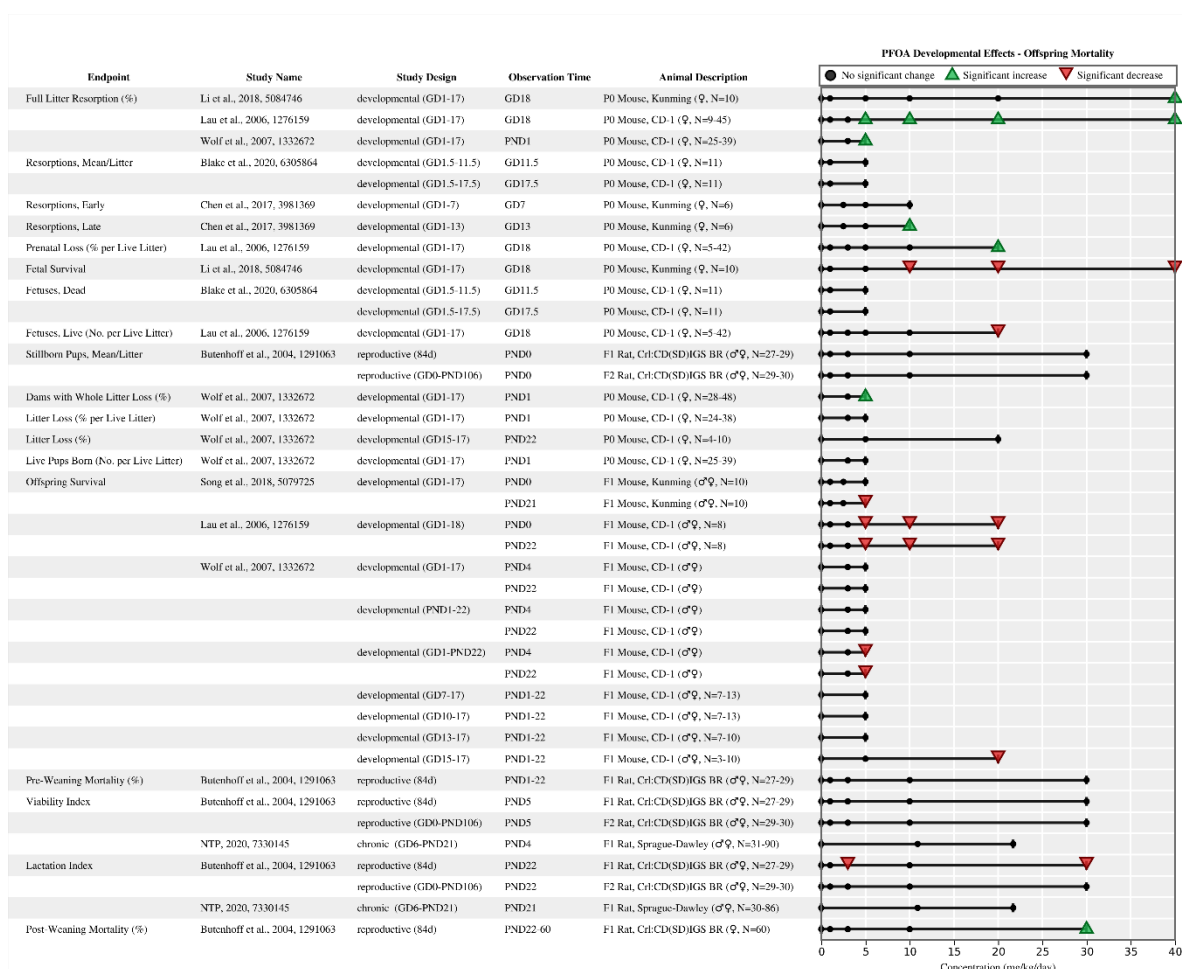


Figure 43. Offspring Mortality in Rodents Following Exposure to PFOA

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

GD = gestation day; PND = postnatal day; P₀ = parental generation; F₁ = first generation; F₂ = second generation; d= day.

3.3.1.2.4 Offspring Body Weight

Available studies of oral gestational PFOA exposure to mice report significant decreases in offspring body weight in prenatal evaluations (at ≥ 5 mg/kg/day) and postnatal evaluations (at dose levels as low as 1 mg/kg/day) {Abbott, 2007, 1335452; Blake, 2020, 6305864; Hu, 2012, 1937235; Lau, 2006, 1276159; Li, 2018, 5084746; Suh, 2011, 1402560; Tucker, 2015, 2851046; White, 2011, 1276150; Wolf, 2007, 1332672; Yahia, 2010, 1332451}. Offspring weight deficits in pups were observed to extend beyond weaning in three studies in CD-1 mice (at 1, ≥ 3 , and 5 mg/kg/day, respectively) {Tucker, 2015, 2851046; Lau, 2006, 1276159; White, 2011, 1276150} and in a multi-generation rat study at 30 mg/kg/day {Butenhoff, 2004, 1291063}. In some studies, decreased fetal and/or pup body weight was observed in the absence of maternal body weight effects.

3.3.1.2.4.1 Mice, Prenatal Evaluations

Blake et al. (2020, 6305864) reported significantly decreased GD17.5 fetal weight at 5 mg/kg/day following gestational exposure in CD-1 mice, despite significantly increased maternal body weight gain. Lau et al. {2006, 1276159} reported a significant decrease in GD18 fetal body weights after gestational exposure of CD-1 mice to 20 mg/kg/day. In pregnant Kunming mice, gestational exposure was associated with significantly decreased GD18 fetal weights at 5–40 mg/kg/day {Li, 2018, 5084746}. Suh et al. (2011, 1402560) reported a significant decrease in GD16 fetal weights at ≥ 10 mg/kg/day after exposure of pregnant CD-1 mice to 0, 2, 10, or 25 mg/kg/day from GD11 to GD16. Body weights of GD18 ICR mouse fetuses were significantly decreased following gestational exposure to 5 or 10 mg/kg/day {Yahia, 2010, 1332451}.

3.3.1.2.4.2 Mice, Postnatal Evaluations

Wolf et al. (2007, 1332672) reported that CD-1 mouse pup BWTs were significantly decreased after gestational exposure to 5 mg/kg/day from GD1 to GD17. The authors also exposed pregnant mice to 20 mg/kg/day from GD15 to GD17 and to 5 mg/kg/day for different lengths of time (GD7–GD17, GD10–GD17, GD13–GD17, or GD15–GD17). After exposure to 5 mg/kg/day from GD7 to GD17 or GD10 to GD17 and to 20 mg/kg/day from GD15 to GD17, male pup BWTs were significantly decreased. Additionally, at 5 mg/kg/day, male and female pup body weights were significantly decreased throughout lactation in all exposure groups, and the magnitude of the effect increased with increasing number of exposure days. Body weight deficits in male pups that had been exposed from GD7 to GD17 or GD10 to GD17 persisted for 10–11 weeks {Wolf, 2007, 1332672}.

Body weights of live pups born to pregnant ICR mice dosed with 5 or 10 mg/kg/day during gestation were significantly reduced {Yahia, 2010, 1332451}. At ≥ 3 mg/kg/day, a dose-related trend in growth retardation (body weight reductions of 25–30%) was observed in neonates at weaning; body weights reached control levels by 6 weeks of age for females and by 13 weeks of age for males {Lau, 2006, 1276159}. Exposure of pregnant C57BL/6N mice to 2 mg/kg/day from mating through lactation resulted in significantly decreased pup weights (32.6% lower than controls, on average) from PND1 to PND21 (there were no effects on maternal body weights) {Hu, 2012, 1937235}. Song et al. (2018, 5079725) observed significantly increased body weights in PND21 male offspring after gestational exposure to 2.5 or 5 mg/kg/day (female data not provided). However, the authors did not report controlling for litter size in this study; the

significantly decreased litter size in the 5 mg/kg/day group could potentially result in increased body weight in those pups due to reduced competition for maternal resources.

In a study in which pregnant 129S1/SvImJ wild-type and PPAR α -null mice were orally exposed from GD1 to GD17 to dose levels ranging from 0.1 to 20 mg/kg/day {Abbott, 2007, 1335452}, decreased offspring body weight was seen in wild-type mice at 1.0 mg/kg/day (highest dose level at which this effect was measured due to extensive litter loss at higher doses) beginning around PND6, and this effect achieved statistical significance on PND9, PND10, and PND22 (males) and PND7–PND10 and PND22 (females). No effects were observed on PPAR α -null offspring body weights. White et al. {2011, 1276150} exposed pregnant CD-1 mice to 0, 1, or 5 mg/kg/day from GD1 to GD17. A separate group of pregnant mice was dosed with either 0 or 1 mg/kg/day from GD1 to GD17 and received drinking water containing 5 ppb PFOA beginning on GD7. F₁ females and F₂ offspring from the second group continued to receive drinking water that contained 5 ppb PFOA until the end of the study, except during F₁ breeding and early gestation, to simulate a chronic low-dose exposure. F₁ offspring body weight at PND42 was significantly reduced at 5 mg/kg/day; at PND63, body weight was significantly reduced for offspring from dams given 1 mg/kg/day plus 5 ppb in the drinking water compared to offspring from dams given only 1 mg/kg/day. For the F₂ pups, a significant reduction in body weight was observed in control plus 5 ppb drinking water PFOA offspring on PND1, but there was no difference by PND3. F₂ offspring from the 1 mg/kg/day and 1 mg/kg/day plus 5 ppb drinking water PFOA groups had increased body weight compared to controls on PND14, PND17, and PND22. Female CD-1 mice that had been exposed gestationally to 1 mg/kg/day had significantly decreased body weights at PND21 and PND35 but not at PND56 {Tucker, 2015, 2851046}. Macon et al. (2011, 1276151) found no effects on offspring body weights following exposure of pregnant CD-1 mice to PFOA from GD1 to GD17 with doses up to 1 mg/kg/day or from GD10 to GD17 with doses up to 3 mg/kg/day.

3.3.1.2.4.3 Rats, Postnatal Evaluations

In two NTP 2-year carcinogenicity studies {NTP, 2020, 7330145}, dietary exposure of pregnant Sprague-Dawley rats to 300 ppm PFOA (approximately 22 mg/kg/day during gestation and 45 mg/kg/day from LD1 to LD14) resulted in significantly decreased pup weights throughout lactation (3–8% lower than controls). In both studies, there were minimal to no effects on maternal body weight. Significantly decreased F₁ pup weight (8–11% lower than controls) during lactation was observed following exposure of pregnant Sprague-Dawley rats to 30 mg/kg/day, in the absence of effects on maternal body weight; F₂ pup weight was slightly decreased at 30 mg/kg/day, but the effect was not statistically significant {Butenhoff, 2004, 1291063}. At 30 mg/kg/day, significant decreases in body weight and body weight gain were seen in F₁ male offspring during the juvenile and peripubertal phases and in F₁ female offspring beginning on day 8 postweaning and continuing through pre-cohabitation, gestation, and lactation (along with decreased food consumption) (Figure 44).

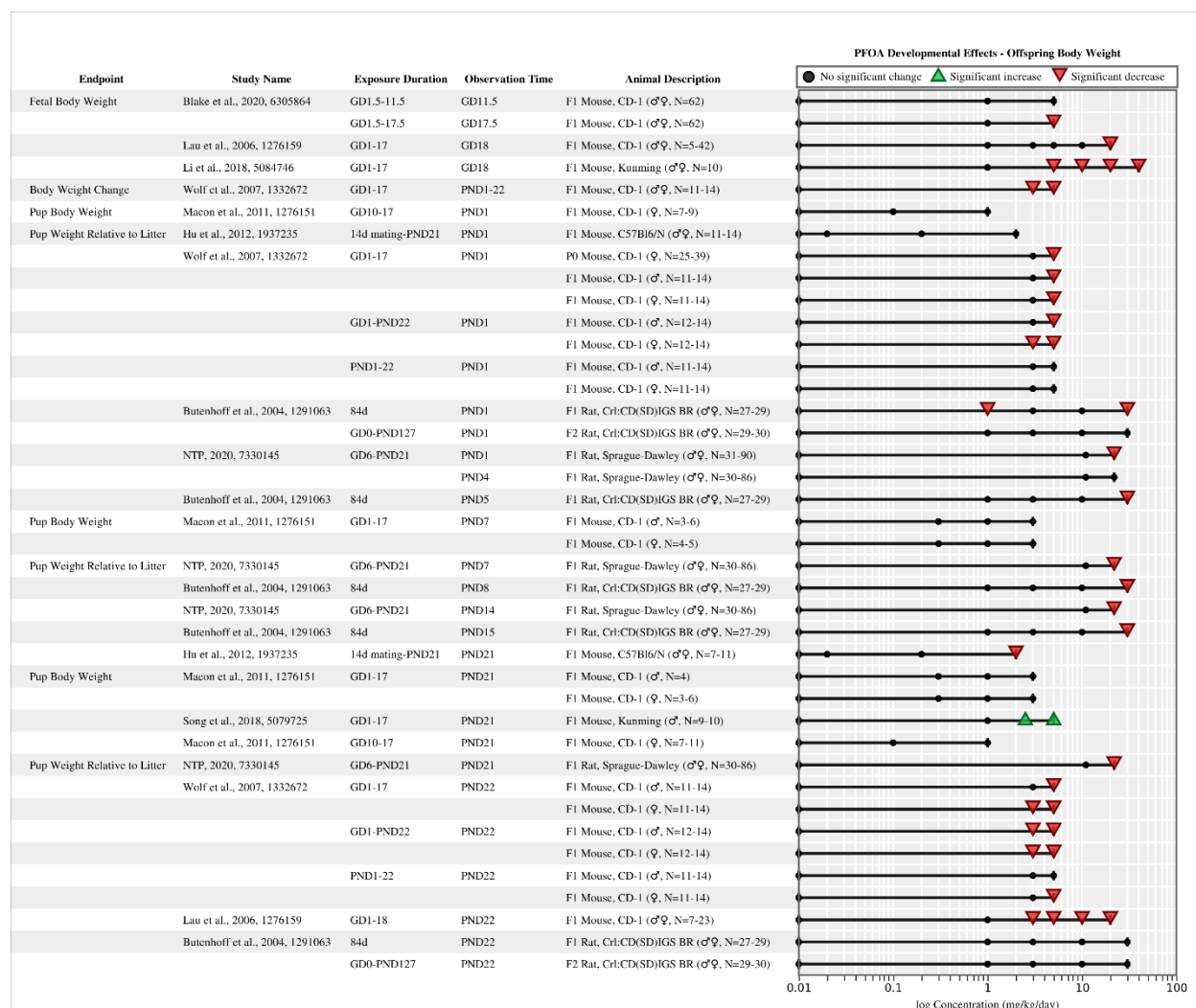


Figure 44. Offspring Body Weight in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA 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; P0 = parental generation; F1 = first generation; F2 = second generation; d = day.

3.3.1.2.5 Skeletal and Visceral Alterations

Following exposure of pregnant CD-1 mice to 1, 3, 5, 10, 20, or 40 mg/kg/day during gestation, Lau et al. (2006, 1276159) reported decreases in ossification of the forelimb proximal phalanges (significant at all dose levels except 5 mg/kg/day), hindlimb proximal phalanges (significant at all dose levels except 3 and 5 mg/kg/day), calvaria (significant at 1, 3, and 20 mg/kg/day), enlarged fontanel (significant at 1, 3, and 20 mg/kg/day), and supraoccipital bone (significant at 10 and 20 mg/kg/day). Significantly reduced ossification of caudal vertebrae, metacarpals, metatarsals, and hyoid was observed at 20 mg/kg/day. Significant increases in minor limb and/or tail defects were observed in fetuses at ≥ 5 mg/kg/day (no defects were observed at 0, 1, or 3 mg/kg/day) and significantly increased incidence of microcardia was observed at 10 and 20 mg/kg/day (no incidences were observed in any other groups). Yahia et al. (2010, 1332451)

dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day from GD0 to GD17 (sacrificed on GD18) and reported a significant increase in the incidence of cleft sternum and ossification delays (phalanges) in GD18 fetuses at 10 mg/kg/day. In the same study, some dams were dosed from GD0 to GD18 and allowed to give birth, and pup lungs and brains were examined at PND4; no abnormalities were reported.

3.3.1.2.6 Altered Developmental Timing

Reduced postnatal growth leading to developmental delays was observed in both rats and mice. Lau et al. (2006, 1276159) and Wolf et al. (2007, 1332672) reported delayed eye opening in CD-1 mice offspring after gestational exposure to ≥ 5 mg/kg/day. Additionally, Wolf et al. (2007, 1332672) observed delayed eye-opening following gestational plus lactational exposure to 3 or 5 mg/kg/day. Wolf et al. (2007, 1332672) also observed delayed body hair emergence following gestational exposure to 5 mg/kg/day or gestational plus lactational exposure to 3 or 5 mg/kg/day. Lau et al. (2006, 1276159) also reported slight but significant delays in vaginal opening at 20 mg/kg/day; in contrast, significant accelerations in sexual maturation were observed in males, with preputial separation occurring 4 days earlier than controls at 1 mg/kg/day and 2–3 days earlier at 3, 5, and 10 mg/kg/day, whereas preputial separation in the 20 mg/kg/day group was slightly but significantly delayed compared to controls. In pregnant 129S1/SvImJ wild-type and PPAR α -null mice orally exposed from GD1 to GD17 to 0.1–20 mg/kg/day {Abbott, 2007, 1335452}, offspring born to wild-type dams showed a dose-related trend for delayed eye opening compared to controls at 0.6 and 1 mg/kg/day (significant at 1 mg/kg/day; extensive litter loss seen at the higher doses). In PPAR α -null offspring, none of the litters from dams exposed to 3 mg/kg/day had eyes open on PND13, but no significant difference between this group and the control was observed by PND14. Yahia et al. (2010, 1332451) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day from GD0 to GD17 (sacrificed on GD18) and reported a significant decrease in the percentage of GD18 fetuses with erupted incisors at 10 mg/kg/day. A two-generation study in Sprague-Dawley rats reported significantly delayed sexual maturation in F₁ males and females at 30 mg/kg/day {Butenhoff, 2004, 1291063}. In a study of direct peripubertal exposure, Yang et al. (2009, 5085085) orally dosed 21-day-old female BALB/c or C57BL/6 mice with 0, 1, 5, or 10 mg/kg/day for 5 days/week for 4 weeks. Vaginal opening was significantly delayed in BALB/c mice dosed with 1 mg/kg/day and did not occur at all at 5 or 10 mg/kg/day. In C57BL/6 mice, vaginal opening was delayed at 5 mg/kg/day and did not occur at 10 mg/kg/day.

3.3.1.2.7 Mammary Gland Development

Altered mammary gland development has been shown to result in later-life functional reproductive consequences, such as reduced lactational efficacy and subsequent pup loss, and has been linked to increased incidence of mammary and breast cancers {Fenton, 2006, 470286; Macon, 2013, 3827893; Birnbaum, 2003, 197117}. Studies examining effects of PFOA exposure on mammary gland development in CD-1 mice reported delayed mammary gland development at dose levels as low as 0.01 mg/kg/day {Macon, 2011, 1276151; Tucker, 2015, 2851046}. However, no differences in response to a lactation challenge were seen in PFOA-exposed CD-1 mouse dams with delayed mammary gland development, and no significant effects on body weight gain were seen in pups nursing from dams with less fully developed mammary glands {White, 2011, 1276150}.

Macon et al. (2011, 1276151) exposed pregnant CD-1 mice to PFOA from GD1 to GD17 (full gestation) or GD10 to GD17 (late gestation) to examine effects of PFOA exposure on mammary gland morphology. Mammary gland whole mounts were scored on a 1 to 4 subjective, age-adjusted, developmental scale. Quantitative measures also were made of longitudinal growth, lateral growth, and number of terminal end buds. At all PFOA exposure levels in both experiments (≥ 0.3 mg/kg/day in the full gestation study and ≥ 0.01 mg/kg/day in the late-gestation study), significantly stunted mammary epithelial growth was observed in female offspring in the absence of effects on offspring body weight. Additionally, there were significant differences from controls in quantitative measures of longitudinal and lateral growth and numbers of terminal end buds at 1 mg/kg/day in the late-gestation experiment. The delayed development was characterized by reduced epithelial growth and the presence of numerous terminal end buds. Photographs of the mammary gland whole mounts at PND21 and PND84 from the full-gestation experiment showed differences in the duct development and branching pattern of offspring from dams given 0.3 and 1 mg/kg/day (offspring from high-dose dams not pictured). At PND21, mammary glands from the 1.0 mg/kg/day late-gestation group had significantly less longitudinal epithelial growth and fewer terminal end buds compared with controls. In the late-gestation experiment, mammary gland development was delayed by exposure to PFOA, especially longitudinal epithelial growth. At PND21, all treatment groups had significantly lower developmental scores. At the highest dose, poor longitudinal epithelial growth and decreased number of terminal end buds were observed. The quantitative measures were statistically significant only for the high dose compared to the controls, whereas the qualitative scores at all doses were significantly different from controls.

CD-1 mice were dosed with 5 mg/kg/day on GD7–GD17, GD10–GD17, GD13–GD17, or GD15–GD17 or with 20 mg/kg/day on GD15–GD17 (controls were dosed GD7–GD17) and mammary gland effects of this study were published by White et al. {2009, 194811}. Mammary gland developmental scores for all offspring of dams exposed to PFOA were significantly lower at PND29 and PND32. Delayed ductal elongation and branching and delayed appearance of terminal end buds were characteristic of delayed mammary gland development at PND32. At 18 months of age, mammary tissues were not scored (due to the lack of a protocol applicable to mature animals) but dark foci (composition unknown) in the mammary tissue were observed at a higher frequency in exposed animals compared to controls (there was no consistent response with respect to dosing interval). Qualitatively, mammary glands from treated dams on LD1 appeared immature compared with control dams {White, 2009, 194811}. The authors also exposed pregnant CD-1 mice to 0, 3, or 5 mg/kg/day from GD1 to GD17 and offspring were cross-fostered at birth to create seven treatment groups: control, *in utero* exposure only (3U and 5U), lactational exposure only (3L and 5L), and *in utero* + lactational exposure (3U+L and 5U+L). Mammary gland whole mounts from female offspring between PND22 and PND63 were scored. With the exception of females of the 3L group, all female offspring of PFOA-exposed dams had reduced mammary gland developmental scores at PND22. At PND42, mammary gland scores from females in the 3U+L group were the only ones not statistically different from control scores. This might have been due to inter-individual variance and multiple criteria used to calculate mammary gland development scores. All offspring of dams exposed to PFOA exhibited delayed mammary gland development at PND63, including those exposed only through lactation (3L and 5L).

White et al. (2011, 1276150) dosed pregnant CD-1 mice with 0, 1, or 5 mg/kg/day from GD1 to GD17. A separate group of pregnant mice was dosed with either 0 or 1 mg/kg/day from GD1 to GD17 and received drinking water containing 5 ppb PFOA beginning on GD7. F₁ females and F₂ offspring from the second group continued to receive drinking water that contained 5 ppb PFOA until the end of the study, except during F₁ breeding and early gestation, to simulate a chronic low-dose exposure. Only the P₀ dams were given PFOA by gavage. P₀ females were sacrificed on PND22. F₁ offspring were weaned on PND22 and bred at 7–8 weeks of age. F₂ litters were maintained through PND63. Groups of F₁ and F₂ offspring were sacrificed on PND22, PND42, and PND63. A group of F₂ offspring also was sacrificed on PND10. A lactational challenge experiment was performed on PND10 with F₁ dams and F₂ offspring to estimate the volume of milk produced during a discrete period of nursing. Mammary glands were evaluated from P₀ dams on PND22, from F₁ dams on PND10 and PND22, and from F₁ and F₂ female offspring on PND10 (F₂ only), PND22, PND42, and PND63. Mammary gland whole mounts were scored qualitatively. At PND22, control P₀ dams displayed weaning-induced mammary involution. At PND22, the mammary glands of all PFOA-exposed P₀ dams, including the control dams receiving 5 ppb PFOA in drinking water, resembled glands of mice at or near the peak of lactation (~PND10). The F₁ dams examined on PND10 and PND22 had significantly lower developmental scores on PND10, but that was no longer evident at PND22, except for those exposed in utero to 5 mg/kg/day. In the F₁ female offspring not used for breeding, the mammary glands of all PFOA-exposed mice were significantly delayed in development on PND22, 42, and 63. For the F₂ female offspring, some differences in mammary gland scores were observed between the groups, but most were not significantly different from controls. No differences in response to a lactational challenge were seen in PFOA-exposed dams with morphologically delayed mammary gland development.

Tucker et al. (2015, 2851046) orally exposed pregnant CD-1 and C57BL/6 mice to 0, 0.01, 0.1, 0.3, or 1 mg/kg/day from GD1 to GD17. After parturition, the number of pups was reduced so that there were ultimately four to eight CD-1 litters and three to seven C57BL/6 litters per treatment. Different treatment blocks monitored for different endpoints at different times. There was a dose-related trend towards decreasing mammary gland developmental scores for both strains of mice. In CD-1 mice, scores were significantly reduced at ≥ 0.01 mg/kg/day on PND35 and at ≥ 0.1 mg/kg/day on PND21. In C57BL/6 mice, scores were significantly reduced at 0.3 and 1.0 mg/kg/day on PND21. At 5 mg/kg/day, in mammary glands of C57BL/6 mice, there was a significant increase in the number of terminal end buds and stimulated terminal ducts; ductal length was not affected. Mammary gland development was inhibited in C57BL/6 mice dosed with 10 mg/kg/day, with no terminal end buds or stimulated terminal ducts present and very little ductal growth.

In a study of direct peripubertal exposure, Yang et al. (2009, 5085085) orally dosed 21-day-old female BALB/c or C57BL/6 mice with 0, 1, 5, or 10 mg/kg/day 5 days/week for 4 weeks. Mammary glands of BALB/c mice treated with 5 or 10 mg/kg/day had reduced ductal length, decreased number of terminal end buds, and decreased stimulated terminal ducts; injection with bromo-2'-deoxyuridine, a marker of cell proliferation, into the mammary gland revealed a significantly lower number of proliferating cells in the ducts and terminal end buds/terminal ducts at 5 mg/kg/day (not examined at 10 mg/kg/day).

3.3.1.2.8 Offspring Liver Weight

Studies report offspring liver weight changes suggestive of hypertrophy. White et al. (2011, 1276150) orally dosed pregnant CD-1 mice with 0, 1, or 5 mg/kg/day from GD1 to GD17. F₁ offspring liver-to-body weight ratios were significantly increased at 1 mg/kg/day on PND22 and at 5 mg/kg/day on PND22 and PND42. Macon et al. (2011, 1276151) exposed pregnant CD-1 mice to PFOA from GD1 to GD17 (full gestation) or GD10 to GD17 (late gestation). At PND7, significantly increased absolute and relative liver weights in offspring were observed as low as 0.3 mg/kg/day after full-gestation exposure; significantly increased absolute and relative liver weights were also observed at the high dose of 1.0 mg/kg/day after late-gestation exposure (PND4 and PND7; relative liver weights were also significantly increased at PND14). Wolf et al. (2007, 1332672) reported that offspring of pregnant CD-1 mice orally dosed with 0 and 5 mg/kg/day on GD7–GD17, GD10–GD17, GD13–GD17, and GD15–GD17 or with 20 mg/kg/day on GD15–GD17 had significantly increased liver-to-body weight ratios at PND22; White et al. (2009, 194811) reported that offspring of CD-1 mice exposed to 5 mg/kg/day during gestation or during gestation plus lactation had significantly increased liver-to-body weight ratios on PND1.

3.3.1.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse developmental outcomes is discussed in Sections 3.2.6, 3.2.7, 3.3.4, 3.4.1, and 3.4.5 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 20 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to developmental effects. A summary of these studies is shown in Figure 45. Additional analysis on the mechanistic actions of PFOA on developmental health outcomes is ongoing 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	0	6	1	7
Cell Growth, Differentiation, Proliferation, Or Viability	5	1	2	8
Cell Signaling Or Signal Transduction	2	2	1	5
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	4	0	0	4
Hormone Function	2	0	0	2
Inflammation And Immune Response	0	1	0	1
Oxidative Stress	2	1	1	4
Xenobiotic Metabolism	3	0	0	3
Other	0	0	1	1
Not Specified (Review Article)	1	0	0	1
Grand Total	8	8	4	20

Figure 45. Summary of Mechanistic Studies of PFOA and Developmental Effects

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

3.3.1.4 Evidence Integration

As noted in the fetal growth restriction summary, there is suggestive evidence that PFOA may impact fetal growth restriction across a variety of BWT-related measures. 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. Collectively, the majority of LBW studies were supportive of an increased risk with increasing PFOA exposures. Five *medium* or *high* confidence studies on LBW showed increased risks with increased PFOA levels. The endpoint of LBW from Chu et al. (2020, 6315711), Sagiv et al. (2018, 4238410), Starling et al. (2017, 3858473), Govarts et al. (2016, 3230364), and Wikström et al. (2020, 6311677) was considered for the derivation of PODs.

Overall, there was less consistent evidence of PFOA 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. Similarly, there was less consistent evidence of an impact of PFOA exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) as many of studies did not show adverse associations.

Collectively, across these various endpoints there is moderate evidence of developmental effects related to PFOA based on the more recent epidemiological literature. However, as noted

previously there is some uncertainty as to what degree the evidence may be impacted by pregnancy hemodynamics 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 PFOA associations. The multipollutant modeling results were often mixed from single pollutant estimates with some estimates increasing and some decreasing. Unlike other PFAS, PFOA was chosen amongst dimension-reducing statistical approaches from models with various PFAS and or other environmental contaminants adjusted for in two different studies {Lenters, 2016, 5617416; Starling et al., 2017}. Although these results are smaller in magnitude, they appear coherent with single exposure model results. 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.

The available animal toxicity data are in concordance with the data in humans and indicate that the developing fetus is a target of PFOA toxicity. Specifically, several studies in rodents show decreased fetal and pup weight with gestational PFOA exposure, similar to the evidence of LBW seen in infants. Oral studies in rodents consistently show that gestational PFOA exposure results in pre- and postnatal effects on offspring, as well as maternal effects in dams. Notably, mice appear to be more sensitive to developmental toxicity as a result of gestational exposure compared to rats. In addition, studies in both rats and mice show that effects on offspring (e.g., decreases in body weight, survival) occur at lower dose levels than those that produced maternal body weight effects. Given the large number of adverse effects identified in the animal toxicity data, EPA considered only the most sensitive effects for derivation of PODs.

Multiple studies observed effects at low dose levels and/or demonstrated a dose-related response and are good candidates for dose-response modeling. The endpoints of placental histopathology (GD 17.5) from Blake et al. (2020, 6305864), decreased fetal body weights from Li et al. (2018, 5084746), decreased pup survival (PND 22) from Song et al. (2018, 5079725), delayed eye opening from Lau et al. (2006, 1276159), and altered mammary gland development from Macon et al. (2011, 1276151), were considered for the derivation of PODs.

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, 3603279} reports identified limited evidence of effects of PFOA on reproductive effects in men and boys. One study {Joensen, 2009, 1405085} of Danish men in the military (n = 105) showed non-significant inverse associations with serum PFOA and semen volume, sperm concentration,

sperm count, sperm motility, and sperm morphology. Comparing men with combined PFOA/PFOS serum levels revealed significantly ($p < 0.05$) less morphologically normal sperm in those men with higher PFOA/PFOS levels compared to those with low PFOA/PFOS levels. No associations were observed for serum sex hormones in this study. In healthy young Danish males Joensen (2013, 2851244) observed no associations with reproductive hormones. Semen parameters were also assessed in men from the Longitudinal Investigation of Fertility and the Environment Study (LIFE) cohort {Buck Louis, 2015, 2851189}, and significant associations were observed for a few morphological parameters, including fewer coiled tails, increased curvilinear velocity, and a larger acrosome area of the head. One prospective birth cohort study {Vested, 2013, 2317339} followed offspring for approximately 20 years after mothers provided a third trimester blood sample. Regarding prenatal PFOA exposure, a significant negative trend was observed for total sperm count with 34% reductions in total count for each of the highest two tertiles compared to the lowest PFOA tertile. Additionally, prenatal PFOA exposure was associated with higher follicle stimulating hormone (FSH) (responsible for stimulating testicular growth) and luteinizing hormone (LH) (responsible for stimulating testosterone production) concentrations in these men after 20 years. Three occupational studies {Olsen, 1998; Sakr, 2007, 1291103; Costa, 2009, 1429922} observed minimal evidence of effects in male employees. A study {Olsen, 1998, 1290857} on male employees ($n = 111$) at a Minnesota PFOA production plant (1993–1995) observed non-significant elevated estradiol (E2) in the highest PFOA exposure group; however, the study authors suggest this may have been confounded by a high correlation between E2 and BMI. A study {Sakr, 2007a, 1291103} of employees at a DuPont facility in West Virginia observed associations for serum E2 and testosterone, but they did not address circadian variations in hormone levels and concluded the biological significance of the result was unclear. No other associations were observed in occupational studies evaluating males.

For this updated review, 21 studies⁶ (22 publications) report on the association between PFOA and endocrine effects since the 2016 document. 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 {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 a reproductive medical center in Nanjing, China {Pan, 2019, 6315783; Cui, 2020, 6833614}. One pair of studies assessed populations from related cohorts belonging to the Hokkaido study on the Environment and Children's Health {Itoh, 2016, 3981465; Goudarzi, 2017}.

Eleven 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}, and the remainder of the publications were on the general population. Different study designs were utilized, including four cohort studies {Ernst, 2019, 5080529; Goudarzi, 2017, 3981462; Itoh, 2016, 3981465; Jensen, 2020, 6311643} with the remainder of the studies following a cross-sectional design. All observational studies measured PFOA in blood components (i.e., blood, plasma, or serum); however, PFOA in semen

⁶ Zhou, 2016, 3856472 and Zhou, 2017, 3858488 use differing methods to analyze participants from the same population using the same health outcome.

was additionally measured in four studies {Cui, 2020 6833614; Di Nisio, 2019, 5080655; Pan, 2019, 6315783; Song, 2018, 4220306}. The studies were conducted in different study populations including populations from Australia, China, Denmark, the Faroe Islands, Greenland, Italy, Japan, Poland, Taiwan, Ukraine, and the United States. While most studies evaluated the relationship between exposure to PFOA and sex hormone concentrations, other male reproductive outcomes investigated included: sex-hormone related steroid hormones (e.g., dehydroepiandrosterone (DHEA)), pubertal markers (e.g., voice break), semen analysis, genomic effects in sperm (e.g., DNA methylation), and anthropometric measurements (e.g., anogenital distance (AGD), penis length, etc.).

3.3.2.1.1.2 Study Quality

Of the 21 studies identified since the 2016 assessment, two studies were classified as *high* confidence, 15 studies as *medium* confidence, three studies as *low* confidence, and one study {Song, 2018, 4220306} was determined to be *uninformative* (Figure 46). Publications from the GBCA {Zhou, 2016, 3856472; Zhou, 2017, 3858488} were considered *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 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*.

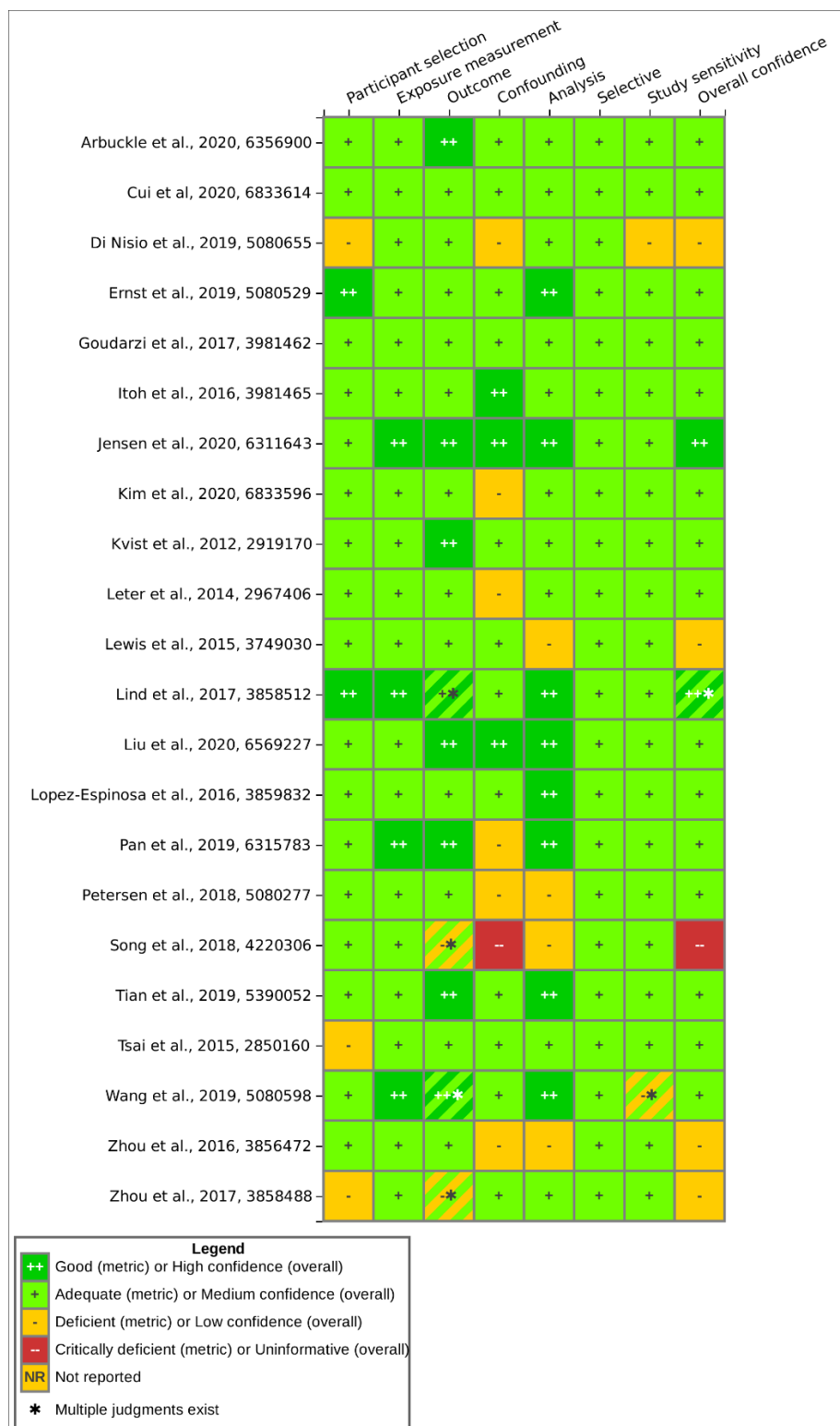


Figure 46. Summary of Study Evaluation for Epidemiology Studies of PFOA and Male Reproductive Effects

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 five observed significant effects (Table C-2). A *high* confidence study {Jensen, 2020, 6311643} in boys from the Odense cohort observed a borderline significant positive association between prenatal PFOA and FSH at four months ($p = 0.06$), but no associations for other serum sex and steroid hormones (i.e., androstenedione, 17-hydroxyprogesterone [17-OHP], and dehydroepiandrosterone sulfate [DHEAS]). A *medium* confidence study {Goudarzi, 2017 3981462} examined male children from the Sapporo cohort, in the Hokkaido Study on the Environment and Children's Health and observed a significant inverse association ($p = 0.025$) with DHEA in cord blood. Associations were not observed among other androgenic hormones. Results from an overlapping *medium* confidence study {Itoh, 2016, 3981465} from the Hokkaido cohort were largely non-significant except for a significant increase in inhibin B in cord blood. Quartile analyses supported this association, but the trend did not reach significance ($p = 0.063$). A *medium* confidence study {Liu, 2020, 6569227} in male infants in China observed a significant positive association with progesterone in cord blood.

A *medium* confidence cross-sectional study {Lopez-Espinosa, 2016, 3859832} of boys (6–9 years) recruited from residents residing near the Mid-Ohio Valley DuPont chemical plant (C8 Health Project) observed a significant inverse association with testosterone, and a significant inverse trend (p for trend = 0.030) by quartiles of PFOA. In contrast, a cross-sectional study {Di Nisio, 2019, 5080655} in Italian high school students examined associations between PFOA levels and possible risk factors for diseases of the male reproductive system and observed significantly increased semen PFOA levels, testosterone, and LH ($p = 0.003$) in exposed individuals compared to unexposed controls. These studies report effects in opposite directions, however, the significance of this conflicting evidence is not entirely clear as each population had reached different points in pubertal development. Additionally, Di Nisio, 2019, 5080655 only controlled for age in all analyses, which may result in some residual confounding by SES or smoking.

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 PFOA 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 significantly increased semen levels and a reduced number of sperm with normal morphology ($p < 0.001$) and a slight increase in semen pH ($p = 0.005$) in exposed individuals compared to controls.

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 non-significant smaller AGD and penile width at three months of age with increasing PFOA. Children from the Shanghai-Minhang Birth Cohort Study {Tian, 2019, 5390052} were evaluated at birth, six months, 12 months of age for changes in AGD. At six months of age, significant decreases were observed for the second

lowest quartile. The effect was consistent in direction for higher quartiles of PFOA exposure but did not reach significance. At 12 months of age, associations were positive, but none were significant. Di Nisio (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 in 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 PFOA were significantly correlated (Spearman's $r = 0.646$, $p < 0.01$). Total and free testosterone were inversely associated ($p < 0.05$) with serum and with semen PFOA levels. E2 and the total testosterone-LH ratio were inversely associated ($p < 0.05$) with semen PFOA, but not with serum PFOA levels. Analyses by quartile agreed and showed significant inverse trends for all outcomes with significant associations in continuous analyses. Analyses stratified by age showed these associations remained in participants 30 years old or younger but were not observed in those participants over 30 years of age. A *medium* confidence cross-sectional study {Petersen, 2018, 5080277} on Faroese men also observed a decrease in free testosterone with increasing serum PFOA levels, however, the association was borderline significant ($p = 0.05$). The free testosterone-E2 ratio was inversely associated ($p = 0.02$) with PFOA levels in this sample. One study {Lewis, 2015, 3749030} analyzed sex hormone concentrations among NHANES participants, but no clear patterns or significant effects were observed.

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). A *medium* confidence study {Pan, 2019, 6315783} in men from Nanjing, China observed significant positive associations ($p < 0.05$) with sperm concentration, total sperm count, and the sperm DNA fragmentation index (DFI)—a measure of the percentage of sperm with damaged DNA. In analyses by quartiles, significant associations were observed for sperm concentration and for the second and fourth quartiles, however, the trend was not significant. Positive associations were observed for sperm DFI among the two highest quartiles of exposure, and the trend was significant (p for trend = 0.03). A significant inverse association ($p = 0.03$) was observed with progressive motility with a significant decreasing trend (p for trend = 0.02). Related motility measures, such as sperm curvilinear velocity and sperm straight-line velocity, did not have significant inverse trends in continuous analyses, however, an inverse association was observed for the highest quartile of exposure for each outcome. No other consistent trends for semen parameters were identified using semen concentrations of PFOA, and no associations were observed with serum PFOA.

One *medium* confidence study {Kvist, 2012, 2919170} evaluating men from the INUENDO cohort from Greenland, Poland, or Ukraine, observed a significant positive association ($p = 0.05$) with the Y:X chromosome ratio in sperm when pooling data across study countries. This association was also observed in the Ukraine 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 LINE-1 loci was significantly increased ($p < 0.05$) in men from Ukraine, but no effect was observed in other INUENDO communities or in the pooled analysis. The LINE-1 loci are a non-transposonic repetitive satellite DNA sequence generally observed in or adjacent to every centromere and was used as a surrogate marker of global DNA methylation.

3.3.2.1.2 Female

3.3.2.1.2.1 Introduction

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

The 2016 HESD for PFOA {U.S. EPA, 2016} concluded that there was suggestive evidence of an association with risk of pregnancy-induced hypertension or preeclampsia based on studies in highly exposed (C8 Health Project) populations {Darrow, 2013, 2850966; Savitz, 2012a, 1276141; Savitz, 2012b, 1424946; Stein, 2009}. There was conflicting evidence from two studies on altered female pubertal onset, and there were suggestive data from two studies on reduced fecundity and fertility. Limited suggestive findings on age at menarche or onset of menopause were hampered by the potential for reverse causation due to PFOA excretion via menstruation. One study examined female reproductive hormone levels in the C8 Health Project {Knox et al., 2011, 1402395} and found no association between PFOA and E2 levels.

For this updated review, 49 studies (53 publications) report on the relationships between PFOA exposure and female reproductive outcomes.⁷ Of these, 21 were cohort studies, 20 cross-sectional studies, and 12 case-control studies. Twenty-one studies were conducted in adults, six were in children and adolescents, 11 were in both adults and children, and 15 were conducted in pregnant women. Most studies assessed exposure to PFOA using biomarkers in blood. Others used amniotic fluid and follicular fluid.

3.3.2.1.2.2 Study Quality

Among the 53 publications available for review, five were classified as *high* confidence, 25 as *medium* confidence, 21 as *low* confidence, and two were considered *uninformative* (Figure 47, Figure 48). Because menstruation is a primary route of PFOA excretion, reverse causality was a specific concern for cross-sectional studies that measured blood PFOA and reproductive hormones with known menstrual fluctuations that failed to report 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}. The *low* confidence studies had deficiencies in participant

⁷ Singular studies with two associated publications include Avanas, 2016, 3981413 and Avanas, 2016, 3981510; Dhingra, 2016, 3981508 and Dhingra, 2017, 3981432; Wang, 2019 5080500 and Wang, 2019, 5080598; Zhou, 2017, 3858488 and Zhou, 2017, 3859799.

selection {Zhang, 2018, 5079665; Heffernan, 2018, 5079713}, exposure measurement methods {Avanasi, 2016, 3981413; Avanasi, 2016, 3981510; Campbell, 2016, 3860110}, reliance on self-reporting for exposure, outcome, or covariate information {Avanasi, 2016, 3981413; Avanasi, 2016, 3981510; Campbell, 2016, 3860110}, and small sample size {Heffernan, 2018, 5079713; McCoy, 2017, 3858475}. Maekawa, 2017, 4238291 was considered *uninformative* for this assessment because of lack of information on participant selection and lack of adjustment for key confounders in the analysis. Lee, 2013, 3859850 was also considered *uninformative* due to lack of consideration of key confounders in analyses.

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. Commonly assessed effects were pregnancy-related outcomes (e.g., preeclampsia, gestational hypertension), menstrual dysfunction (e.g., endometriosis, cycle irregularity), female fertility indicators, and female reproductive hormone levels (e.g., E2, testosterone, sex hormone binding globulin (SHGB)). Other female reproductive outcomes discussed in this review include spontaneous abortion, stillbirth, breastfeeding duration, genital tract infection rate, and female pubertal milestones.

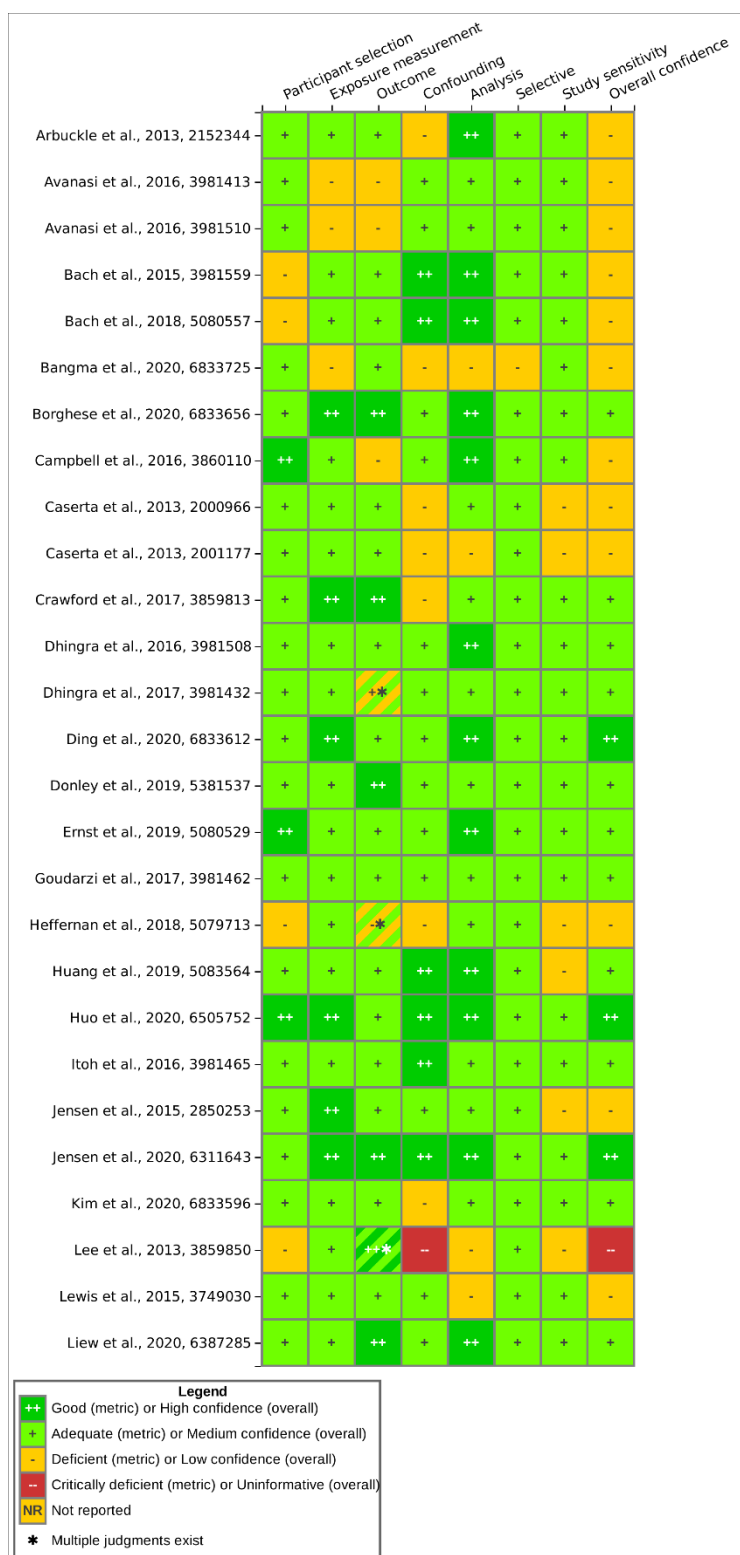


Figure 47. Summary of Study Evaluation for Epidemiology Studies of PFOA and Female Reproductive Effects

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



Figure 48. Summary of Study Evaluation for Epidemiology Studies of PFOA 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 two *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 {Jensen, 2020, 6311643; Yao, 2019, 5187556} and four *medium* confidence {Liu, 2020, 6569227; Itoh, 2016, 3981465; Goudarzi, 2017, 398146; Wang, 2019, 5080598} studies examined the association between PFOA exposure and female reproductive hormones in female infants. One *medium* cross-sectional analysis reported a significant positive association between cord blood PFOA and cord blood estriol in female infants (beta: 0.29, 95% CI: 0.02, 0.56) {Wang, 2019, 5080598}. Two *high* {Jensen, 2020, 6311643; Yao, 2019, 5187556} and three *medium* confidence studies {Liu, 2020, 6569227; Itoh, 2016, 3981465; Goudarzi, 2017, 398146} observed no significant associations between maternal serum or cord blood PFOA levels and reproductive hormones, such as 17-OHP, DHEA, FSH, and LH {Jensen, 2020, 6311643}, E2, testosterone, or testosterone-to-E2 ratio {Yao, 2019, 5187556} progesterone {Liu, 2020, 6569227}, prolactin, SHBG, testosterone, DHEA, androstenedione {Itoh, 2016, 3981465; Goudarzi, 2017, 3981462}.

Three *medium* confidence studies and one *low* confidence study examined the effects of PFOA exposure on female reproductive hormone levels in female adolescents with mixed results. Two *medium* confidence studies observed positive associations with E2 in a high exposed population {Lopez-Espinosa, 2016, 3859832} and testosterone {Maisonet, 2015, 3859841}. As part of the C8 Health Project, Lopez-Espinosa, 2016, 3859832 observed significantly increased E2 levels in serum PFOA quartile 2 compared to quartile 1 (percent difference = 12.6; 95% CI: 3.0, 23.1), but smaller non-significant, positive associations were observed for girls in the two highest PFOA quartiles. In daughters from the Avon Longitudinal Study of Parents and Children (ALSPAC), Maisonet, 2015, 3859841 reported a positive association for total testosterone at age 15 when analyzed by maternal serum PFOA tertiles (beta for maternal PFOA tertile 2 vs tertile 1: 0.15, 95% CI: -0.02, 0.32; beta for tertile 3 vs tertile 1: 0.24, 95% CI: 0.05, 0.43). Maternal serum PFOA was not significantly associated with daughter's SHBG levels. No associations were observed for follicular stimulating hormone or SHBG in a *medium* confidence study {Tsai, 2015, 2850160} or for E2 or testosterone in a *low* confidence study {Zhou, 2016, 3856472}.

One *medium* confidence study and one *low* confidence study reported no evidence of an association between prenatal PFOA exposure and pubertal milestones in female adolescents. Breast development, pubic hair development, axillary hair development, and age at menarche were not associated with maternal blood PFOA during pregnancy in 555 adolescent girls from the Danish National Birth Cohort (DNBC) {Ernst, 2019, 5080529}. Zhou (2017, 3859799) reported positive associations between PFOA and risk of hypomenorrhea (OR for PFOA quantile 3 (Q3) vs quantile 1 (Q1): 2.68, 95% CI: 1.24, 5.78), irregular menstrual cycle (OR for PFOA quantile 4 (Q4) vs Q1: 1.99, 95% CI: 1.22, 3.24; OR per log increase PFOA: 1.52, 95% CI: 1.08, 2.15), and long menstrual cycle (OR for PFOA Q4 vs Q1: 1.95, 95% CI: 1.21, 3.14; OR per log increase PFOA: 1.5 (1.06, 2.1) among female adolescents aged 10–15 years. However, the analyses were not adjusted for key confounders in this *low* confidence study.

3.3.2.1.2.4 Findings from Pregnant Women

Six studies examined the relationship between PFOA exposure and preeclampsia (Table C-4). Of these, five observed positive non-significant associations {Huang, 2019, 5083564; Borghese, 2020, 6833656; Rylander, 2020, 6833607; Wikstrom, 2019, 5387145; Avanas, 2016, 3981510; Avanas, 2016, 3981413} and one observed a negative non-significant association {Huo, 2020, 6505752}. Huo, 2020, 6505752, a *high* confidence cohort study of 3,220 pregnant women, observed non-significant decreased odds of preeclampsia in women with higher serum PFOA levels (OR for women in the 80th percentile or higher for serum PFOA (ln-ng/mL) versus women below the 80th percentile = 0.92; 95% CI: 0.5, 1.7; OR per unit increase in serum PFOA (ln-ng/mL) = 0.89; 95% CI: 0.5, 1.57). All four *medium* confidence studies observed, positive non-significant associations between PFOA exposure and preeclampsia, in cross-sectional {Huang, 2019, 5083564}, case-control {Rylander, 2020, 6833607} and cohort studies {Wikstrom, 2019, 5387145; Borghese, 2020, 6833656}. One *low* confidence study re-analyzed data from a study reviewed in the 2016 HESD, Savitz et al., 2012, and observed non-significant, positive associations between modeled serum PFOA levels and odds of preeclampsia {Avanas, 2016, 3981510; Avanas, 2016, 3981413}.

One *high* confidence study {Huo, 2020, 6505752} and two *medium* confidence studies examined the relationship between PFOA exposure and gestational hypertension reporting non-significant mixed effects. Huo, 2020, 6505752, a *high* confidence cohort study of 3,220 pregnant women, observed non-significant increased odds of gestational hypertension in women with higher serum PFOA levels. Similarly, Borghese, 2020, 6833656 found non-significant increased odds of gestational hypertension for women in plasma PFOA tertile 3 versus tertile 1 and per log₂-ng/mL unit increase in plasma PFOA. In contrast, Huang, 2019, 5083564 reported non-significant reduced odds of gestational hypertension with increasing maternal plasma PFOA levels in both tertile and continuous analyses. When exploring the association between PFOA exposure and impacts on blood pressure, Borghese, 2020, 6833656 found a significant positive association between first trimester plasma PFOA (ug/L) and systolic blood pressure (SBP) (beta: 0.82; 95% CI: 0.23, 1.42; p = 0.006) and diastolic blood pressure (DBP) (beta: 0.64; 95% CI: 0.24, 1.05; p = 0.002). A significant relationship was also observed between continuous plasma PFOA (ug/L) measured at delivery and SBP (beta: 1.52; 95% CI: 0.52, 2.50; p = 0.002) as well as DBP (beta: 1.11; 95% CI: 0.44, 1.78; p = 0.001). Results were less consistent when stratified by infant sex.

Two *medium* confidence studies {Louis, 2016, 3858527; Liew, 2016, 6387285} and two *low* confidence studies {Jensen, 2015, 2850253; Wu, 2012, 2919186} investigated the effect of PFOA exposure on pregnancy loss/miscarriage and reported mixed results. In a case-control study nested within the DNBC, Liew, 2016, 6387285 observed a positive trend across maternal plasma PFOA levels for odds of miscarriage, with odds being significantly increased for quartile 4 compared to quartile 1 (OR = 2.2; 95% CI: 1.2, 3.9). Odds of miscarriage also significantly increased per doubling of maternal plasma PFOA (OR = 1.4; 95% CI: 1.0, 1.9). Jensen, 2015, 2850253 observed significantly increased risk of miscarriage for pregnant women in PFOA tertile 2 compared to tertile 1, but not for women in PFOA tertile 3. Wu, 2012, 2919186 reported significantly higher mean PFOA in women with pregnancies ending in stillbirth compared to women with pregnancies ending in live birth. In a *medium* confidence cohort study of 501 couples, Louis, 2016, 3858527 reported a non-significant, negative association between serum PFOA levels and pregnancy loss during the first seven weeks of pregnancy.

Two *medium* confidence studies assessed the relationship between serum PFOA levels in pregnancy and breastfeeding duration and both reported significant, inverse associations between the two {Timmermann, 2017, 3981439; Romano, 2016, 3981728}. Using data from two Faroese birth cohorts (N = 1,130), one study observed significant, negative associations between maternal serum PFOA (ng/mL) and both exclusive (regression coefficient per doubling of serum PFOA (ng/mL): -0.5 months; 95% CI: -0.7, -0.3 months) and total (regression coefficient per doubling of serum PFOA (ng/mL): -1.3 months; 95% CI: -1.9, -0.7 months) breastfeeding duration {Timmermann, 2017, 3981439}. These observations were supported by a prospective birth cohort study which observed a consistent, positive trend between increasing serum PFOA quartile and relative risk of breastfeeding duration at three and six months postpartum. Relative risk of breastfeeding termination at three months postpartum was significantly increased for women in serum PFOA quartiles 3 (risk ratio (RR) = 1.63; 95% CI: 1.16, 2.28) and 4 (RR = 1.77; 95% CI: 1.23, 2.54) compared to quartile 1. Relative risk of breastfeeding termination at six months postpartum was also significantly increased for women in serum PFOA quartiles 3 (RR = 1.38; 95% CI: 1.06, 1.79) and 4 (RR = 1.41; 95% CI: 1.06, 1.87) compared to quartile 1.

One *high* confidence study examined SHBG measured three years postpartum in 812 women enrolled in the Project Viva birth cohort {Mitro, 2020, 6833625}. The study observed a negative non-significant association between early pregnancy plasma PFOA and SHBG. These findings were consistent in analyses stratified by age at pregnancy (<35 years versus ≥35 years).

One *medium* confidence study {Lyngsø, 2014, 2850920} examined the effects of serum PFOA levels on pre-pregnancy menstruation. The study reported significantly increased odds of long menstrual cycles for women in the highest PFOA tertile compared to the lowest (OR: 1.8, 95% CI 1.0, 3.3) and when analyzing PFOA as a continuous variable (OR: 1.5 (95% CI 1.0, 2.1). Significant results persisted when analyses were restricted to nulliparous women.

3.3.2.1.2.5 Findings from the General Adult Population

One *high* confidence, eight *medium* confidence, and eleven *low* confidence studies assessed relationships between PFOA exposure and female reproductive outcomes in non-pregnant adult women (Table C-5). Assessed outcomes included various fertility indicators, age at natural menopause, and reproductive hormone levels.

Five *medium* confidence studies and eight *low* confidence studies examined female fertility indicators and no clear associations or dose-response trends were observed. A cohort study of 501 couples attempting to conceive observed positive significant associations but no trend across baseline serum PFOA tertiles for day-specific probability of pregnancy or menstrual cycle length {Lum, 2017, 3858516}. Crawford, 2017, 3859813 observed positive association with cycle-specific time to pregnancy and anti-Müllerian hormone (AMH), a biomarker of ovarian reserve, and a negative association with day-specific time to pregnancy, but the associations were non-significant. A *low* confidence study examining time to pregnancy {Bach, 2018, 5080557} reported a positive association. Another study of AMH examined levels in female adolescents in the ALSPAC and found a significant positive association between maternal serum PFOA during pregnancy and AMH concentration (beta: 0.05; 95% CI 0.01-0.09). This association was not significant after missing data imputation {Donley, 2019, 5381537}. A *low* confidence study investigated PFOA exposure and premature ovarian insufficiency (POI), reporting no significant associations {Zhang, 2018, 5079665}, while another *low* confidence study found positive

associations between the highest PFOA tertile and polycystic ovary syndrome when compared to the lowest PFOA tertile {Vagi et al., 2014, 2718073}. Wang, 2017, 3856459 observed no associations and no trend in odds of endometriosis-related infertility across plasma PFOA tertiles. Campbell (2016, 3860110A) reported increased odds of endometriosis only for the third PFOA exposure quartile compared to the lowest PFOA quartile (OR: 5.45; 95% CI: 1.19, 25.04), while another *low* confidence study did not observe an association with endometriosis diagnosis {Louis, 2012, 159740}. Kim, 2020, 6833596 observed a positive non-significant association between PFOA in follicular fluid and fertilization rate. Other *low* confidence studies examining fertility-related outcomes reported non-significant positive associations between PFOA exposure and percent fertilization {McCoy, 2017, 3858475}, minimal correlation with expression of nuclear receptors when examined by fertility status {Caserta et al., 2013, 2000966}, and no association between maternal serum PFOA and infertility {Bach et al., 2013, 3981559}.

The two studies (3 publications) examined age at natural menopause, and all observed positive associations. A *high* confidence study of premenopausal women aged 45-56 in the Study of Women's Health Across the Nation (SWAN) cohort {Ding, 2020, 6833612} reported a significantly increased risk of natural menopause for women in the highest exposure tertile (HR = 1.31; 95% CI: 1.04, 1.65), but no significant association per doubling of serum PFOA. A *medium* confidence study (2 publications) {Dhingra, 2016, 3981508; Dhingra, 2017, 3981432} of women ages 30-65 years in the high exposed Mid-Ohio Valley cohort assessed associations between both measured and modeled PFOA exposure and self-reported menopause). Menopause was significantly associated with serum PFOA (p-trend = 0.04), but not modeled PFOA exposure (p-trend = 0.90) {Dhingra, 2017, 3981432}. However, the findings might be hampered by reverse causation, likely due to reduced kidney function, as urine is a primary route of PFOA excretion.

One *medium* confidence study and four *low* confidence studies assessed the relationship between serum PFOA levels and reproductive hormone levels in non-pregnant adult women. In the *medium* confidence study, no clear dose-response trends were observed for either FSH or SHBG across quartiles by age category {Tsai, 2015, 2850160}. While one *low* confidence study observed mixed associations between PFOA levels and increased testosterone, with a significant positive association reported for controls {Heffernan, 2018, 5079713}, another {Zhang, 2018, 5079665} observed no significant associations between PFOA and any female reproductive hormone outcomes, including E2, prolactin, testosterone, LH, and FSH. Two other *low* confidence studies, Lewis et al., 2015, 3749030 and Petro et al., 2014, 2850178, reported no association for total testosterone or E2, respectively.

3.3.2.2 Animal Evidence

There are 8 studies from the most recent literature search conducted in 2020 and 5 key studies from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and reproductive effects. Study quality evaluations for these 13 studies are shown in Figure 49.

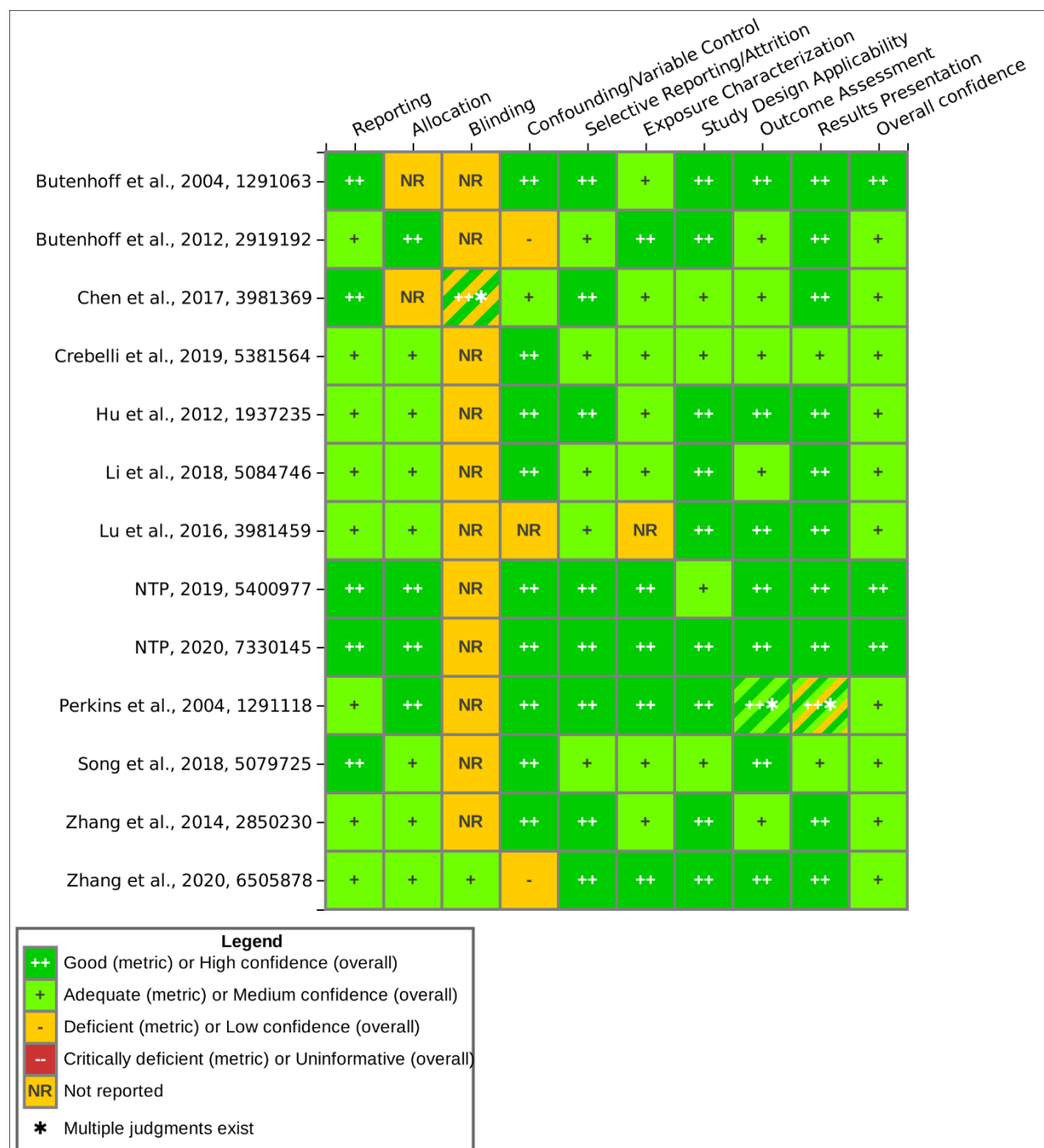


Figure 49. Summary of Study Evaluation for Toxicology Studies of PFOA and Reproductive Effects

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

Several animal studies report significant effects on reproductive endpoints following oral exposure to PFOA; however, the evidence is not consistent across species with effects observed in mice more frequently than in rats or monkeys. In addition, the effects were observed at dose levels that have been shown to reduce growth and body weight in several studies, which may

explain the effects observed on reproductive endpoints. Effects observed in male rodents include reduced fecundity (mice only), decreased epididymal weights, decreased sperm count and quality, and morphological changes in the testes and epididymides. Female rodents exposed to PFOA have displayed prolonged diestrus and reduced number and size of corpora lutea compared to vehicle controls. In addition, alterations in reproductive hormone levels have been observed in male and female rodents. Developmental studies in mice have reported adverse effects on the weight and histopathology of the placenta (see Section 3.3.1.2), and there have been cancers observed in reproductive organs that are discussed in Section 3.3.17.2.

3.3.2.2.1 Reproductive Performance

One standard two-generation reproduction study is available for PFOA that reported no effects on mating or fertility in rats administered PFOA by gavage for 10 weeks prior to mating with doses ranging from 1 to 30 mg/kg/day {Butenhoff et al., 2004, 1291063; York et al., 2010, 2919279}. Reproductive endpoints including number of days in cohabitation, fertility index, pregnancy, implantation, and length of gestation were not affected in either generation. Although F₁ pups exposed to 30 mg/kg/day had decreased BWTs, survival, and delayed sexual maturation (Section 3.3.1.2), no effects were observed on reproductive performance or fertility in these animals as adults.

Information on the reproductive performance of mice exposed to PFOA prior to and during mating is available from two studies. Fecundity was decreased in male BALB/c mice following exposure to 5 mg/kg/day PFOA by gavage for 28 days when mated to untreated females, shown by reductions in the numbers of mated females per male mouse and pregnant females per male mouse {Lu et al. 2016, 2850390}. The authors did not measure body weight or sperm parameters in the treated males and did not report if any clinical signs of toxicity were observed, therefore it is difficult to interpret the toxicological significance of the effect on reproductive performance. In contrast, Hu et al. {2012, 1937235} administered PFOA (0.02, 0.2, or 2 mg/kg/day) to female C57BL/6N mice by daily gavage from the day they were paired with untreated males through weaning of offspring. On average, females were dosed for 12.9 (± 7.3) days prior becoming pregnant. No effects were observed in the number of days to pregnancy or the number of dams that became pregnant between treated groups and controls {Hu et al. 2012, 1937235}.

3.3.2.2.2 Sperm Parameters

Sperm parameters were quantitatively measured in two studies in rats {NTP 2019, 5400977; Butenhoff et al. 2004, 1291063; York et al. 2010, 2919279} and two studies in mice {Zhang et al. 2014, 2850230; Li et al. 2011, 1294081}. Overall, the findings were not consistent between rats and mice and therefore do not provide clear evidence of an adverse effect on spermatogenesis.

In a short-term study by NTP, male Sprague Dawley rats were administered 0.625, 1.25, 2.5, 5, or 10 mg/kg/day PFOA by gavage for 28 days and sperm parameters were evaluated in the control and three highest dose groups at the end of the treatment period (sample size n = 10) {NTP 2019, 5400977}. Cauda epididymal sperm count was significantly decreased (24%) in the high-dose group compared to controls, but when normalized to sperm count per gram of cauda epididymis, the difference was no longer statistically significant. No effects were observed on epididymal sperm motility or testicular spermatid counts. Histopathological examination of the epididymis revealed hypospermia and exfoliated germ cells in one rat each in the 5 and 10

mg/kg/day groups, though the findings were not significantly different from the control group. Body weight was significantly reduced in males treated with dose levels ≥ 2.5 mg/kg/day and the highest dose group weighed 19% less than controls at necropsy. This could explain the reduction in sperm count observed at that dose level. A two-generation reproduction study in Sprague Dawley rats with doses up to 30 mg/kg/day PFOA found no treatment-related effects on epididymal sperm count, density, motility, or morphology, as well as testicular spermatid count or density (sample size $n = 28-30$) {Butenhoff et al. 2004, 1291063; York et al. 2010, 2919279}. The incidences of hypospermia and exfoliated germs cells in the epididymis were slightly higher for P_0 males treated with 30 mg/kg/day versus controls (2/14 vs. 0/13 for each finding); however, it is not clear if statistical analyses were performed for those results.

Zhang et al. {2014, 2850230} administered 0.31, 1.25, 5, or 20 mg/kg/day PFOA to adult male BALB/c mice by gavage for 28 days, but sperm parameters were only evaluated in the control and 5 mg/kg/day groups (sample size $n = 5$). At the end of the treatment period, epididymal sperm count was significantly decreased (32%) in the 5 mg/kg/day group compared to controls. Sperm motility and progressiveness were also significantly reduced. In addition, the rates of head and neck teratosperm were significantly increased as was the overall rate of teratosperm.⁸ Body weights were not reported in this study, and it is unclear if the mice in the 5 mg/kg/day group experienced concurrent systemic toxicity.

Li et al. {2011, 1294081} also evaluated sperm parameters in a study designed to examine the involvement of mouse and human PPAR α in male reproductive effects induced by PFOA. Adult male wild-type, PPAR α -humanized, and PPAR α -null mice of a 129/Sv background were administered 1 or 5 mg/kg/day PFOA by daily gavage for 6 weeks. At the end of the treatment period, body weights did not differ between the control and treated groups. Epididymal sperm count and motility were unaltered by treatment (sample size $n = 8-10$); however, the percentage of sperm abnormalities was significantly increased in both treated groups of wild-type and humanized PPAR α mice, but not in PPAR α -null mice. Therefore, the effects observed in this particular study are potentially related to PPAR α .

The overall evidence is suggestive of an effect on PFOA on spermatogenesis, but there are several limitations with the dataset that make interpretation difficult. The studies that observed adverse effects on sperm parameters did not evaluate fertility or fecundity, while the only study that found an effect on fecundity did not measure sperm parameters or report if overt toxicity occurred in the males. Furthermore, the studies in mice used relatively small sample sizes ($n = 5-10$), while a comprehensive two-generation study in rats with large sample sizes ($n = 28-30$) observed no effects on sperm parameters or male fertility {Butenhoff et al. 2004, 1291063; York et al. 2010, 2919279}. Epididymal sperm concentration was reduced by 24% in rats treated with 10 mg/kg/day {NTP 2019, 5400977} and by 32% in mice treated with 5 mg/kg/day {Zhang et al. 2014, 2850230}; however, the reduction observed in rats was negated when normalized to weight of the cauda epididymis {NTP 2019, 5400977}. The study in mice did not normalize sperm count to organ weight to determine if the effect remained significant. Furthermore, body weights of rats were significantly reduced at the same dosage that caused reduced sperm

⁸The text of Zhang et al. (2014, 2850230) reports that sperm motility and progressiveness were both significantly reduced and the overall rate of teratosperm was significantly increased in treated rats, but the results in figures 1D(b), (c), and (d) show the opposite effects. It appears that the figures are mislabeled, and the results were switched. The corresponding author was contacted for clarification, but no response was received.

concentration, which could explain the effect on sperm. Body weights were not reported by Zhang et al. {2014, 2850230} to determine if that was also a confounding factor in mice. Increased rates of sperm abnormalities were reported in two studies with mice {Zhang et al. 2014, 2850230; Li et al. 2011, 1294081}, but not observed in the two-generation study in rats {York et al. 2010, 2919279}. In summary, it is unclear if the effects on spermatogenesis observed in mice are the result of direct toxicity to reproductive processes or a reflection of PFOA's effects on body weight or other systemic effects. Figure 50 summarizes the effects of PFOA on sperm counts observed in animal studies.

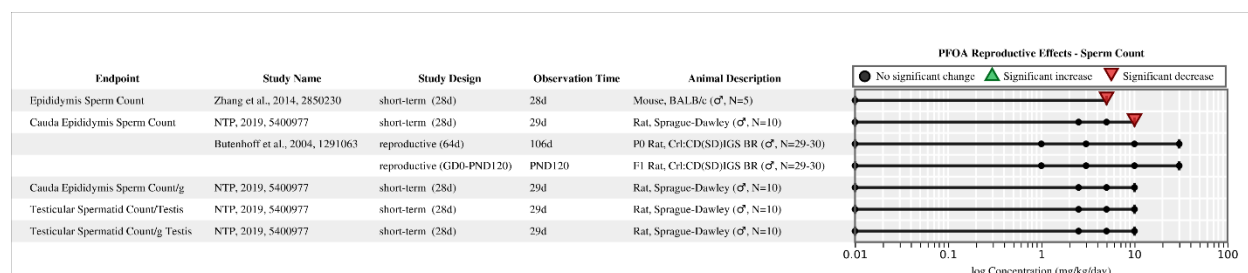


Figure 50. Sperm Counts in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA 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; F1 = first generation; PND = postnatal day; d = day.

3.3.2.2.3 Estrous Cyclicity and Ovarian Function

A small number of studies have evaluated estrous cyclicity and effects on corpora lutea following oral exposure to PFOA, and some significant effects have been observed.

A tendency toward prolonged diestrus was reported in one study with rats {NTP 2019, 5400977} and in one study with mice {Zhang et al. 2020, 6505878}. In the study by NTP, adult female rats were treated for 28 days with doses up to 100 mg/kg/day and estrous cyclicity was evaluated daily during the last 16 days of treatment. The cycles of treated rats were observed to be mostly similar to controls; however, rats dosed with 100 mg/kg/day spent around 20% more time in diestrus than controls (62.5% vs. 51.9% of the cycle). Markov analyses indicated that high-dose females had a higher probability than control animals to transition from a regular cycle to a cycle with extended diestrus ($p < 0.001$). No effects were observed in the mean estrous cycle length or the lengths of time spent in other estrous stages. The body weights of females were not significantly altered by treatment {NTP 2019, 5400977}.

A two-generation reproduction study in rats {Butenhoff et al. 2004, 1291063} found no evidence of extended diestrus in P0 or F1 female rats, but the doses were lower than the NTP study and the authors did not specifically evaluate the proportion of time spent in diestrus. The study authors observed a significant increase in the number of estrous stages per 21 days in the high-dose (30 mg/kg/day) F1 females compared to controls (5.4 vs. 4.7 estrous stages/21 days); however, there were no significant differences observed in the incidences of rats displaying prolonged diestrus or estrus (defined as >6 days for each), and no significant changes were observed in the estrous cycles of females in the P generation. The slight increase observed in number of estrous stages per 21 days was most likely due to the different stages the rats entered the measurement period and was probably not related to PFOA treatment.

A study conducted with mice observed significant effects on the estrous cycle at doses much lower than those causing alterations in the NTP study in rats. Zhang et al. {2020, 6505878} administered 0.5–5 mg/kg/day PFOA to adult female mice for 28 days by gavage and monitored daily vaginal cytology throughout the study (sample size n = 8). The number of days spent in diestrus was significantly increased in females treated with 2 or 5 mg/kg/day, and the authors noted that the mice in those groups were rarely observed to enter the estrus phase of the cycle after the second week of exposure to PFOA; however, the durations of estrus and proestrus were not significantly altered by treatment. Body weight was significantly reduced in the 5 mg/kg/day group on days 24 and 28 (by 11%) but not significantly affected in the 2 mg/kg/day group.

In the same study, the numbers of corpora lutea were significantly reduced in mice administered 2 or 5 mg/kg/day PFOA for 28 days; however, no effects were observed on the antral follicle count per ovary {sample size n = 8; Zhang et al. 2020, 6505878}. Decreases in the number and size of corpora lutea were also observed in pregnant mice administered PFOA (2.5, 5 or 10 mg/kg/day) beginning on GD1 {sample size n = 6; Chen et al. 2017, 3981369}. The numbers of corpora lutea were significantly decreased in the low- and mid-dose groups on GD7 and in the mid- and high-dose groups on GD13. The ratio of corpora lutea to ovarian areas was also significantly decreased at both time points in a dose-dependent manner. The results of this study suggest that PFOA treatment can significantly impair ovarian function during pregnancy and the authors also found evidence of increased oxidative stress and apoptosis in the ovaries of treated mice. Maternal body weights were not reported in this study.

The overall evidence for adverse effects of PFOA on ovarian function is suggestive but inconclusive because the effects were mainly observed in mice and in studies with small sample sizes (n = 6–8). It is likely that prolonged diestrus and reduced corpora lutea observed in mice were treatment-related effects because they followed a clear dose response, and the effects were observed at dose levels lower than those causing decrements in body weight (when reported). Rats also demonstrated a slight increase in the time spent in diestrus, but only at a very high dosage (100 mg/kg/day) {NTP 2019, 5400977}. Only one study was identified that evaluated effects on corpora lutea in rats {Staples et al. 1984, 1332669}, and that study found no difference between the number of corpora lutea in control rats and those treated with 100 mg/kg/day PFOA from GD6–GD15.

3.3.2.2.4 Reproductive Hormone Levels

3.3.2.2.4.1 Males

Several studies have reported significant alterations in reproductive hormone levels in male animals following oral exposure to PFOA, but the results are not consistent across species or study durations. Figure 51 summarizes the effects of PFOA on reproductive hormone levels observed male rodents.

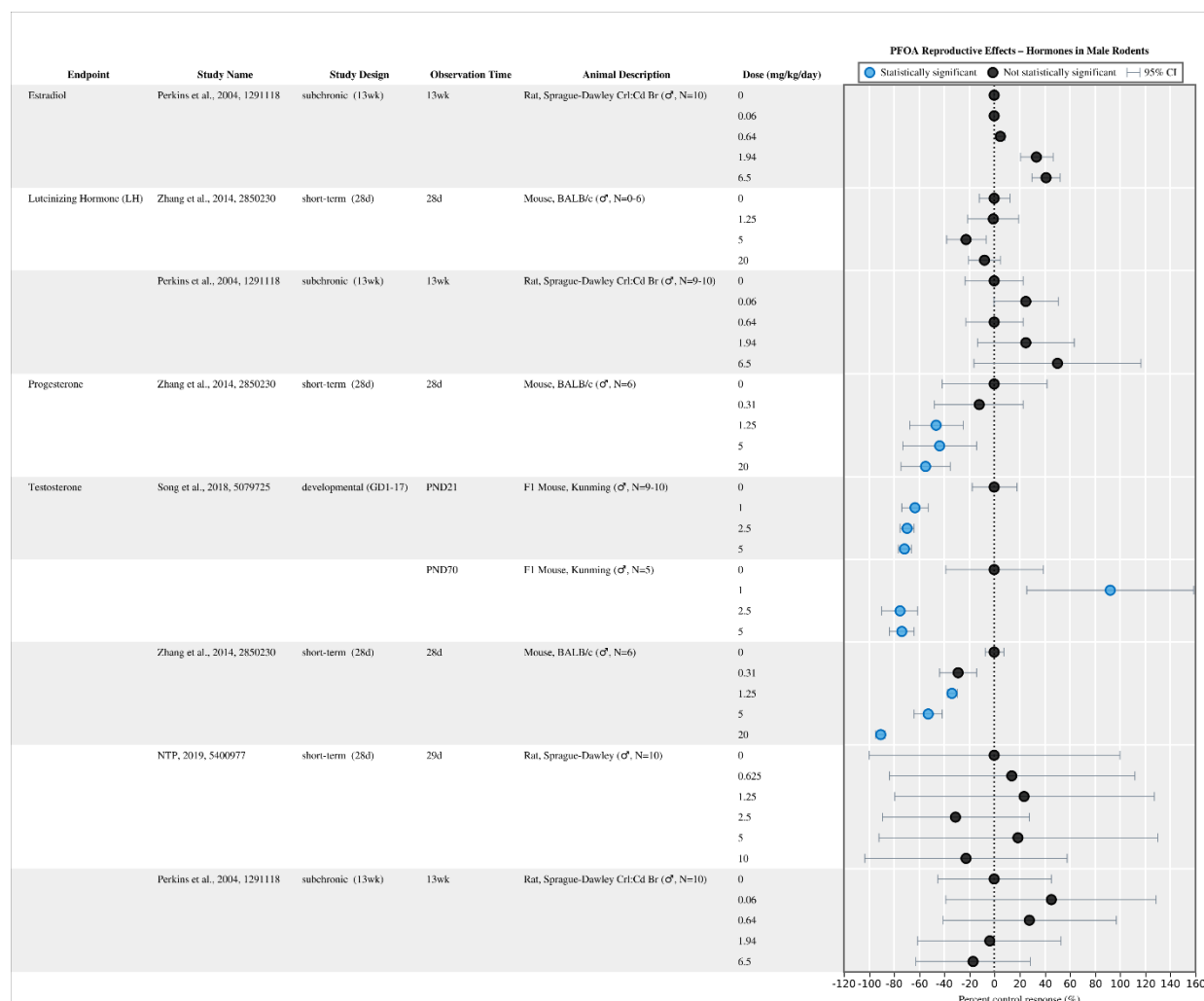


Figure 51. Percent Change in Male Reproductive Hormone Levels Relative to Controls in Rodents Following Exposure to PFOA

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

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

Testosterone levels were measured in several studies, and significant reductions were reported in two-week studies in rats {Cook et al. 1992, 1306123; Biegel et al. 1995, 1307447} and several studies in mice {Li et al. 2011, 1294081; Zhang et al. 2014, 2850230; Song et al. 2018, 5079725}. However, a 28-day study in rats {NTP 2019, 5400977}, a 13-week study in rats {Perkins et al. 2004, 1291118}, and a 6-month study in male monkeys {Butenhoff et al. 2002, 1276161} all observed no significant effects or consistent patterns of alterations in testosterone levels during or after exposure to PFOA. Several studies reported increased serum E2 concentrations in male rats during or after exposure to PFOA {Cook et al. 1992, 1306123; Biegel et al. 1995, 1307447; Liu et al. 1996, 1307751; Biegel et al. 2001, 673581}. However, another study in rats {Perkins et al. 2004, 1291118} and one study in monkeys {Butenhoff et al. 2002, 1276161} found no significant effects of PFOA on male E2 levels. The results for other male reproductive hormones measured in serum shown no clear dose-related trends, including LH,

FSH, and prolactin {Biegel et al. 2001, 673581; Perkins et al. 2004, 1291118; Zhang et al. 2014, 2850230}.

NTP {2019, 5400977} administered 0.625–10 mg/kg/day PFOA for 28 days to male rats and found no significant differences in serum testosterone levels between treated groups and controls at the end of the treatment period. The high-dose group had serum testosterone levels 22% lower than controls, but the difference did not attain statistical significance. Likewise, a subchronic dietary study in rats found no significant treatment-related alterations in serum testosterone, E2, or LH levels measured after 4, 7, and 13 weeks of exposure with up to 100 ppm PFOA in the diet (equivalent to 6.5 mg/kg/day) {Perkins et al. 2004, 1291118}. Biegel et al. {2001, 673581} measured hormones at 3-month intervals in male rats fed 300 ppm PFOA for two years (equivalent to 13.6 mg/kg/day), and no apparent treatment-related trends were observed in serum testosterone, prolactin, LH, or FSH levels. Serum FSH was significantly increased only at 6 months, and LH was significantly increased at 6 and 18 months; however, serum E2 levels were consistently increased at the 1-, 3-, 6-, 9-, and 12-month time points compared to controls {Biegel et al. 2001, 673581}.

Serum testosterone was significantly reduced in the male offspring of Kunming mice administered PFOA (1, 2.5, or 5 mg/kg/day) from GD1–GD17 {Song et al. 2018, 5079725}. On PND21, serum testosterone levels were reduced in a dose-dependent fashion in all treated groups (by 63–71%); however, on PND70, there was no clear dose-response trend (serum testosterone was increased by 92% in the low-dose group and decreased in the mid- and high-dose groups by 74–75%). Zhang et al. {2014, 2850230} administered 0.31, 1.25, 5, or 20 mg/kg/day PFOA to adult male mice for 28 days, and no significant differences were observed in serum LH levels. Testicular testosterone and progesterone concentrations were both significantly reduced at dose levels ≥ 1.25 mg/kg/day at the end of the treatment period. Testicular testosterone was decreased by 34–91% in a dose-dependent manner, and testicular progesterone was decreased by 44–55%. In addition, intratesticular cholesterol was significantly reduced (by 39–44%) at ≥ 5 mg/kg/day.

In the 6-week mechanistic study by Li et al. {2011, 1294081}, plasma testosterone levels measured at the end of treatment were decreased in wild-type mice administered 1 mg/kg/day (by 37%), and significantly decreased in wild-type mice administered 5 mg/kg/day (by 57%) compared to controls. Plasma testosterone was also significantly decreased in low- and high-dose humanized PPAR α mice (by 29% and 31%, respectively). In PPAR α -null mice, plasma testosterone was slightly reduced in a dose-related manner, but statistical significance was not attained.

Overall, there are no clear treatment-related trends in male reproductive hormone levels across species and study durations. Serum, plasma, or intratesticular testosterone levels were all decreased in treated mice {Song et al. 2018, 5079725; Zhang et al. 2014, 2850230; Li et al. 2011, 1294081}, but similar effects on testosterone were not observed in rats after 28 days or longer exposures. Testosterone in males is pulsatile and can display large random peaks, therefore studies measuring hormones at various time points over the course of a study are more useful for determining treatment-related effects than studies that measured concentrations at a single time point, for example at necropsy. The studies that measured male hormone levels at various times throughout treatment reported no consistent changes in testosterone {Perkins et al. 2004, 1291118; Biegel et al. 2001, 673581}. Two studies reporting reduced testosterone in mice also observed adverse effects on sperm concentration and/or quality following exposure to PFOA

{Zhang et al. 2014, 2850230; Li et al. 2011, 1294081}; however, because of the limited number of studies available and the lack of reproducibility in rats, no firm conclusions can be made about the adversity of these findings. The 22% decrease in testosterone that was observed in high-dose male rats of the 28-day study by NTP {2019, 5400977} was not large enough to be considered adverse given the inherent variability in testosterone levels with a male and between males.

Serum E2 levels were consistently increased at multiple time points in one chronic study in male rats {Biegel et al. 2001, 673581}; however, the concentrations were very low (in the range of pg/mL), and it has been shown that estrogen levels are too low to be accurately measured using radioimmunoassay kits, which was the method used in that study. Therefore, no firm conclusions can be made about the relevance of those findings as well.

3.3.2.2.4.2 Females

Figure 52 summarizes the effects of PFOA on reproductive hormone levels observed female rodents.

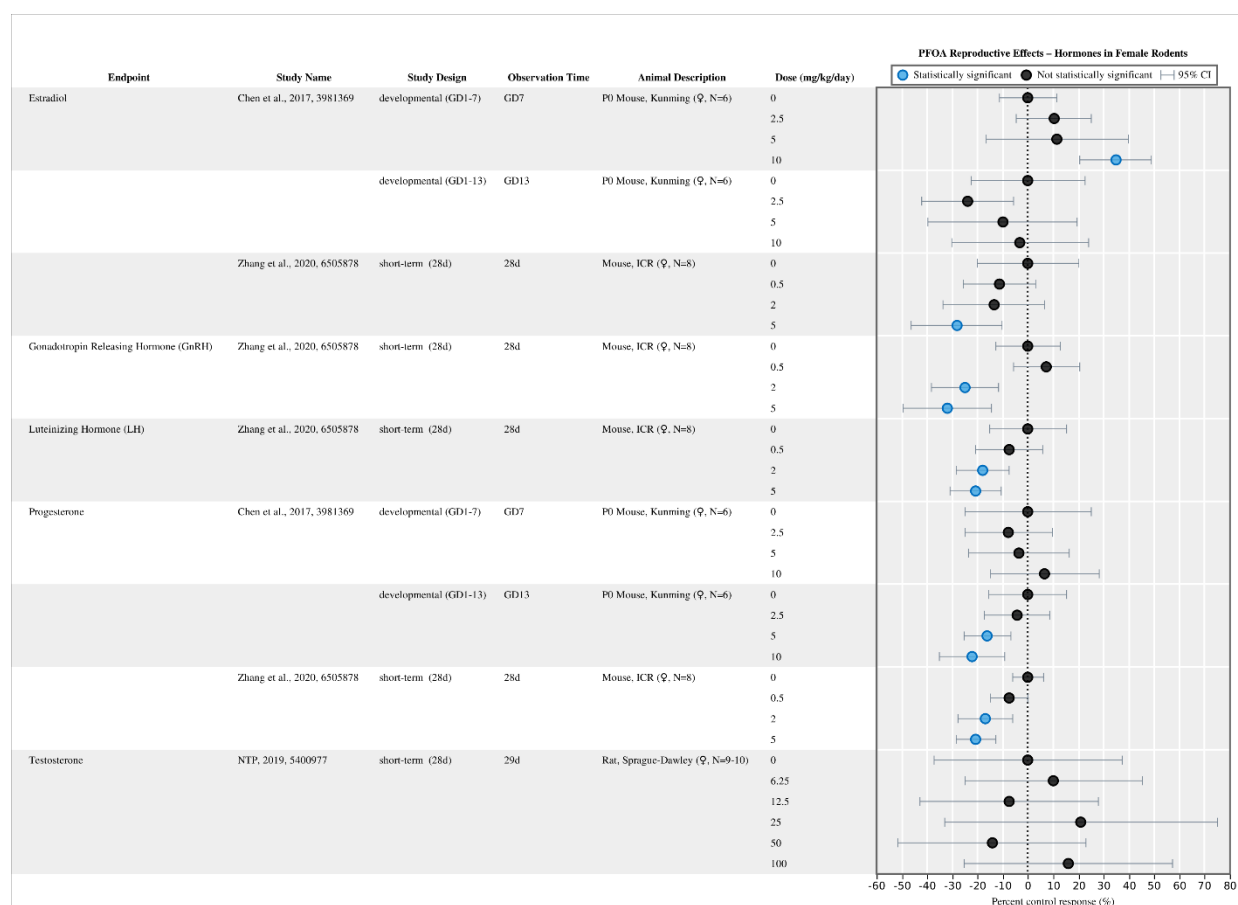


Figure 52. Percent Change in Female Reproductive Hormone Levels Relative to Controls in Rodents Following Exposure to PFOA

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

Only three studies measured female reproductive hormones following oral exposure to PFOA, and the only effect observed in more than one study was slightly reduced progesterone levels {Chen et al. 2017, 3981369; Zhang et al. 2020, 6505878}.

No significant differences were observed in serum testosterone levels of adult female rats administered 6.25–100 mg/kg/day PFOA for 28 days {NTP, 2019, 5400977}, but no other reproductive hormones were measured in that study. A 28-day study in adult female mice observed significant reductions in several hormone levels following administration of 2 or 5 mg/kg/day, including reduced serum progesterone (17–21%), gonadotrophin-releasing hormone (GnRH) (25–32%), and LH (18–21%). Serum E2 was also significantly reduced (28%) at 5 mg/kg/day {Zhang et al. 2020, 6505878}. In contrast, when pregnant female mice were administered PFOA beginning on GD1 {Chen et al., 2017, 3981369}, serum E2 was slightly increased on GD7 but unaltered on GD13. Meanwhile, serum progesterone was unaltered on GD7 but was significantly reduced on GD13 at 5 and 10 mg/kg/day (by 16–22%).

Due to the small dataset and the small percent changes from controls, no firm conclusions can be made about the effects of PFOA on female reproductive hormones in animals.

3.3.2.2.5 Reproductive Organ Weights and Histopathology

3.3.2.2.5.1 Males

Some studies in rats and mice indicate that PFOA exposure can result in changes in the normal structure of the testes and epididymides; however, the overall body of evidence is inconsistent with several other studies reporting no histological changes in male reproductive organs.

Absolute weights of the testes were either significantly decreased {NTP 2020, 7330145; Zhang et al. 2014, 2850230} or unaltered {NTP 2019, 5400977; Crebelli et al. 2019, 5381564; Butenhoff et al. 2012, 2919192; Butenhoff et al. 2004, 1291063} in adult male rodents following exposure to PFOA. Meanwhile, relative weights of the testes were either significantly increased {NTP 2019, 5400977; NTP 2020, 7330145; Butenhoff et al. 2004, 1291063} or unaltered {Zhang et al. 2014, 2850230; Butenhoff et al. 2012, 2919192}. The decreases observed in absolute testicular weights in conjunction with unaltered or increased relative weights appear to be secondary to body weight changes and therefore unrelated to treatment with PFOA.

Several studies observed no histological changes in the testes, including a 28-day study in rats {NTP 2019, 5400977}, a 13-week dietary study in rats {Perkins et al. 2004, 1291118}, a two-generation reproduction study in rats {Butenhoff et al. 2004, 1291063}, a 26-week study in monkeys {Butenhoff et al. 2002, 1276161}, and a two-year study in rats (see study design details in Section 3.3.1.2.1.2) {NTP 2020, 7330145}. However, there is some evidence in mice that suggests developmental exposure can alter the normal structure of the testes. Song et al. {2018, 5079725} exposed pregnant mice to 1, 2.5, or 5 mg/kg/day PFOA from GD1–GD17 and evaluated testicular weights and histopathology in the male offspring on PND21 and PND70. Absolute testis weights were significantly increased in the high-dose group on PND21, but the effect was not observed on PND70. There were no significant differences in relative testis weights at either time point and the change in absolute weight appeared to be related to increased body weights also observed in the high-dose group. Histopathological examination revealed significant changes in the testes of the 2.5 and 5 mg/kg/day groups on both PND21 and PND70. Effects that were reported quantitatively were decreased numbers of Leydig cells on PND21 (by

25–27%) and PND70 (by 17–25%) and increased intercellular substance areas on PND21 (by 105–111%) and PND70 (by 9–13%). Other microscopic changes were reported qualitatively only and included atrophy of the spermatogenic epithelium, reduction in spermatogenic cells, vacuolization of Sertoli cells and decrease or disappearance of spermatozoa at 5 mg/kg/day. With increasing dose to the dam, the degree of damage to the testes was noted to increase. From 2.5 to 5 mg/kg/day, the intercellular substance in the testes of offspring became larger and the interstitial cells gradually decreased. The spermatogenic cells of all levels were arranged in an irregular pattern; however, vacuolization was not observed on PND70 indicating some recovery had occurred since PND21.

Zhang et al. {2014, 2850230} also reported damage to the testes in adult male mice treated for 28 days, but results were reported qualitatively without incidence data. The findings in rats treated with 5 or 20 mg/kg/day included atrophy of the seminiferous tubule epithelia, lack of germ or Sertoli cells between basal membrane and adluminal portions, and detached germ cells sloughed off into the tubular lumen. In the 6-week mechanistic study in mice by Li et al. {2011, 1294081}, histopathological examination of the testes revealed abnormal seminiferous tubules with vacuoles or lack of germ cells in wild-type and humanized PPAR α mice administered 5 mg/kg/day (reported qualitatively without incidence data), but these changes were not observed in PPAR α -null mice. Necrotic cells in testes and significantly reduced weights of the epididymis and seminal vesicle plus prostate gland were also observed in the 5 mg/kg/day wild-type mice only.

At the 1-year sacrifice of a chronic dietary study in rats {Butenhoff et al. 2012, 2919192}, testicular tubular atrophy with marked aspermatogenesis was observed in 2/15 (13%) of high-dose (300 ppm; 14.2 mg/kg/day) males but not in any of the controls (statistical significance not reported). At the terminal evaluation, there were no significant differences in the incidences of tubular atrophy, but the incidence of vascular mineralization in the testes was significantly increased in high-dose males. The incidences of the lesion in the control, 30, and 300 ppm (0, 1.3, and 14.2 mg/kg/day) groups were 0%, 6%, and 18%, respectively. In contrast, a two-year dietary study conducted by NTP {2020, 7330145} found no treatment-related effects in the testes of rats fed PFOA at concentrations up to 300 ppm (32 mg/kg/day) for 16 weeks or 80 ppm (4.6 mg/kg/day) for 2 years (including groups that were also exposed during gestation; see further study design details in Section 3.3.1.2.1.2).

Effects on the epididymis have also been observed following PFOA exposure. Absolute weights of the epididymis or cauda epididymis were significantly reduced in a few studies {NTP 2019, 5400977; Lu et al. 2016, 3981459; Butenhoff et al. 2004, 1291063}, and relative epididymis weight was also significantly reduced in one of those studies {Lu et al. 2016, 3981459}.

In the two-generation reproduction study in rats, absolute weights of several male reproductive organs were significantly decreased in the high-dose males of the P₀ (i.e., right and left epididymis, cauda epididymis, seminal vesicles with and without fluid, and prostate) while the relative weights of those organs were all significantly increased (except for the prostate) {Butenhoff et al. 2004, 1291063; York et al. 2010, 2919279}. The patterns observed were consistent with significant decrements in body weights that were also observed in male groups treated with ≥ 1 mg/kg/day, and there were no treatment-related changes observed in histopathology of those organs.

NTP {2019, 5400977} observed hypospermia and exfoliated germ cells in the epididymis of one rat each in the 5 and 10 mg/kg/day groups following 28 days of oral exposure, although the incidences were not statistically different from controls (n = 10 per group evaluated). This coincided with significantly reduced absolute weights of the left cauda epididymis (≥ 5 mg/kg/day) and left epididymis (10 mg/kg/day) as well as reduced epididymal sperm count (10 mg/kg/day). However, relative epididymal weights were not reported in this study. No treatment-related effects were observed in the testes, seminal vesicles, or accessory sex glands.

In a 28-day study in mice, absolute weights of the epididymis were reduced in mice treated with 5 or 20 mg/kg/day and relative epididymis weights were also reduced at 20 mg/kg/day {Lu et al. 2016, 3981459}. Histopathological examination revealed empty spaces in the tubules of cauda epididymis of mice treated with 5 or 20 mg/kg/day and a lack of normal sperm (reported qualitatively without incidence data). In addition, the levels of triglycerides in the epididymis were significantly reduced at 5 and 20 mg/kg/day and the cholesterol content of the epididymis was significantly reduced at 20 mg/kg/day.

In contrast to the results observed in 28-day studies, chronic studies have reported no treatment-related changes in the epididymis or accessory sex glands of treated rats or monkeys {NTP 2020, 7330145; Butenhoff et al. 2012, 2919192; Butenhoff et al. 2002, 1276161}.

Overall, the evidence for adverse effects on the male reproductive system is inconsistent for PFOA. Some studies have reported damage to the testes including atrophy of the seminiferous tubule epithelia {Song et al. 2018, 5079725; Zhang et al. 2014, 2850230; Butenhoff et al. 2012, 2919192}; however, two comprehensive studies conducted by NTP {2019, 5400977; 2020, 7330145} and a two-generation reproduction study {Butenhoff et al. 2004, 1291063} all reported no significant changes in the histopathology of male reproductive organs and glands. The two-year study by Butenhoff et al. {2012, 2919192} reported a small but statistically significant increase in the incidence of vascular mineralization in the testes of high-dose males. The toxicological significance of that finding is unclear as the study authors did not evaluate any parameters related to fertility including any hormone levels nor did they see any effects on testes weights. In addition, this lesion was not observed in another chronic rat study {NTP 2020, 7330145} or in any of the shorter duration mouse studies where there were suggestive effects on sperm parameters and fecundity. When mice were exposed to PFOA in utero, the numbers of Leydig cells in the testes were decreased and there was evidence of dose-dependent testicular damage on PND21 and PND70 {Song et al. 2018, 5079725}. Leydig cells are the primary site of testicular steroidogenesis in males {Huhtaniemi, 1995, 7420539}. No other studies evaluated the number of Leydig cells following developmental exposure to PFOA. BWTs of the pups and growth during the lactation period were not reported; therefore, it is unclear whether these effects reflect a specific toxicity to the testes or if they resulted from delayed growth and systemic toxicity. Body weights were not reduced compared to control on PND21 or PND70; therefore, a direct effect on the developing testes cannot be ruled out.

Reduced epididymal weights were reported in two studies along with reduced epididymal sperm concentration and/or observations of hypospermia {NTP 2019, 5400977; Lu et al. 2016, 3981459}. It is also unclear if these effects resulted from a specific toxicity to the epididymis or from concurrent systemic toxicity as effects were observed in conjunction with decrements in body weight {NTP 2019, 5400977} or body weights were not reported {Lu et al. 2016, 3981459}.

3.3.2.2.5.2 Females

Histopathological changes in the uterus and ovary have been observed following exposure to PFOA; however, comprehensive studies with chronic exposure durations do not provide evidence of increased nonneoplastic lesions in female reproductive organs.

Li et al. {2018, 5084746} administered PFOA (1, 5, 10, 20, or 40 mg/kg/day) to pregnant Kunming mice from GD1–GD17 and measured apoptosis in the uterine tissue on GD18. The number of apoptotic cells was significantly increased for females dosed with 5 mg/kg/day or higher in a dose-dependent manner compared to controls, and embryo survival was significantly decreased at doses ≥ 10 mg/kg/day (see Section 3.3.1.2). The uterus was examined in several other studies with no significant changes reported in organ weight or incidences of nonneoplastic lesions, including a 28-day study in rats {NTP 2019, 5400977}, a two-generation reproduction study in rats {Butenhoff et al. 2004, 1291063} and a 2-year dietary study in rats {Butenhoff et al. 2012, 2919192}. No significant differences in uterine weights were observed at the 16-week interim evaluation of the NTP 2-year dietary study in rats (see study design details in Section 3.3.1.2.1.2){NTP, 2020, 7330145}; however, the terminal evaluation found that females treated with PFOA had a higher incidence of uterine adenocarcinoma that may have been related to exposure (see Section 3.3.17.2). The incidences of nonneoplastic lesions of the uterus were not significantly increased in any of the PFOA exposure groups {NTP 2020, 7330145}.

As mentioned above, Chen et al. {2017, 3981369} and Zhang et al. {2020, 6505878} both observed significant changes in the ovaries of adult female mice administered PFOA, including reductions in the number of corpora lutea and the ratio of corpora lutea to ovarian areas. However, the NTP chronic dietary study {NTP 2020, 7330145} and a two-generation reproduction study {Butenhoff et al. 2004, 1291063} both found no treatment-related effects in the ovaries of treated rats. Butenhoff et al. {2012, 2919192} observed a significant, dose-related increase in the incidences of ovarian tubular hyperplasia in rats exposed for 2 years to PFOA in the diet. The incidences of this lesion in the control, 30, and 300 ppm groups were 0%, 14%, and 32%, respectively. The tissues were subjected to a pathology peer review using updated diagnostic nomenclature and no statistical differences were found between treated groups and controls {Mann and Frame, 2004, 6569580}.

3.3.2.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse reproductive outcomes is discussed in Sections 3.2.2, 3.2.7, 3.3.3, 3.3.4, and 3.4.3 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 44 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to reproductive effects. A summary of these studies is shown in Figure 53. Additional analysis on the mechanistic actions of PFOA on reproductive health outcomes is ongoing 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	1	2
Big Data, Non-Targeted Analysis	1	0	5	5
Cell Growth, Differentiation, Proliferation, Or Viability	8	0	21	25
Cell Signaling Or Signal Transduction	3	1	16	20
Extracellular Matrix Or Molecules	0	0	3	3
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	2	0	2	3
Hormone Function	6	1	18	22
Inflammation And Immune Response	1	0	0	1
Oxidative Stress	2	0	6	8
Xenobiotic Metabolism	1	0	6	6
Other	0	0	1	1
Not Specified (Review Article)	1	0	0	1
Grand Total	15	2	33	44

Figure 53. Summary of Mechanistic Studies of PFOA and Reproductive Effects

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

3.3.2.4 Evidence Integration

3.3.2.4.1 Reproductive Effects in Males

In summary, studies evaluating children and adults observed suggestive evidence of an effect for testosterone, but limited epidemiological evidence was observed for other effects of PFOA on the male reproductive system. There was inconsistent evidence for the relationship between PFOA and testosterone in cross-sectional studies {Lopez-Espinosa, 2016, 3859832; Di Nisio, 2019, 5080655} on children and young adults. The inconsistent relationships were observed in populations at different stages of pubertal development, and the one positive association was observed in a *low* confidence study {Di Nisio, 2019, 5080655}. One *medium* confidence study (Liu, 2020, 6569227) observed a positive association for progesterone in male infants. Studies in adolescents did not identify effects on pubertal development, but negative associations were observed for testicular volume, penis length, penis circumference, and number of sperm with normal morphology {Di Nisio, 2019, 5080655}. In adults, there was evidence in two studies {Cui, 2020, 6833614; Petersen, 2018, 5080277} of inverse associations between serum PFOA and testosterone (total and free), and these associations were also observed using semen PFOA. Inverse associations were also seen for E2 and the total testosterone-LH ratio. Regarding semen and sperm characteristics in adults, associations were observed for several parameters in analyses of semen PFOA, including increased sperm concentration and total sperm count, decreased

motility and number of morphologically normal sperm, and increased sperm DNA fragmentation. Other results for markers of genotoxic effects (e.g., sperm Y:X chromosome ratio, sperm DNA methylation, etc.) in sperm were inconsistent. Overall, these studies provide additional evidence of potential effects on testosterone levels in adult males.

Similar to the findings from the 2016 Health Advisory the recent evidence is suggestive of some male reproductive toxicity of PFOA, especially for sperm parameters. However, the associations are inconsistent across various parameters, and it is at times difficult to assess the impact on health outcomes of these alterations. Therefore, no endpoints from the epidemiological literature were considered for POD derivation

The evidence from animal studies also indicates that PFOA can adversely affect the male reproductive systems; however, the evidence from animal studies is similarly inconsistent as in epidemiological studies. Despite this, some studies observed significant alterations in reproductive hormone levels and adverse effects on sperm parameters.

Exposure during development or for short durations in adult rodents has resulted in changes in the normal structure of the testis and epididymis {Song et al. 2018, 5079725; NTP 2019, 5400977; Lu et al. 2016, 3981459; Zhang et al. 2014, 2850230; Li et al. 2011, 1294081}. However, studies with chronic exposures generally found no histological changes in the testes except for one two-year study that reported an increased incidence of vascular mineralization in the testes of the high-dose group {Butenhoff et al. 2012, 2919192}. The toxicological significance of that finding is unclear and it was not observed in another two-year study by NTP {2020, 7330145}. EPA concluded that the observed changes in the testes and epididymis represent toxicities possibly relevant to humans. In particular, alterations in Leydig cell structure or physiology may be driving the reductions in testosterone and effects on sperm parameters seen in both humans and animals {Zirkin, 2018}. Therefore, the endpoint of reduced number of Leydig cells on PND21 observed by Song et al. {2018, 5079725} was considered for POD derivation.

3.3.2.4.2 Reproductive Effects in Females

As in the 2016 Health Assessment, there is suggestive evidence of an association between PFOA 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).

Results for female fertility are mixed. In the 2016 Health Advisory, two studies reported correlations between higher PFOA levels and infertility {Fei et al., 2009, 1291107; Velez et al., 2015, 2851037}. Studies published since the 2016 HESD have observed no clear dose-response trends or directionality for a potential relationship {Lum, 2017, 3858516; Crawford, 2017, 3859813; Wang, 2017, 3856459; Kim, 2020, 6833596}. However, Kim, 2020, 6833596 did observe some non-significant, positive associations between follicular fluid PFOA and fertility etiology factors for other gynecologic pathologies, including endometriosis, polycystic ovarian syndrome (PCOS), genital tract infections, and idiopathic infertility.

There is limited evidence of an inverse association between serum PFOA levels in pregnancy and breastfeeding duration. Timmermann, 2017, 3981439 observed negative associations

between PFOA exposure and exclusive and total breastfeeding duration, while Romano, 2016, 3981728 observed increased relative risk of breastfeeding termination with increasing PFOA exposure.

Evidence of a relationship between PFOA exposure and the female reproductive milestones of age at menarche and menopause is mixed. In the 2016 Health Assessment, Kristensen et al., 2013, 2321268 reported a positive association between prenatal PFOA exposure and later age at menarche, while Christensen et al., 2011, 1290803 reported no association between the two. Since the 2016 Health Assessment, Ernst (2019, 5080529) observed a non-significant, negative association between prenatal PFOA exposure and age at menarche. Other studies have investigated relationships between the menarche as well as menopause and concurrent PFOA exposure. In the 2016 Health Assessment, Lopez-Espinosa et al., 2011, 1424973 observed a positive association between concurrent PFOA exposure and age at menarche. More recently, Ding, 2020, 6833612 observed an inverse relationship between PFOA levels and age at menopause. However, findings from studies concurrently assessing menstruation events and PFOA levels in blood must be interpreted with caution due to potential reverse causality, as menstruation is a primary route of PFOA excretion for people who menstruate.

Two studies have reported on the relationship between PFOA exposure and pregnancy loss since the 2016 HESD, reporting mixed results. Louis et al. {2016, 3858527} observed no association between serum PFOA levels and pregnancy loss in the first 7 weeks following conception, while Liew et al. {2016, 6387285} observed a positive dose-response trend for increased odds of miscarriage across plasma PFOA tertiles in pregnant women.

Since the 2016 PFOA Health Assessment, 11 studies have assessed relationships between PFOA exposure and various female reproductive hormones, nine of which studied female infants and adolescents. Most studies did not report significant associations or consistent trends between PFOA exposure and reproductive hormones including 17-OHP, DHEA, E2, FSH, SHBG, and testosterone. *Medium* confidence studies have observed significant, positive associations between cord blood PFOA and estriol in female infants {Wang, 2019, 5080598}, concurrent PFOA exposure and serum E2 in female adolescents {Lopez-Espinosa, 2016, 3859832}, and maternal serum PFOA during pregnancy and AMH concentrations in adolescent daughters {Donley, 2019, 5381537}. There were few studies assessing relationships between PFOA exposure and female reproductive hormone levels in adult women (both pregnant and non-pregnant), and those identified did not report consistent evidence of relationships between PFOA exposure and these outcomes. Evidence of relationships between PFOA exposure and human female reproductive hormonal outcomes remains inconsistent.

Overall, the recent evidence is suggestive of an association between PFOA and preeclampsia and gestational hypertension; there is conflicting evidence on altered puberty onset and limited data suggesting reduced fertility and fecundity. The associations are inconsistent across reproductive hormone parameters, and it is difficult to assess the adversity of these alterations. Given the suggestive findings from the epidemiological studies, no endpoints were considered for POD derivation.

The evidence from animal studies also indicates that PFOA can adversely affect the female reproductive system; however, it is often unclear if alterations seen in animal studies reflect specific toxicity to the reproductive system or if they result from concurrent systemic toxicity.

Despite this, some studies observed significant alterations in ovarian physiology which were not confounded by alterations in body weight.

Specifically, effects of PFOA on the ovary included altered estrous cyclicity and number of corpora lutea. In female mice, effects on the estrous cycle (lengthened diestrus phase) were observed at doses that did not significantly reduce body weight {Zhang et al, 2020, 6505878}. These results in mice are supported by a study in female rats that similarly found slightly lengthened diestrus phase, though with a much higher PFOA dose {NTP, 2019, 5400977}. Altered ovarian physiology was also evidenced by two studies (short-term and gestational) in adult female mice showing reduced numbers of corpora lutea with increasing PFOA doses {Zhang et al., 2020, 6505878; Chen et al., 2017, 3981369} and one study in female rats (chronic) showing increased tubular hyperplasia of the ovarian stroma {Butenhoff et al., 2012, 2919192}. EPA concluded that these effects represent toxicities possibly relevant to humans as they both may indicate a potential reduction in fertility, an outcome that also had mixed results from epidemiological studies. The reduced number of corpora lutea observed by Zhang et al. {2020, 6505878} on exposure day 28 and Chen et al. {2017, 3981369} on GD 13 were considered for POD derivation. In addition, the increased duration of diestrus observed by Zhang et al. {2020, 6505878} was also considered for POD derivation.

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}. Elevated liver serum biomarkers are frequently an indication of liver injury, though not as specific as functional tests, such as histology findings and liver disease.

In the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}, evidence was generally consistent showing of an association between PFOA exposure and increases in serum ALT in multiple populations, including occupational and highly exposed communities. Increases were observed for AST and GGT as well, though less consistently. The associations were not large in magnitude but indicate the potential of PFOA to affect liver function. Only one study, an occupational cohort, of functional hepatic endpoints was available; no association was observed with hepatitis or fatty liver disease, but there was a positive association with non-hepatitis liver disease with a 10-year lag {Steenland, 2015, 2851015}.

3.3.3.1.2 Study Quality

For this updated review, 20 new epidemiology studies (22 publications)⁹ report on the relationship between PFOA exposure and liver effects. Of these, 10 were classified as *medium* confidence, five as *low* confidence, and five were considered *uninformative* due to potential confounding {Jiang, 2014, 2850910; Predieri, 3889874; Abraham, 2020, 6506041} or use of

⁹ Multiple publications of the same data: Jain (2019, 5381566); Jain (2019, 5080621); Jain (2019, 5381541).

PFAS as the dependent variable {Jain, 2020, 6833623; Fan, 2014, 2967086} (Figure 54). Seven of the 20 informative studies examined liver enzymes in adults, including four cross-sectional studies {Wang, 2012, 2919184; Jain, 2019, 5381541; Nian, 2019, 5080307; Liu, 2018, 4238514}, one cohort with retrospective exposure assessment {Darrow, 2016, 3749173}, and one prospective cohort {Salihovic, 2018, 5083555}, one open-label controlled trial (Convertino, 2018, 5080342) and one occupational cohort {Olsen, 2012, 2919185}. Most of these were in general population adults, but specific populations examined include elderly adults {Salihovic, 2018, 5083555} and fluorochemical plant workers {Wang, 2012, 2919184; Olsen, 2012, 2919185}. In addition, one occupational cohort {Girardi, 2019, 6315730} and three cross-sectional studies {Darrow, 2016, 3749173; Rantakokko, 2015, 3351439; Liu, 2018, 4238396} examined functional liver endpoints in adults (histology, liver disease, hepatic fat mass). 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 histology endpoints {Jin, 2020, 6315720}. With the exception of Darrow et al. (2016, 3749173), all of the studies of general population adults and children and one of the occupational cohorts {Olsen, 2012, 2919185} measured exposure to PFOA using biomarkers in blood. Darrow et al. (2016, 3749173) modeled exposure based on residential history, drinking water sources, and consumption rates. The other occupational cohorts estimated PFOA exposure based on job duties {Girardi, 2019, 6315730}.

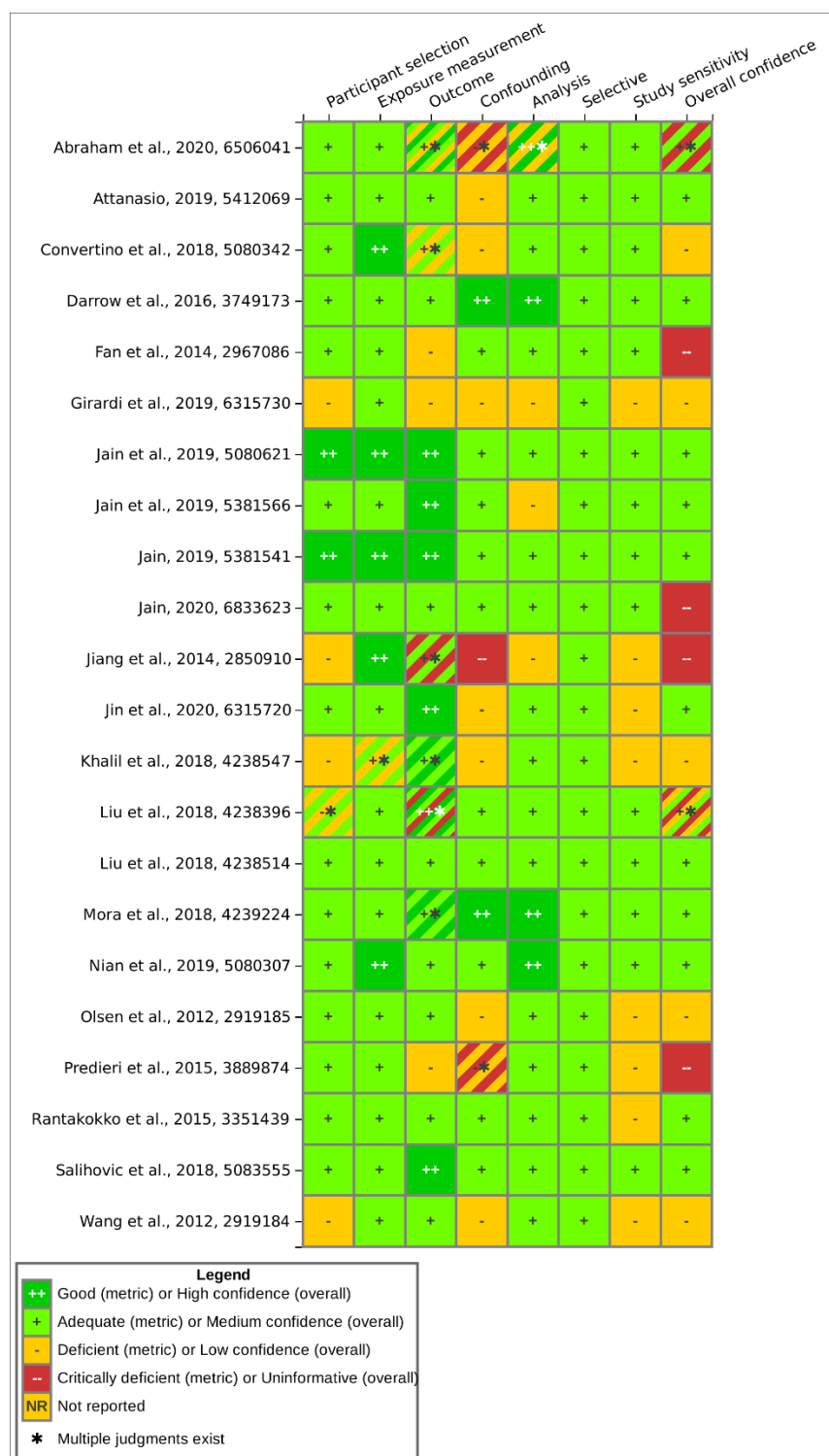


Figure 54. Summary of Study Evaluation for Epidemiology Studies of PFOA and Hepatic Effects^a

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

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

3.3.3.1.3 Findings

Results for the studies that examined ALT are presented in Table C-6. In adults, statistically significant positive associations between ALT and PFOA (i.e., increased ALT as a continuous measure with higher PFOA exposure levels) were observed in four of seven studies {Salihovic, 2018, 5083555; Nian, 2019, 5080307; Darrow, 2016, 3749173; Jain, 2019, 5381541}, including all the *medium* confidence studies. In addition, an exposure-response gradient was observed in the single study that examined quintiles of exposure {Darrow, 2016, 3749173}. This study additionally examined elevated ALT as a dichotomous outcome and reported an OR of 1.16 (95% CI 1.02-1.33) in the highest vs. lowest quintiles of exposure. The positive associations in Jain et al. (2019, 5381541) were observed only in certain sub-groups (e.g., by renal function (i.e., glomerular filtration stage) and obesity status), with no clear pattern. Among *low* confidence studies in adults, Olsen et al. (2012, 2919185) reported an inverse association ($p < 0.05$), however this analysis differed from the other studies in that the exposure measure used was change in PFOA levels during the study period. In the other *low* confidence study, Wang et al. (2012, 2919184) detected associations in opposite directions in fluorochemical plant workers (positive association) versus area residents (inverse association). In children and adolescents, positive associations were observed in girls (with exposure-response gradient across quartiles) in the *medium* confidence study by Attanasio et al. (2019, 5412069) and in the *low* confidence study in obese children {Khalil, 2018, 4238547}. However, inverse associations were observed in boys in Attanasio et al. (2019, 5412069) and in Mora et al. (2018, 4239224), so associations in this age group are less consistent than in adults. Insufficient data are available to assess the potential for effect modification by sex.

The studies that examined AST are presented in Table C-6. In adults, positive associations were observed in the two *medium* confidence studies {Jain, 2019, 5381541; Nian, 2019, 5080307}. In the two *low* confidence studies of fluorochemical plant workers {Olsen, 2012, 2919185; Wang, 2012, 2919184}, no associations were observed. In children and adolescents, the *medium* confidence study {Attanasio, 2019, 5412069} reported a positive association in girls but an inverse association in boys. In the *low* confidence study {Khalil, 2018, 4238547}, the direction of association was inverse, but the result was extremely imprecise. For the other liver enzymes (bilirubin, GGT), results were generally consistent with ALT and AST, with the exception of inverse associations for bilirubin in some studies {Salihovic, 2018, 5083555; Darrow, 2016, 3749173}.

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 PFOA exposure levels were associated with extremely reduced odds of lobular inflammation (OR=0.02, $p < 0.05$), while Jin et al. (2020, 6315720) reported the opposite direction of association, though the results in the latter study were non-monotonic and not statistically significant. Jin et al. (2020, 6315720) additionally reported lower odds of ballooning and portal inflammation, but higher odds of steatosis (association non-monotonic) and nonalcoholic steatohepatitis. Three studies examined some form of liver disease. The only *medium* confidence study of liver disease {Darrow, 2016, 3749173} reported no increases in any liver disease or specifically enlarged, fatty, or cirrhosis. In contrast, the *low* confidence study reported more liver disease with higher exposure. In Girardi et al. (2019, 6315730), workers at a PFAS production plant had higher mortality from liver cancer

or cirrhosis when compared to regional mortality statistics and a control group of non-chemical workers ($p < 0.05$ for some comparisons).

3.3.3.2 *Animal Evidence*

There are 12 studies from the most recent literature search conducted in 2020 and 7 key studies from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and hepatic effects. Study quality evaluations for these 19 studies are shown in Figure 55.

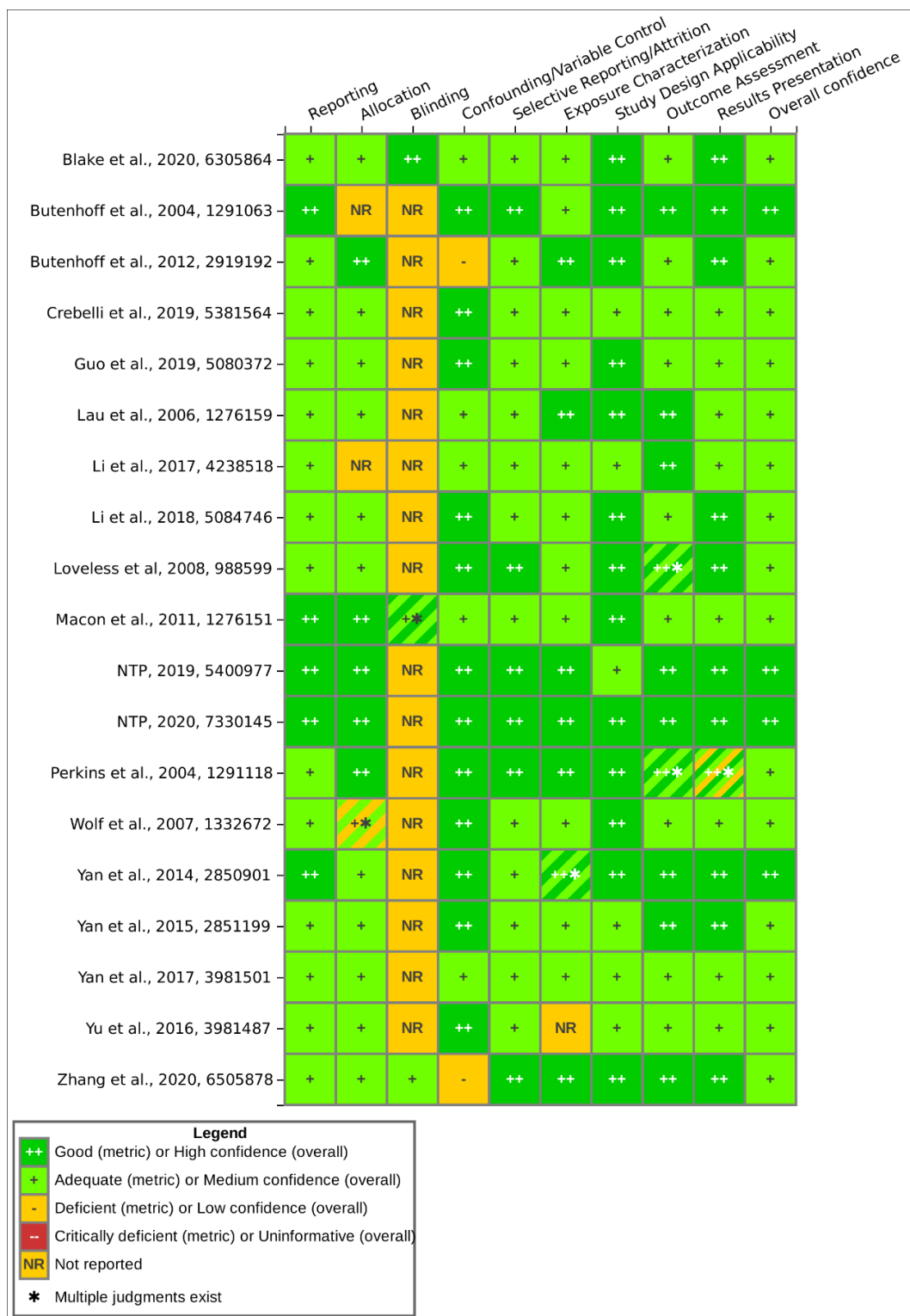


Figure 55. Summary of Study Evaluation for Toxicology Studies of PFOA and Hepatic Effects

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 PFOA 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 PFOA toxicity.

3.3.3.2.1 Liver Weight

Generally, increases in absolute and relative liver weight were observed across all available PFOA animal studies, regardless of species, sex, life stage, and exposure paradigm (Figure 56). Significant increases in absolute and relative liver weight were reported at doses as low as 0.05 mg/kg/day and 0.31 mg/kg/day, respectively {Li et al., 2017, 4238518; Yan, 2014, 2850901}, and were often present at the lowest dose administered in each study. In male mice, significant increases in both absolute and relative liver weights were observed with doses ranging from 0.31–30 mg/kg/day after 4–5 weeks of exposure {Loveless, 2008, 988599; Minata, 2010, 1937251; Yan, 2014, 2850901; Yu, 2016, 3981487; Li, 2017, 4238518; Crebelli, 2019, 5381564; Guo, 2019, 5080372}. Similarly, significant increases in absolute and relative liver weights were reported in male rat short-term/subchronic studies with doses between 0.625–30 mg/kg/day {Perkins, 2004, 1291118; Loveless, 2008, 988599; Cui, 2009, 757868; NTP, 2019, 5400977}. Two subchronic dietary studies in male rats with exposures lasting for 13–16 weeks reported significantly increased absolute and relative liver weights at doses as low as 1 mg/kg/day {Perkins, 2004, 1291118; NTP, 2020, 7330145}. Male cynomolgus monkeys orally administered PFOA capsules for 26 weeks also had significantly increased absolute liver weights at doses ≥ 3 mg/kg/day, though the increase in relative liver weight was only statistically significant in the highest dose group (30/20 mg/kg/day) {Butenhoff, 2002, 1276161}.

Several systemic toxicity studies evaluating liver weight in female mice and rats after short-term, subchronic, or chronic PFOA exposures are also available {Butenhoff, 2012, 2919192; Li, 2017, 4238518; NTP, 2019, 5400977; NTP, 2020, 7330145; Zhang, 2020, 6505878}. Two 28-day studies in female mice reported significant increases in absolute liver weight at doses ranging from 0.05–5 mg/kg/day (relative liver weight not reported) {Li, 2017, 4238518; Zhang, 2020, 6505878}. NTP (2019, 5400977) conducted a 28-day gavage study in female rats and reported significant increases in both absolute and relative liver weights at doses ≥ 25 mg/kg/day. In a chronic feeding study (see study design details in Section 3.3.1.2.1.2), NTP (2020, 7330145) reported significant increases in absolute and relative liver weight in female rats after 16 weeks of exposure to 63.4 but not 18.2 mg/kg/day PFOA. A 2-year feeding study in female rats similarly found no significant difference in absolute or relative liver weight with doses of 1.6 or 16.1 mg/kg/day PFOA compared to controls {Butenhoff, 2012, 2919192}.

There are also multiple reproductive and developmental toxicity studies that report maternal and/or offspring liver weight in female mice after gestational PFOA exposures. Blake et al. (2020, 6305864) reported significant increases in absolute and relative liver weights in dams exposed to PFOA at doses of 1 or 5 mg/kg/day from GD1.5–11.5 or GD1.5–17.5. Yahia et al. (2010, 1332451) similarly reported significant increases in maternal mouse absolute liver weights at PFOA doses ≥ 5 mg/kg/day and relative liver weights at PFOA doses ≥ 1 mg/kg/day. In a 2-generation reproductive toxicity study in rats {Butenhoff, 2004, 1291063}, P₀ dams dosed with 1, 3, 10, or 30 mg/kg/day PFOA at least 70 days prior to mating through lactation did not show consistent alterations in absolute or relative liver weights at the time of sacrifice on

PND22. However, significantly increased absolute and relative liver weights were observed in P₀ males and male F₁ offspring starting at the lowest dose of 1 mg/kg/day. No statistical differences in absolute or relative liver weights were reported for female F₁ offspring. Several other developmental toxicity studies reported significantly increased maternal, fetal, and/or pup liver weights associated with gestational PFOA exposure but the authors did not further examine tissue or serum samples for hepatic effects {Lau, 2006, 1276159; Wolf, 2007, 1332672; White, 2009, 194811; Macon, 2011, 1276151; White, 2011, 1276150; Tucker, 2015, 2851046; Li, 2018, 5084746}.

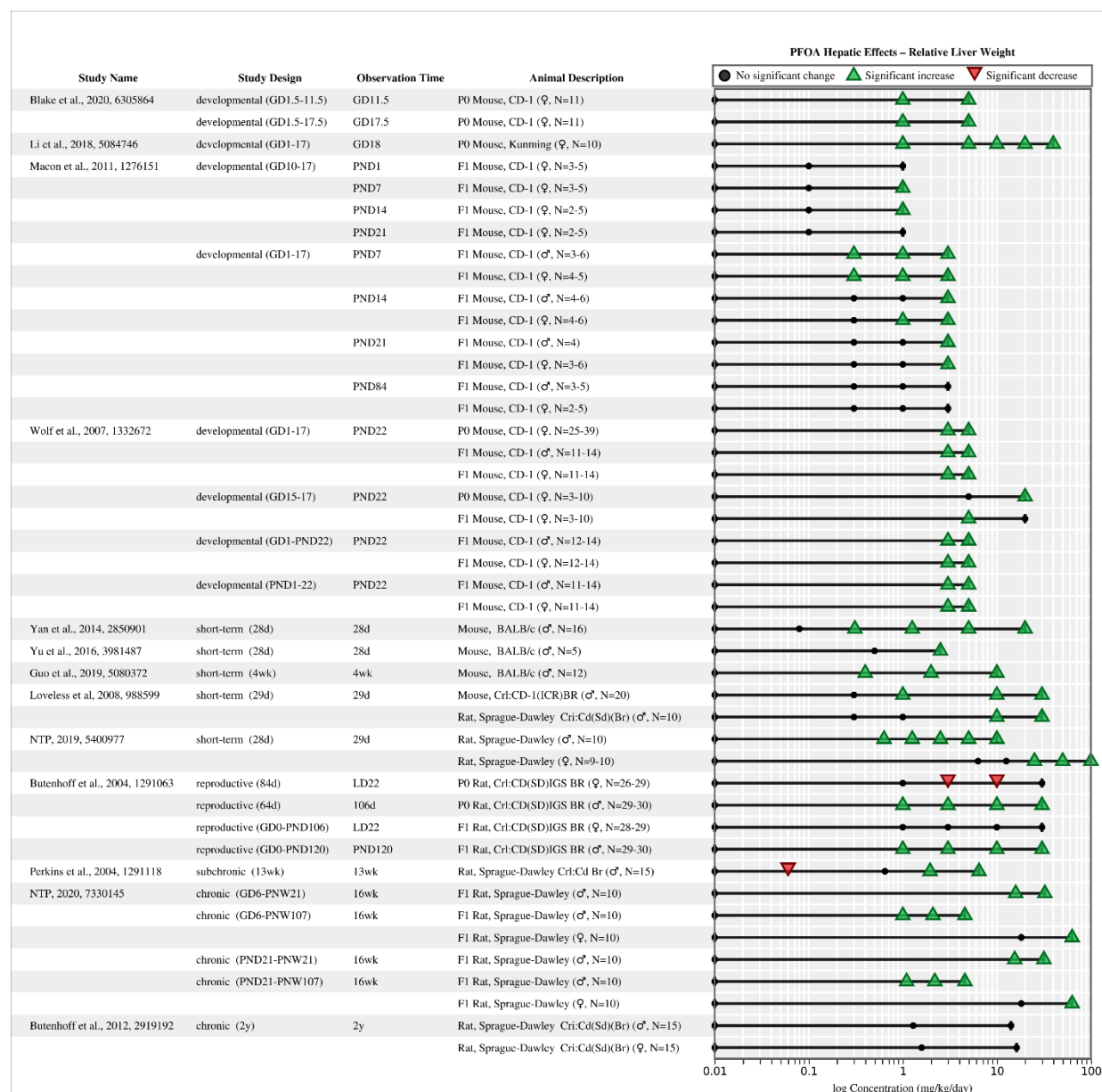


Figure 56. Relative Liver Weight in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA 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; PNW = postnatal week; LD = lactational day; P₀ = parental generation; F₁ = first generation; d = day; wk = week; y = year.

3.3.3.2.2 Clinical Chemistry

Albumin, a blood protein that plays a major role in PFOA toxicokinetics (Section 3.2), is synthesized by the liver. Increases in serum albumin were reported in several short-term and chronic studies in male rodents, with increases seen at doses as low as 0.4 and 1.3 mg/kg/day, in mice and rats respectively {Butenhoff, 2012, 2919192; Yan, 2014, 2850901; Guo, 2019, 5080372; NTP, 2020, 7330145}. Females appeared to be less sensitive, with increased albumin only at doses ≥ 25 mg/kg/day in rats after short-term or chronic exposures and no significant differences or inconsistent decreases in pregnant mice after gestational exposures {Yahia, 2010, 1332451; Butenhoff, 2012, 2919192; NTP, 2019, 5400977; Blake, 2020, 6305864; NTP, 2020, 7330145}.

Increases in enzymes including ALT, alkaline phosphatase (ALP), and AST following PFOA exposures were observed across multiple species, sexes, and exposure paradigms (Figure 57 [male mice], Figure 58 [male rats], Figure 59 [female rodents]). 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 {EPA, 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 controls) {EPA, 2002, 625713; Hall et al., 2012, 2718645}.

In male mice dosed with PFOA for 4–5 weeks, statistically significant increases in ALT and/or AST were observed at levels ranging from 2–21.6 mg/kg/day {Minata, 2010, 1937251; Yan, 2014, 2850901; Crebelli, 2019, 5381564; Guo, 2019, 5080372}. Increases in ALT were $\geq 100\%$ change at doses as low as 1.25 mg/kg/day {Yan et al., 2014, 2850901}. Biologically significant increases in AST were only observed in two of these studies with doses ≥ 20 mg/kg/day {Minata, 2010, 1937251; Yan et al., 2014, 2850901}. In the only study examining ALP in male mice, ALP was reported to significantly increase at concentrations of 5 and 20 mg/kg/day after 28-day exposures {Yan, 2014, 2850901}; serum ALP levels were $\geq 100\%$ change at doses of 1.25 mg/kg/day and higher.

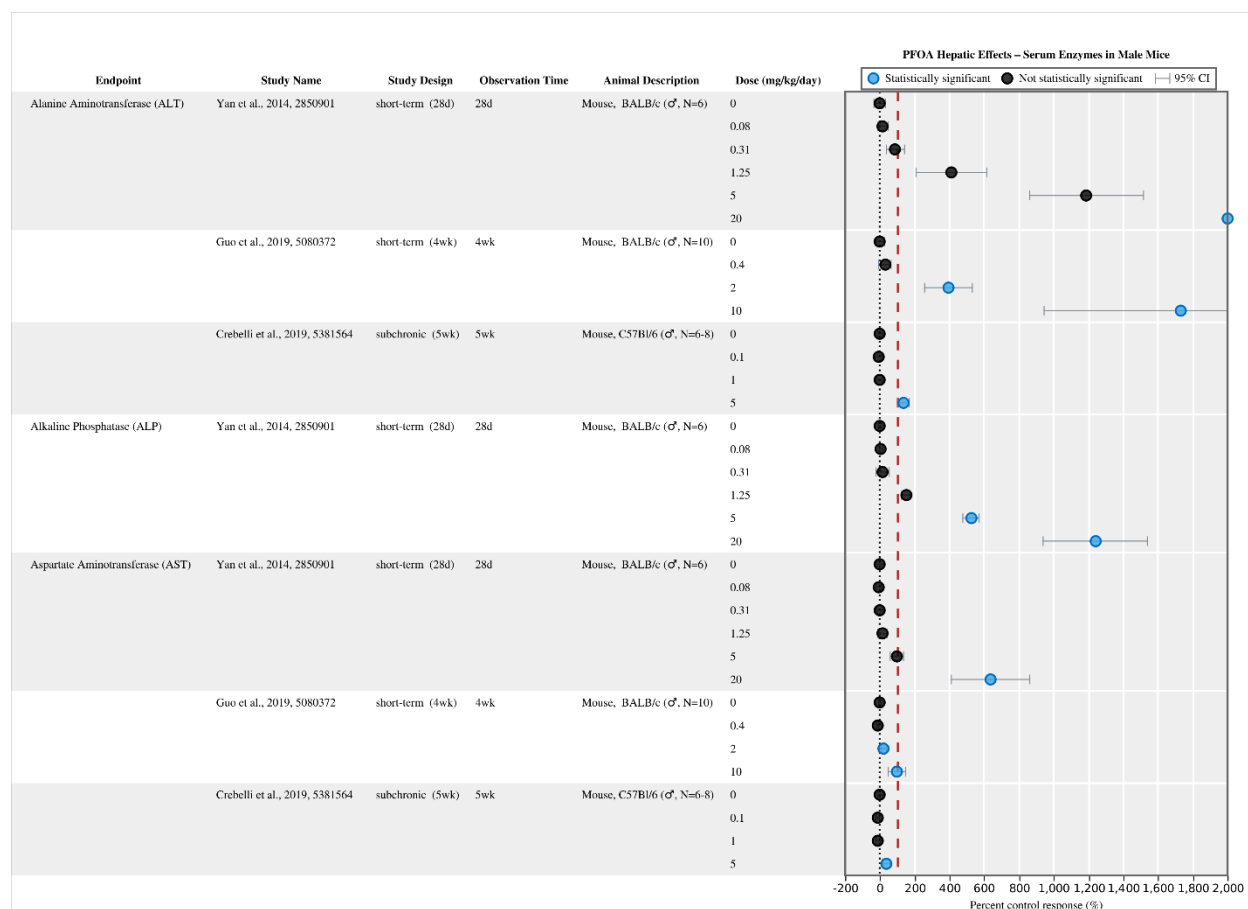


Figure 57. Percent Change in Serum Enzyme Levels Relative to Controls in Male Mice Following Exposure to PFOA^{a,b}

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

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

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

^bResults for Yan et al., 2014 are presented for 6 doses (0, 0.08, 0.31, 1.25, 5, and 20 mg/kg-day), and a statistically significant response of 7,000% occurs at the highest dose for the endpoint alanine aminotransferase (ALT). The axis has been truncated at 2,000% to allow results at lower doses for other studies and endpoints to be legible.

NTP (2019, 5400977; 2020, 7330145) reported significantly increased ALT and ALP at all doses tested in the 28-day and 16-week exposures of male rats to PFOA (dose range of 0.625–32.1 mg/kg/day). However, increases in ALT did not exceed 100% change in either study. Similarly, increases in ALP did not exceed 100% change in the 28-day gavage study {NTP, 2019, 5400977} and only exceeded 100% change with doses ≥ 15.6 mg/kg/day at the 16-week interim time point of the chronic dietary study {NTP, 2020, 7330145}. In another chronic dietary study, Butenhoff et al. (2012, 2919192) generally observed increased ALT and ALP in male rats dosed with 1.3 and 14.2 mg/kg/day PFOA at time points ranging from 3 months to 2 years of administration. Increases in ALT were above or approximately 100% change in both dose groups at 6, 12, and 18 months of exposure. ALP levels were elevated at all time points with 14.2 mg/kg/day PFOA, but were only above 100% change at the 18-month time point. AST was also less sensitive than ALT or ALP in male rats. NTP (2019, 5400977) observed statistically

significant but not biologically significant increases in AST at doses of 2.5 mg/kg/day and higher (up to 10 mg/kg/day) after 4 weeks. Butenhoff et al. (2012, 2919192) did not observe biologically significant increases in AST at any time point during the 2-year feeding study.

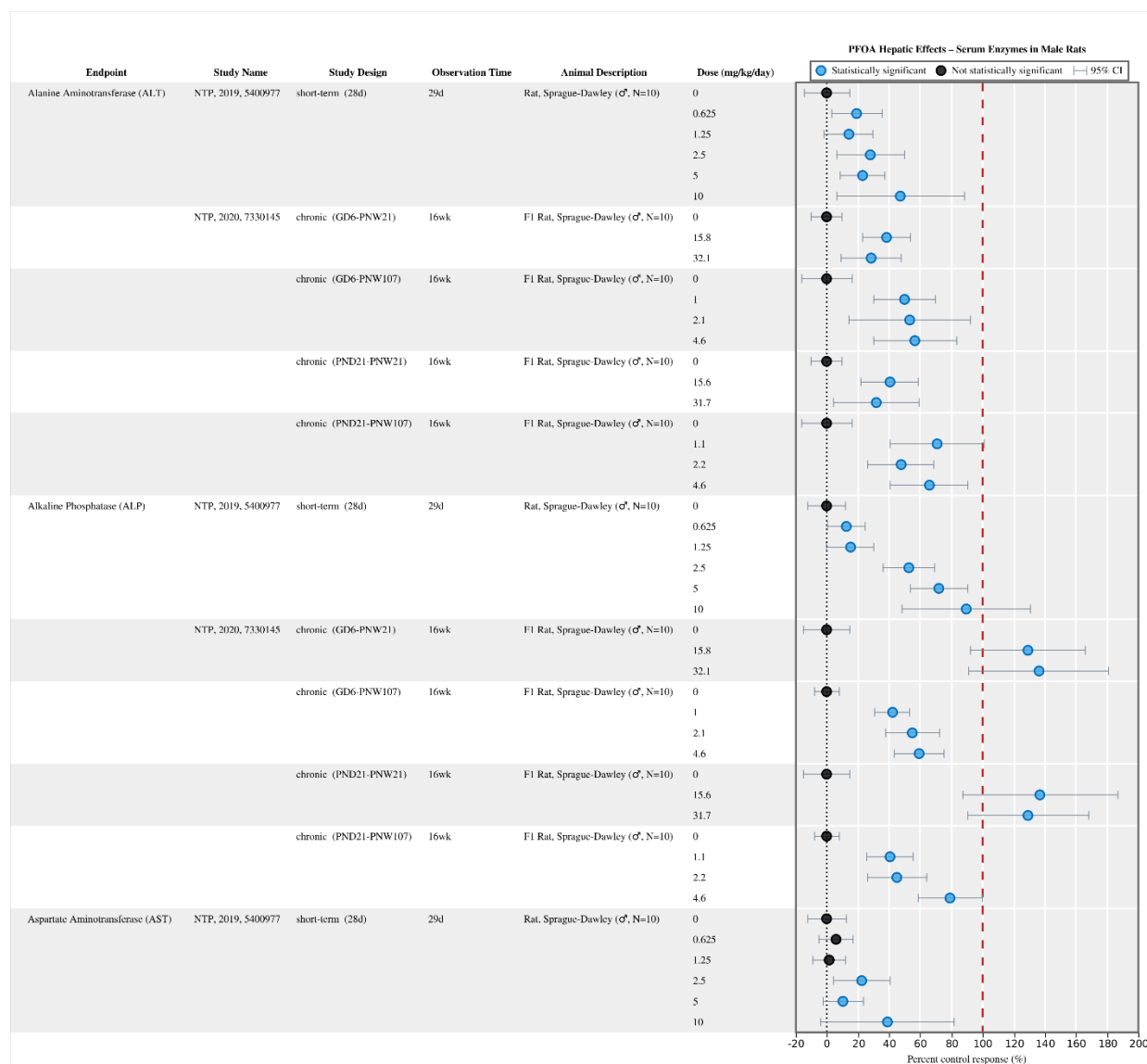


Figure 58. Percent Change in Enzyme Levels Relative to Controls in Male Rats Following Exposure to PFOA^a

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

ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GD = gestation day; PND = postnatal day; PNW = postnatal week; F1 = first generation; d = day; wk = week; CI = confidence interval.

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

In addition to the findings in rodents, no consistent responses of serum enzymes were observed in male cynomolgus monkeys dosed with PFOA for 26 weeks {Butenhoff, 2002, 1276161}.

The only available studies measuring ALT, AST, or ALP in female mice were after gestational exposures in dams. Blake et al. (2020, 6305864) reported no altered responses of ALT or ALP

with gestational PFOA exposures, and significantly increased AST (113% control) only after exposures of 5 mg/kg/day from GD1.5–17.5. In contrast, Yahia et al. (2010, 1332451) reported biologically significant increases in ALT and AST in dams with gestational exposure of 5 or 10 mg/kg/day (150% and 372% control ALT levels, respectively; 312% and 813% control AST levels, respectively); statistically significant increases in ALT, ALP (296% change), and AST were only observed at the highest dose of 10 mg/kg/day.

Short-term and chronic studies reported statistically but not biologically significant increases in ALT in female rats after 4- or 16-week PFOA exposures between 50–100 mg/kg/day {NTP, 2019, 5400977; NTP, 2020, 7330145}. The 4- and 16-week studies also reported no biologically significant changes in ALP with any PFOA dose, though PFOA exposures resulted in statistically significant ALP increases at gavage doses as low as 6.25 mg/kg/day after 4 weeks {NTP, 2019, 5400977; NTP, 2020, 7330145}. NTP (2019, 5400977) found no statistical or biological differences in AST in female rats following 4-week PFOA gavage dosing. Butenhoff et al. (2012, 2919192) also did not observe significant changes in ALT, AST, or ALP in female rats exposed to 1.6 or 16.1 mg/kg/day PFOA for up to 2 years.

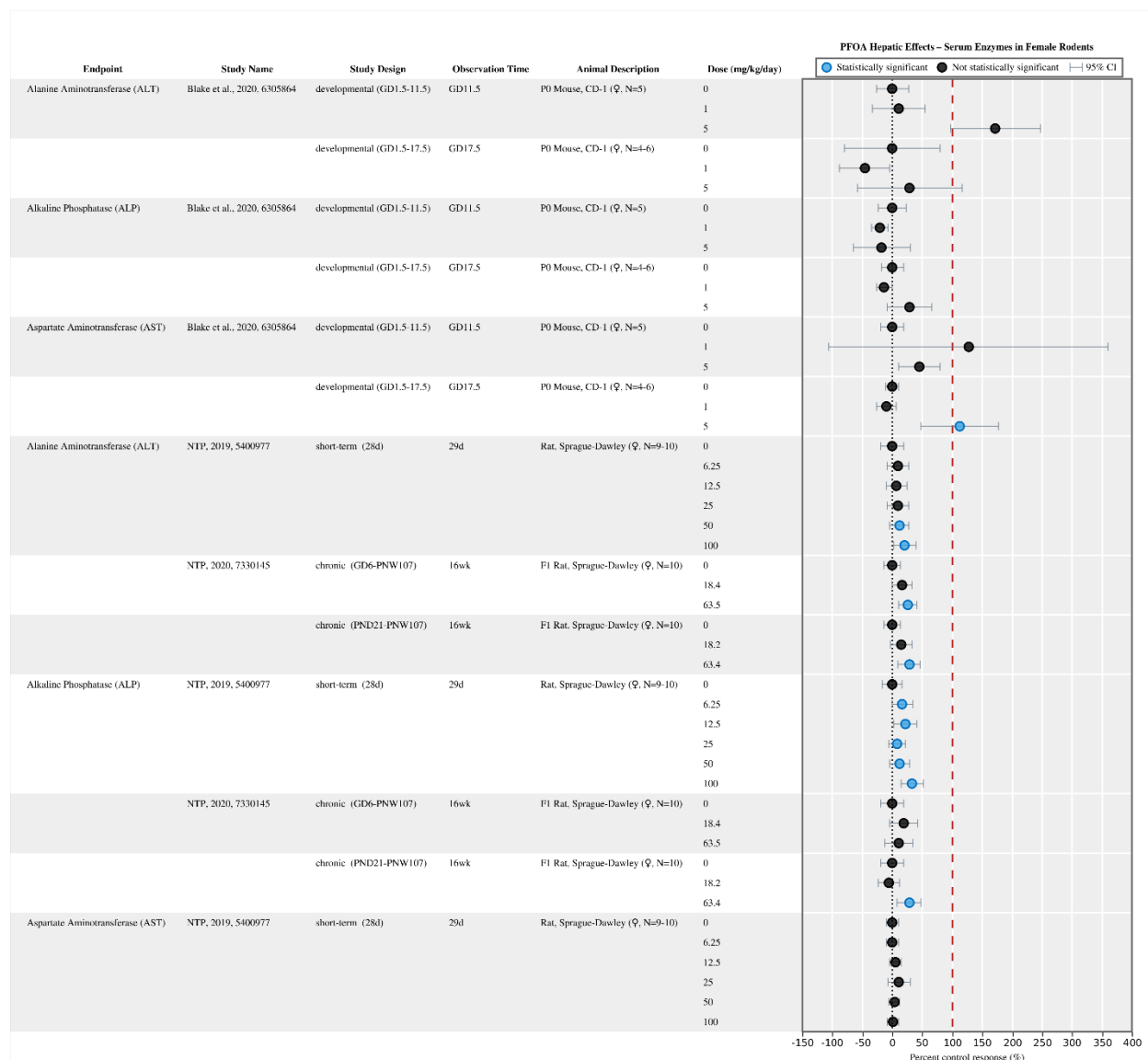


Figure 59. Percent Change in Enzyme Levels Relative to Controls in Female Rodents Following Exposure to PFOA^a

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

ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GD = gestation day; PND = postnatal day; PNW = postnatal week; P₀ = parental generation; F₁ = first generation; d = day; wk = week; CI = confidence interval.

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

3.3.3.2.3 Histopathology

Alterations in liver histopathology with PFOA exposure were observed in the available literature. Increased cell proliferation/division, hyperplasia, and hepatocellular hypertrophy were common responses across multiple studies. Loveless et al. (2008, 988599) reported increased incidence and severity of hepatocellular hypertrophy with increasing doses of PFOA (0.3–30 mg/kg/day) in male mice dosed for 29 days (incidences of 0/19, 20/20, 20/20, 20/20, and 19/19 (all severity grades combined) in the 0, 0.3, 1, 10, and 30 mg/kg/day groups, respectively). Several other 28-

day studies in male mice provided qualitative descriptions and images as evidence of increased hypertrophy, though results were not quantitatively reported {Minata, 2010, 1937251; Yan, 2017, 3981501; Li, 2017, 4238518; Guo, 2019, 5080372}.

Doses as low as 0.3 mg/kg/day resulted in increased incidence and severity of hypertrophy in male rats dosed with PFOA for 28 or 29 days {Perkins, 2004, 1291118; Loveless, 2008, 988599; NTP, 2019, 5400977}; female rats dosed for 28 days showed slight increases at 50 mg/kg/day (20%) and a 100% hypertrophy incidence rate at 100 mg/kg/day compared to 0% incidence at all lower doses (6.25, 12.5, or 25 mg/kg/day) and in controls (n = 10) {NTP, 2019, 5400977}. Butenhoff et al. (2012, 2919192) reported significant increases in the incidence of hypertrophy in male and female rats administered PFOA for 1 or 2 years at the highest dose tested for each sex (14.2 and 16.1 mg/kg/day for males and females, respectively). NTP (2020, 7330145) also reported increased incidence of hepatocellular hypertrophy in male and female rats dosed with PFOA for 16 or 107 weeks (see study design details in Section 3.3.1.2.1.2). At the 16-week interim necropsy, males had significantly increased incidences of hypertrophy at all doses tested (1–32.1 mg/kg/day); significantly increased incidences of hypertrophy were only observed in females at the highest doses tested (63.4/63.5 mg/kg/day) at 16 weeks. At 107-weeks, significantly increased incidences of hypertrophy were observed in males and females at doses ≥ 1.1 mg/kg/day and ≥ 18.2 mg/kg/day, respectively.

In a developmental toxicity study, Blake et al. (2020, 6305864) observed 100% incidence of hepatocellular hypertrophy with decreased glycogen and intensely eosinophilic granular cytoplasm at both the GD11.5 and GD17.5 time points with doses of 1 and 5 mg/kg/day compared to 0% incidence in controls (all n = 5–6); however, control dams at the GD17.5 time point did exhibit what the authors characterized as hepatocellular hypertrophy consistent with pregnancy at that stage of gestation. Quist et al. (2015, 6570066) similarly reported increased severity of hepatocellular hypertrophy with increasing PFOA doses (0.01–1 mg/kg/day) in PND91 female offspring exposed from GD1–17. In a standard 2-generation reproductive toxicity study, significant increases in the incidence of diffuse hepatocellular hypertrophy was reported for male F₁ offspring at doses of 3 mg/kg/day and higher {Butenhoff, 2004, 1291063}.

In addition to hepatocellular hypertrophy, significantly increased incidences of mitotic figures and bile duct hyperplasia were observed in male mice exposed to 10 or 30 mg/kg/day PFOA for 29 days {Loveless, 2008, 988599}. NTP (2020, 7330145) reported significantly increased incidences of mitoses and bile duct hyperplasia in female rats dosed with 63.5 mg/kg/day PFOA for 2 years, but not in males. In contrast, Filgo et al. (2015, 2851085) reported the incidence and severity of bile duct hyperplasia in two strains of 18-month-old wild-type female mice exposed to PFOA during gestation and found no alterations in one strain and a significant decrease in the severity of bile duct hyperplasia in the other. However, increased mitoses were observed (data not provided) in mouse dams exposed to 1–10 mg/kg/day PFOA during gestation {Yahia, 2010, 1332451}.

Several studies reported cytoplasmic alterations including cytoplasmic vacuolization resulting from PFOA exposures. Male mice dosed with PFOA for 28 days were reported to have increased vacuolation at doses between 5.4–21.6 mg/kg/day (incidence data not provided) and significantly decreased numbers of nuclei per unit area with 28-day exposures to ≥ 0.4 mg/kg/day {Minata, 2010, 1937251; Guo, 2019, 5080372}. Male rats were particularly susceptible to cytoplasmic alterations; NTP (2019, 5400977; 2020, 7330145) reported incidences of 90–100% in animals

receiving doses ≥ 1 mg/kg/day for 4 or 16 weeks compared to 0% incidences in controls (all $n = 10$). In the 2-year study, males receiving ≥ 2.1 mg/kg/day showed a 58% or greater incidence rate compared to 0% incidence rates in controls (all $n = 50$) {NTP, 2020, 7330145}.

Female rats receiving doses ≥ 25 mg/kg/day for 4, 16, or 107 weeks had 98–100% incidence rates of cytoplasmic alterations compared to 0% incidence rates in controls {NTP, 2019, 5400977; NTP, 2020, 7330145}. In mouse dams, 100% incidence rates of cytoplasmic vacuolization were observed only at the highest dose of 5 mg/kg/day but at both gestational time points (GD11.5 and GD17.5) compared to 0% incidence rates in controls ($n = 5-6$) {Blake, 2020, 6305864}. In this study, the vacuoles frequently contained remnant membrane material as myelin figures.

Cell and tissue death and degeneration was the final category of hepatic histological effects observed across multiple studies, species, and sexes (Table 7). Incidence rates of individual cell necrosis in male mice dosed with PFOA for 29 days were above 50% at doses ≥ 1 mg/kg/day {Loveless, 2008, 988599}. There was similarly a significantly increased percentage of necrotic liver cells, analyzed by flow cytometry, in male mice administered 5 mg/kg/day PFOA in drinking water for 5 weeks {Crebelli, 2019, 5381564}. Significantly increased incidences of single cell death were observed in male rats after 16 weeks of exposure to doses as low as 1 mg/kg/day but were not increased in females at this time point {NTP, 2020, 7330145}. Incidence rates of single cell death in male and female rats after 2-year exposures as reported in NTP (2020, 7330145) are provided in Table 7 (see further study design details in Section 3.3.1.2.1.2). Apoptosis and single-cell necrosis were also observed in livers of pregnant mice after gestational exposures of 1 and 5 mg/kg/day, with increasing length of exposure resulting in increased incidence rates {Blake, 2020, 6305864}.

In male mice exposed to PFOA for 29 days, the incidence of hepatic focal necrosis significantly increased with increasing PFOA doses between 1–30 mg/kg/day {Loveless, 2008, 988599}. In the same study, significantly increased incidences of necrosis were reported in male rats only with the highest dose tested (30 mg/kg/day) (Loveless et al., 2008, 988599). Inconsistent incidences of hepatic necrosis were observed in male and female rats administered PFOA in feed for 16 weeks, though there were increases reported after 2 years {NTP, 2020, 7330145}. Table 7 depicts the 2-year data for males and females. In a separate 2-year study, there were no significant differences in the incidence of hepatic necrosis in male or female rats {Butenhoff, 2012, 2919192}. Blake et al. (2020, 6305864) did not observe consistent increases in the incidence of focal necrosis in mouse dams dosed with PFOA during gestation. However, Butenhoff et al. (2004, 1291063) reported significant increases in focal and multifocal necrosis in F₁ generation male rats in a 2-generation reproductive toxicity study (data not provided).

Table 7. Associations Between PFOA Exposure and Cell Death or Necrosis in Rodents

Reference	Study Design	Endpoint Name	Incidence
Males			
NTP (2019, 5400977)	28-day Sprague Dawley rat oral gavage dosing; 0, 0.625, 1.25, 2.5, 5, 10 mg/kg/day	Focal Hepatocellular Necrosis	0/10, 0/10, 0/10, 0/10, 1/10, 0/10

Reference	Study Design	Endpoint Name	Incidence
Loveless (2008, 988599)	29-day Crl:CD(SD)IGS BR rat oral gavage dosing; 0, 0.3, 1, 10, 30 mg/kg/day	Focal Necrosis	0/10, 0/10, 0/10, 1/10, 4/10
	29-day Crl:CD-1(ICR)BR mouse oral gavage dosing; 0, 0.3, 1, 10, 30 mg/kg/day	Individual Cell Necrosis	0/19, 0/20, 11/20, 20/20, 19/19
	29-day Crl:CD-1(ICR)BR mouse oral gavage dosing; 0, 0.3, 1, 10, 30 mg/kg/day	Focal Necrosis	0/19, 1/20, 3/20, 4/20, 7/19
Perkins (2004, 1291118) ^a	4-week Crl:CD@BR rat feeding study; 0, 0.06, 0.64, 1.94, 6.5 mg/kg/day	Coagulative Necrosis	0/15, 0/15, 0/15, 1/15, 2/14
	7-week Crl:CD@BR rat feeding study; 0, 0.06, 0.64, 1.94, 6.5 mg/kg/day	Coagulative Necrosis	0/15, 0/15, 0/15, 0/15, 1/15
	13-week Crl:CD@BR rat feeding study; 0, 0.06, 0.64, 1.94, 6.5 mg/kg/day	Coagulative Necrosis	0/15, 1/15, 0/15, 1/15, 0/15
Butenhoff (2012, 2919192)	2-year Crl:COBS® CD(SD)BR rat feeding study; 0, 1.3, 14.2 mg/kg/day	Focal Hepatocellular Necrosis	3/50, 5/50, 5/50
NTP (2020, 7330145)	16-week Hsd:Sprague Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/150, 0/300, 150/150, and 300/300 ppm	Hepatocellular Single Cell Death	0/10, 10/10, 10/10, 9/10, 10/10
		Necrosis	0/10, 6/10, 2/10, 2/10, 4/10
	16-week Hsd:Sprague Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/20, 0/40, 0/80, 300/0, 300/20, 300/40, 300/80 ppm	Hepatocellular Single Cell Death	0/10, 7/10, 9/10, 10/10, 0/10, 5/10, 8/10, 10/10
		Necrosis	1/10, 1/10, 6/10, 4/10, 0/10, 2/10, 3/10, 1/10
	2-year Hsd:Sprague Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/20, 0/40, 0/80, 300/0, 300/20, 300/40, 300/80 ppm	Hepatocellular Single Cell Death	1/50, 1/50, 11/50, 24/50, 1/50, 3/50, 5/50, 29/50
		Necrosis	2/50, 17/50, 23/50, 20/50, 1/50, 11/50, 14/50, 21/50
Females			
NTP (2019, 5400977) ^b	28-day Hsd:Sprague Dawley SD rat oral gavage dosing; 0, 6.25, 12.5, 25, 50, 100 mg/kg/day	Focal Hepatocellular Necrosis	0/10, 0/10, 0/10, 0/10, 0/10, 0/10
Butenhoff (2012, 2919192)	2-year Crl:COBS@ CD(SD)BR rat feeding study; 0, 1.6, 16.1 mg/kg/day	Focal Hepatocellular Necrosis	5/50, 6/50, 2/50
		Focal Necrosis	1/5, 0/5, 2/5

Reference	Study Design	Endpoint Name	Incidence
Blake (2020, 6305864)	Gestational CD-1 mouse gavage dosing from GD1.5–GD11.5 (dams); 0, 1, 5 mg/kg/day	Cell Death (including apoptosis and single-cell necrosis of individual hepatocytes)	0/5, 1/5, 3/5
	Gestational CD-1 mouse gavage dosing from GD1.5–GD17.5 (dams); 0, 1, 5 mg/kg/day	Focal Necrosis	0/5, 0/5, 0/6
		Cell Death (including apoptosis and single-cell necrosis of individual hepatocytes)	0/5, 5/5, 6/6
NTP (2020, 7330145)	16-week Hsd:Sprague Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/300, 0/1,000 150/300, and 300/1,000 ppm	Hepatocellular Single Cell Death	0/10, 0/10, 1/10, 0/10, 0/10
		Necrosis	0/10, 0/10, 2/10, 0/10, 0/10
	2-year Hsd:Sprague Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/300, 0/1,000 150/300, and 300/1,000 ppm	Hepatocellular Single Cell Death	0/50, 4/50, 29/50, 5/50, 32/50
		Necrosis	0/50, 1/50, 8/50, 4/50, 5/50

GD = gestation day.

^aIncidence data as reported by Perkins et al. (2004, 1291118) were split into severity categories within the original study. For the purposes of this table, all non-grade 0 severities were considered an incidence (results for severity grades 1–3 were combined).

^bIncidence data not explicitly reported by NTP (2019, 5400977).

Cystoid degeneration was also observed across two chronic feeding studies in male rats. Butenhoff et al. (2012, 2919192) reported incidences of cystoid degeneration characterized as areas of multilocular microcysts in the liver parenchyma in 4/50 (8%), 7/50 (14%), and 28/50 (56%) male rats dosed for 2 years with 0, 1.3, or 14.2 mg/kg/day, respectively. NTP (2020, 7330145) similarly reported increases in the incidence of cystic degeneration in the liver of male rats administered 4.6 mg/kg/day PFOA for 107 weeks.

3.3.3.2.4 Additional Endpoints

A suite of other liver effects was observed but were either not included as endpoints of interest across multiple studies or had inconsistent results between studies, sexes, and/or species. These included serum measures of bile acids (generally no response or increases at high doses; Butenhoff, 2002, 1276161; Yan, 2014, 2850901; NTP, 2019, 5400977; Blake, 2020, 6305864; NTP, 2020, 7330145), bilirubin (no change or increases at high doses; Butenhoff, 2002, 1276161; Yahia, 2010, 1332451; NTP, 2019, 5400977), and histopathological findings such as hepatic inflammation (increased incidence/severity, decreased incidence, or no response; Filgo, 2015, 2851085; Quist, 2015, 6570066; NTP, 2020, 7330145) and hepatocytomegaly {Zhang, 2020, 6505878}. NTP (2020, 7330145) also reported on a variety of other histopathological outcomes including eosinophilic or mixed-cell foci (significant increases in males) and pigmentation (significant increases in males and females). Butenhoff et al. (2004, 1291063) similarly reported increased discoloration of the liver in male F₁ rats analyzed during a standard 2-generation reproductive toxicity study.

3.3.3.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse hepatic outcomes is discussed in Sections 3.2.1, 3.2.2, 3.2.3, 3.2.7, 3.2.8, 3.2.9, 3.3.2, 3.3.3, 3.3.4, 3.4.1, 3.4.2, 3.4.3, 3.4.4, and 4.2 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 76 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to hepatic effects. A summary of these studies is shown in Figure 60. Additional analysis on the mechanistic actions of PFOA on hepatic health outcomes is ongoing and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	7	0	9	15
Cell Growth, Differentiation, Proliferation, Or Viability	14	1	31	42
Cell Signaling Or Signal Transduction	10	1	15	24
Extracellular Matrix Or Molecules	1	0	1	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	20	0	17	33
Hormone Function	6	1	2	9
Inflammation And Immune Response	5	1	2	8
Oxidative Stress	7	0	11	17
Xenobiotic Metabolism	7	1	12	19
Other	2	0	3	5
Not Specified (Review Article)	2	0	1	2
Grand Total	40	2	44	76

Figure 60. Summary of Mechanistic Studies of PFOA and Hepatic Effects

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

3.3.3.4 Evidence Integration

Consistent with the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}, the human epidemiological data provide consistent evidence of a positive association between PFOA exposure and ALT in adults. 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). An exposure-response gradient observed in one study that examined categorical exposure in adults {Darrow, 2016, 3749173} also increases certainty in the association. However, the associations were not large in magnitude, and it is unclear whether the observed changes are clinically adverse. One study reported higher odds of elevated ALT with PFOA exposure {Darrow, 2016, 3749173}, but due to the lack of this type of analysis in other studies, it is not possible to assess consistency. Evidence in children and adolescents is less consistent.

Results for functional measures of liver toxicity, including histology and liver disease, are mixed. There is some indication of higher risk of liver disease with higher exposure, coherent with the liver enzyme findings. However, the strongest associations were observed in low confidence 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 et al., 2018, 5083555}; PFOA and PFOS were moderately correlated in both studies ($r = 0.4$ – 0.5). Jin et al. (2020, 6315720), reported positive associations with histology and fairly low correlations between PFOS and PFOA ($r = 0.14$), which reduces the concern for confounding in that population. In addition, studies in populations where PFOA exposure predominates due to contamination or occupational exposure {Steenland, 2015, 2851015; Wang, 2012, 2919184; Girardi, 2019, 6315730} are unlikely to suffer from notable confounding by other PFAS. Thus, there is minimal concern for confounding by other PFAS in this association. Because the r values were not large in magnitude, and it is unclear whether the observed changes are clinically adverse the increases in serum ALT levels were not considered for POD derivation.

The animal toxicity data provides additional evidence of PFOA-induced hepatic damage. 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; 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}. Many of the studies discussed in this section reported dose-dependent increases in liver weight and hepatocellular hypertrophy in rodents of both sexes. However, a limited number of these studies additionally examined functional or histopathological hepatic impairment to provide evidence that the enlargement of hepatic tissue was an adverse, and not adaptive, response {Minata, 2010, 1937251; Yan, 2014, 2850901; Crebelli, 2019, 5381564; Guo, 2019, 5080372; Blake et al., 2020, 6305864; Loveless et al., 2008, 7330145; NTP, 2020, 7330145}.

EPA identified two studies in male rodents, NTP (2020, 7330145), a chronic dietary study in Sprague Dawley rats (see study design details in Section 3.3.1.2.1.2), and Loveless et al. (2008, 988599), a 29-day gavage dosing study in CD-1 mice, as providing the most comprehensive evidence of dose-dependent hepatotoxicity resulting from oral PFOA exposure. NTP (2020, 7330145) conducted histopathological examinations of liver tissue in male rats and reported dose-dependent increases in the incidence of hepatocellular hypertrophy and hepatocellular cytoplasmic vacuolation, as well as increases in the incidence of hepatocellular single cell death and hepatocellular necrosis at the same dose levels. As this is one of the few available chronic PFOA toxicity studies with a large sample size ($n = 50$), numerous and relatively low dose levels, and a comprehensive suite of endpoints, both the single cell death and necrosis endpoints from the 107-week time point were considered for derivation of PODs.

Loveless et al. (2008, 988599) similarly provides concurrent evidence of liver enlargement and hepatic lesions in male mice gavaged with PFOA for 29 days. Increases in the incidence and severity of hepatocellular hypertrophy were dose-dependent, as well as increases in individual cell or focal cell necrosis. Similar to the NTP study (2020, 7330145), Loveless et al. (2008,

988599) provides a comprehensive report of hepatotoxicity, with a low dose range resulting in dose-dependent increases in histopathological outcomes indicating adversity. Therefore, the incidences of focal cell necrosis and individual cell necrosis in male mice from Loveless et al. (2008, 988599) were also considered for the derivation of PODs.

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}.

The 2016 Health Assessment for PFOA {U.S. EPA, 2016, 3603365} found consistent evidence of an association between PFOA exposure and immunosuppression. Three studies reported decreases in response to one or more vaccines in relation to higher exposure to PFOA in children {Grandjean et al., 2012, 1248827; Granum et al., 2013, 1937228} and adults {Looker et al., 2014, 2850913}. In the studies of children, there was concern that the associations were also seen with other correlated PFAS, but this was not considered a limitation in the study in adults, which was a high-exposed population (the C8 study).

For this updated review, associations between prenatal, childhood, or adult PFOA exposure and immunosuppression, specifically infectious disease incidence and antibody response to vaccination, were examined in 16 studies. A summary of the study evaluation of these studies is provided in Figure 61. One study from the 2016 assessment {Grandjean et al., 2012, 1248827} was updated during this period, and the update was included in the systematic review {Grandjean et al., 2017, 3858518}.

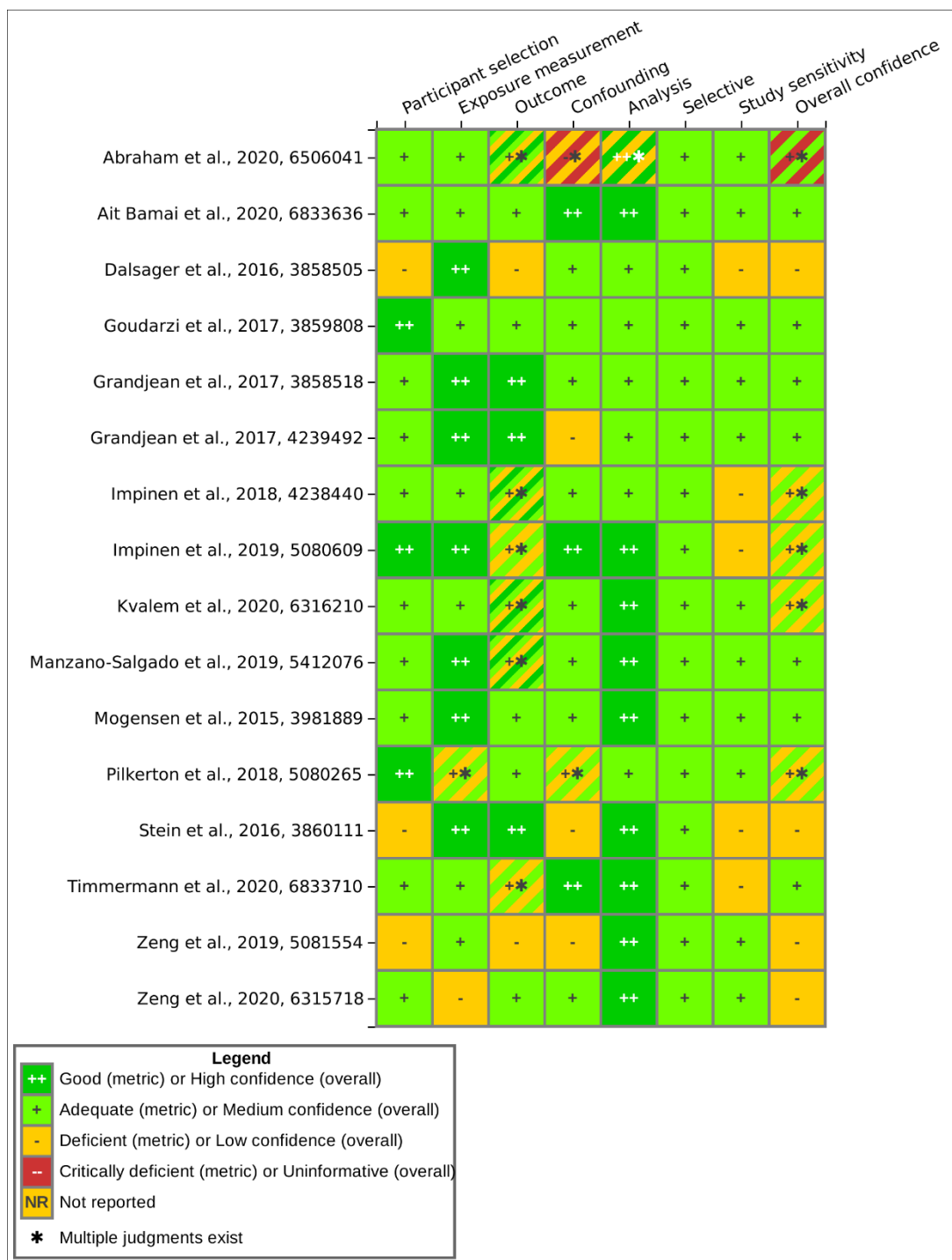


Figure 61. Summary of Study Evaluation for Epidemiology Studies of PFOA and Immunosuppression Effects

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

Seven studies studied the relationship between antibody response to vaccination and PFOA exposure. Five of these studies investigated antibody response to vaccination in children

{Timmermann et al., 2020, 6833710; Abraham et al., 2020, 6506041; Grandjean et al., 2017, 3858518; Grandjean et al., 2017; 4239492; Mogensen, 2015, 3981889}, one study investigated adult flu vaccine response {Stein et al., 2016, 3860111}, and one study measured rubella antibodies in both adolescents (aged 12 and older) and adults {Pilkerton et al., 2018, 5080265}. In addition, one study {Zeng et al., 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, six studies were *medium* confidence {Abraham et al., 2020, 6506041; Grandjean et al., 2017, 3858518; Grandjean et al., 2017, 4239492; Timmermann et al., 2020, 6833710; Mogensen, 2015, 3981889; Pilkerton et al., 2018, 5080265} and two were *low* confidence {Stein et al., 2016, 3860111, Zeng et al., 2019, 5081554}.

Of the studies that measured antibody response to vaccination in children, four studies were cohorts {Timmermann et al., 2020, 6833710; Grandjean et al., 2017, 3858518; Grandjean et al., 2017; 4239492; Mogensen, 2015, 3981889}, and one was cross-sectional {Abraham et al., 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, depending on the length of follow-up {Grandjean et al., 2017, 3858518; Grandjean et al., 2017; 4239492; Mogensen, 2015, 3981889}. Mogensen (2015, 3981889) provided additional analyses to Grandjean (2012, 1248827) using serum PFOA 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 in 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 PFOA at study inclusion (when children were aged one year).

Four studies measured antibody response to tetanus vaccination {Abraham et al., 2020, 6506041; Grandjean et al., 2017; 3858518; Grandjean et al., 2017; 4239492; Mogensen, 2015, 3981889}; four studies measured antibody response to diphtheria vaccination {Abraham et al., 2020, 6506041; Grandjean et al., 2017, 3858518; Grandjean et al., 2017; 4239492; Mogensen, 2015, 3981889}; one study measured antibody response to measles antibodies {Timmermann et al., 2020, 6833710}, and one study to *Haemophilus influenza* type b (Hib) antibodies {Abraham et al., 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 et al., 2012, 1248827; Grandjean et al., 2017, 3858518; Grandjean et al., 2017; 4239492; Mogensen, 2015, 3981889} observed associations between elevated levels of PFOA 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 years, age 7 years, and age 13 years. There are a few results in the opposite direction for sub-analyses of the Faroe Island cohorts {Grandjean et al., 2017, 3858518; Grandjean et al., 2017; 4239492}, such as maternal exposure with tetanus antibodies at 7 years. No biological rationale has been identified as to whether one time period for exposure or outcome measurement is more predictive of an overall immune response. Timmermann et al. (2020, 6833710) observed non-significant associations between elevated

levels of PFOA and decreased adjusted antibody levels against measles across time in the group with no measles vaccination at age 9 months. This association was not seen in the group with one measles vaccination. The same pattern was observed at the 2-year follow-up. Abraham et al. (2020, 6506041) also observed statistically significant correlations between adjusted tetanus, Hib, and diphtheria antibody levels and PFOA 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 et al., 2016, 3860111}, and one was a cross-sectional analysis {Pilkerton et al., 2018, 5080265}. Both studies measured PFOA 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 adolescent antibody response to vaccinations. In adults, results were less consistent than in children. Pilkerton et al. (2018, 5080265) observed statistically significant associations between high-quartile PFOA levels and decreased rubella IgA levels compared with low-quartile PFOA levels in adult men. Stein et al. (2016, 3860111) reported no immunosuppression based on seroconversion following FluMist vaccination. No association with rubella antibody levels was observed in adolescents in Pilkerton et al. (2018, 5080265).

It is plausible that the observed associations with PFOA 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 PFOA and PFOS, PFHxS, and perfluorononanoic acid (PFNA) (0.50, 0.53, 0.54, respectively) {Grandjean et al., 2017, 3858518; Grandjean et al., 2017, 4239492}. However, the authors assessed the possibility of confounding in a follow-up paper {Budtz-Jorgensen and Grandjean, 2018, 5083631} where estimates were adjusted for PFOS 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. However, as described above, one study {Looker et al., 2014, 2850913} included in the 2016 Health Assessment of PFOA observed an association with PFOA in a population where PFOA exposure predominated (the C8 Health Study population), and this is not likely to be confounded by other PFAS. Overall, while it is not possible to completely rule out confounding across PFAS, the available evidence suggests that it is unlikely to explain the observed effects.

Despite the imprecision (i.e., wide CIs) of some of the exposure-outcome analysis pairs, the findings are generally consistent with an association between PFOA exposure and immunosuppression. 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}. 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 evidence. Overall, the evidence indicates an association between increased serum levels of PFOA and decreased antibody production following routine vaccinations, particularly in children.

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 et al., 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 PFOA 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 ($p > 0.05$) between serum PFOA 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 PFOA 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 (LTRIs), respiratory syncytial virus, 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 PFOA exposure, six were cohorts {Ait Bamai et al., 2020, 6833636; Dalsager et al., 2016, 3858505; Kvale et al., 2020, 6316210; Manzano-Salgado et al., 2019, 5412076; Gourdazi et al., 2017, 3859808; Impinen et al., 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 et al., 2020, 6506041}. Five studies measured PFOA concentrations from mothers during pregnancy {Ait Bamai et al., 2020, 6833636; Dalsager et al., 2016, 3858505; Manzano-Salgado et al., 2019, 5412076; Gourdazi et al., 2017, 3859808; Impinen et al., 2019, 5080609}. Impinen et al. (2018, 4238440) measured PFOA concentrations from cord blood at delivery. Two studies measured PFOA concentrations in children's serum at age one year {Abraham et al., 2020, 6506041} and at age 10 years {Kvale et al., 2020, 6316210}.

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

Increased incidence of some infectious diseases in relation to PFOA 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 et al., 2017, 3859808} reported higher odds of total infectious diseases in girls ($p > 0.05$) but not in boys. Two studies, one *medium* and one *low* confidence, observed statistically significant associations between elevated PFOA concentration and increased risk of developing pneumonia in 0 to 3-year-old children {Impinen et al., 2019, 5080609} and 7-year-old children {Ait Bamai et al., 2020, 6833636}; however, one other *medium* confidence study observed a null association {Abraham et al., 2020,

6506041}. Two *low* confidence studies observed statistically significant associations or non-significant trends between elevated PFOA concentrations and higher incidence of LTRIs {Kvalem et al., 2020, 6316210 and Impinen et al., 2018, 4238440}, but results were null in Manzano-Salgado et al. (2019, 5412076), a *medium* confidence study. There were also statistically significant associations seen for PFOA in relation to respiratory syncytial virus, rhinitis, pseudocroup, and gastric flu {Ait Bamai et al., 2020, 6833636; Kvalem et al., 2020, 6316210; Impinen et al., 2019, 5080609}, but findings were inconsistent across studies. One cohort study {Dalsager et al., 2016, 3858505} measured common infectious disease symptoms in children aged 1 to 4 years. Overall, the observed associations provide some coherence with the associations observed with vaccine response, but inconsistency across studies reduces confidence in the evidence.

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. While these responses are mediated by circulating factors such as T-cells, IgE, and inflammatory cytokines, there are many health effects associated with hypersensitivity and allergic response. Functional measures of sensitivity and allergic response consist of health effects such as allergies or asthma and skin prick tests.

In the 2016 HESD for PFOA, two studies reported higher odds of asthma with higher PFOA exposure in children {Dong et al., 2013, 1937230 and Humblet et al., 2014, 2851240}. For this updated review, associations between PFOA exposure and immune hypersensitivity, specifically asthma, allergy, and eczema were examined in 22 studies. Results of the risk of bias to studies measuring immunosuppression can be seen in Figure 62.

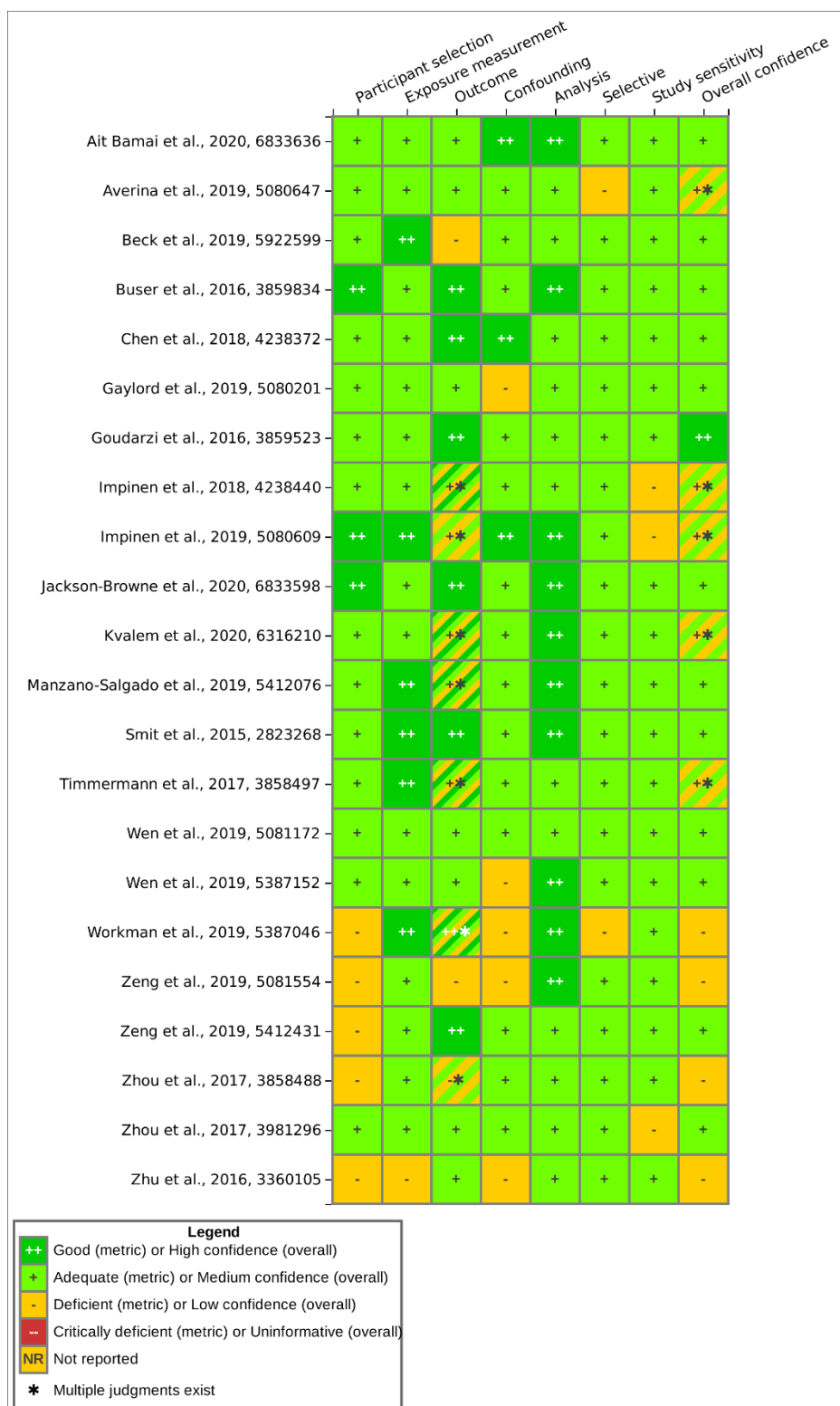


Figure 62. Summary of Study Evaluation for Epidemiology Studies of PFOA and Immune Hypersensitivity Effects

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

Thirteen studies¹⁰ (fifteen publications) examined asthma (or asthma symptoms) and PFOA exposure. Nine of these studies were cohorts {Averina et al., 2019, 5080647; Beck et al., 2019, 5922599; Kvaalem et al., 2020, 6316210; Manzano-Salgado et al., 2019, 5412076; Zeng et al., 2019, 5412431; Impinen et al., 2019, 5080609; Smit et al., 2015, 2823268; Timmermann et al., 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 et al., 2019, 5080201; Impinen et al., 2018, 4238440}; and one was a cross-sectional analysis {Jackson-Browne et al., 2020, 6833598}. Seven studies measured the prevalence of “current” asthma for at least one time point {Averina et al., 2019, 5080647; Beck et al., 2019, 5922599; Manzano-Salgado et al., 2019, 5412076; Kvaalem et al., 2020, 6316210; Impinen et al., 2018, 4238440; Impinen et al., 2019, 5080609; Zeng et al., 2019, 5412431}. Nine studies measured “ever” asthma for at least one time point {Averina et al., 2019, 5080647; Kvaalem et al., 2020, 6316210; Manzano-Salgado et al., 2019, 5412076; Jackson-Browne et al., 2020, 6833598; Gaylord et al., 2019, 5080201; Impinen et al., 2018, 4238440; Impinen et al., 2019, 5080609; Smit et al., 2015, 2823268; Timmermann et al., 2017, 3858497}. Incident or recurrent wheeze was examined in one study {Workman, 2019, 5387046}. Overall, nine publications were rated *medium* confidence, and six publications were *low* confidence for asthma (Figure 62). 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), and few statistically significant results were observed. Several studies observed positive associations with ORs greater than 1.2 between PFOA concentration levels and increased “current” or “ever” asthma {Beck et al., 2019, 5922599; Timmermann et al., 2017, 3858497; Jackson-Browne et al., 2020, 6833598; Kvaalem et al., 2020, 6316210; Zeng et al., 2019, 5412431; Averina et al., 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. Beck et al. (2019, 5922599) observed statistically significant increased odds of self-reported asthma per PFOA increase in boys, 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. Kvaalem et al. (2020, 6316210) reported increased odds of asthma in girls at 10 ($p < 0.05$) and 10-16 years of age, but null associations at 16 years, while the opposite was true for boys. Zeng et al. (2019, 5412431) observed a positive association in girls and an inverse association in boys (both $p > 0.05$). Jackson-Browne et al. (2020, 6833598) also observed statistically significant increased odds of “ever” asthma from increased PFOA concentrations in children aged 3 to 5. However, these associations were null in other age groups and in sex and race categories. Gaylord et al. (2019, 5080201) reported non-

¹⁰ Three publications {Zhou, 2016, 3981296; Zhou, 2017, 3858488; Zhu, 2016, 3360105} reported on the same cohort (Genetic and Biomarker study for Childhood Asthma) and outcome and are considered one study.

significant positive associations in 13–22 years old youth. The *low* confidence study, Timmermann et al. (2017, 3858497), observed positive associations ($p < 0.05$) between increased asthma odds and elevated PFOA 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, an inverse association was observed ($p > 0.05$). *Low* confidence studies from the GBCA study {Zhou, 2016, 3981296; Zhou, 2017, 3858488; Zhu, 2016, 3360105} observed elevated PFOA levels ($p < 0.001$) 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 PFOA exposure {Zhu, 2016, 3360105}. Two other studies {Impinen et al., 2018, 4238440; Impinen et al., 2019, 5080609} observed small positive associations (OR: 1.1); in Impinen et al. (2019, 5080609), this was only observed for current asthma in boys. Two studies reported non-significant inverse associations with asthma {Manzano-Salgado et al., 2019, 5412076; Smit et al., 2015, 2823268}, and one *low* confidence study did not observe a significant effect for recurrent wheeze {Workman, 2019, 5387046}.

Overall, there is some evidence of an association between PFOA exposure and asthma, but there is considerable uncertainty due to inconsistency across studies and sub-populations. 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.

Seven studies observed associations between PFOA exposure and allergies, specifically allergic rhinitis or rhinoconjunctivitis, skin prick test, and food or inhaled allergies. Five of these studies were cohorts {Goudarzi et al., 2016, 3859523; Ait Bamai et al., 2020, 6833636; Kvale et al., 2020, 6316210; Impinen et al., 2019, 5080609; Timmermann et al., 2017, 3858497}, one study was a case-control analysis {Impinen et al., 2018, 4238440}, and one study was a cross-sectional study using data from NHANES 2005–2010 {Buser et al., 2016, 3859834}. All studies were considered *medium* confidence for allergy outcomes. PFOA concentrations were measured at a variety of time points. Three studies measured PFOA during pregnancy {Goudarzi et al., 2016, 3859523; Ait Bamai et al., 2020, 6833636; Impinen et al., 2019, 5080609}. Three studies measured PFOA concentrations from children at age 5 years {Timmermann et al., 2017, 3858497}, age 10 years {Kvale et al., 2020, 6316210}, age 13 years {Timmermann et al., 2017, 3858497} and ages 12–19 years {Buser et al., 2016, 3859834}. Impinen et al. {2018, 4238440} and one {Timmermann et al., 2017, 3858497} measured PFOA concentrations from cord blood at delivery. Results for these outcomes are presented in Table C-10.

Results were generally inconsistent across studies. Three studies conducted skin prick tests on participants to determine allergy sensitization at age 10 years {Kvale et al., 2020, 6316210 and Impinen et al., 2018, 4238440}, at age 13 years {Timmermann et al., 2017, 3858497}, and at age 16 years {Kvale et al., 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. Kvale et al. (2020, 6316210) reported a statistically significant but small association (OR: 1.1) with a positive skin prick test at ages 10 and 16 years. 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

results in Impinen et al. (2018, 4238440) were null. Five studies measured symptoms of “current” or “ever” allergic rhinitis or rhinoconjunctivitis {Goudarzi et al., 2016, 3859523; Ait Bamai et al., 2020, 6833636; Impinen et al., 2018, 4238440; Kvaalem et al., 2020, 6316210; Timmermann et al., 2017, 3858497}. *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. Rhinitis was increased with exposure at age 16 years ($p < 0.05$) but decreased at age 10 years in Kvaalem et al. (2020, 6316210). Non-significant increases in rhinitis were also reported in Impinen et al. (2018, 4238440) and Timmermann et al. (2017, 3858497), but results were null in Ait Bamai et al. (2020, 6833636) and Goudarzi et al. (2016, 3859523) for rhinoconjunctivitis. Impinen et al. (2019, 5080609) measured parent-reported, doctor-diagnosed “current” or “ever” allergy symptoms at age 7 years, in addition to known food and inhaled allergies and reported higher odds of current food allergies and ever inhaled allergies (both $p > 0.05$), but not ever 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.

Eight studies measured the association between PFOA concentration and eczema (described by some authors as atopic dermatitis). Seven of these studies were cohorts {Goudarzi et al., 2016, 3859523; Wen et al., 2019, 5387152; Wen et al., 2019, 5081172; Manzano-Salgado et al., 2019, 5412076; Chen et al., 2018, 4238372; Timmermann et al., 2017, 3858497}, and one was a case-control analysis {Impinen et al., 2018, 4238440}. Four studies measured PFOA concentrations in cord blood at delivery {Wen et al., 2019, 5387152; Wen et al., 2019, 5081172; Chen et al., 2018, 4238372; Impinen et al., 2018, 4238440}, three studies measured PFOA concentrations in pregnancy {Goudarzi et al., 2016, 3859523; Manzano-Salgado et al., 2019, 5412076; Timmermann et al., 2017, 3858497}, and one study measured child blood at age 5 and 13 years {Timmermann et al., 2017, 3858497}. All the studies were considered *medium* confidence for eczema. Results are presented in Table C-11.

Three studies observed statistically significant associations between increased odds of atopic dermatitis within the highest quantiles of PFOA exposure {Wen et al., 2019, 5387152; Wen et al., 2019, 5081172; and Chen et al., 2018, 4238372}, however both associations were non-monotonic across categories of exposure. Impinen et al. (2018, 4238440) observed a non-significant association between higher PFOA concentrations and “ever” atopic dermatitis at age 2 years; however, results were null for “current” atopic dermatitis at age 10 years. Results from Goudarzi et al. (2016, 3859523), Manzano-Salgado et al. (2019, 5412076) and Timmermann et al. (2017, 3858497) were null.

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 PFOA {U.S. EPA, 2016, 3603279} identified one occupational study {Steenland, 2015, 2851015} reporting significant positive trends for rheumatoid arthritis and ulcerative colitis with increasing cumulative PFOA exposure. The C8 Science Panel concluded there was no probable link between PFOA and autoimmune disease {C8 Science Panel, 2012, 1430770}.

Four studies examined PFOA exposure and autoimmune disease (Table C-12; Figure 63). One study examined the association between PFOA exposure multiple autoimmune conditions (rheumatoid arthritis, lupus, MS, ulcerative colitis, and Crohn's disease) in the combined C8 Health Project occupational and community cohort {Steenland, 2013, 1937218}. Two case-control studies examined MS {Ammitzbøll, 2019, 5080379} and ulcerative colitis {Steenland, 2018, 5079806} in adults, and one case-control study examined celiac disease in children and young adults {Gaylord, 2020, 6833754}. The combined occupational and community study used modeled PFOA exposure based on serum concentrations and historical data on residences and drinking water quality {Steenland, 2013, 1937218}, and the case-control studies measured PFOA in serum only {Ammitzbøll, 2019, 5080379}. Two studies were without notable deficiencies and considered *medium* confidence {Gaylord, 2020, 6833754; Steenland, 2013, 1937218}. 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. Information on participant selection, particularly control selection, was not reported in Ammitzbøll (2019, 5080379). Additionally, PFOA was evaluated as a dependent rather than independent variable, making no informative determinations about associations between PFOA exposure and risk of MS. (Figure 63).

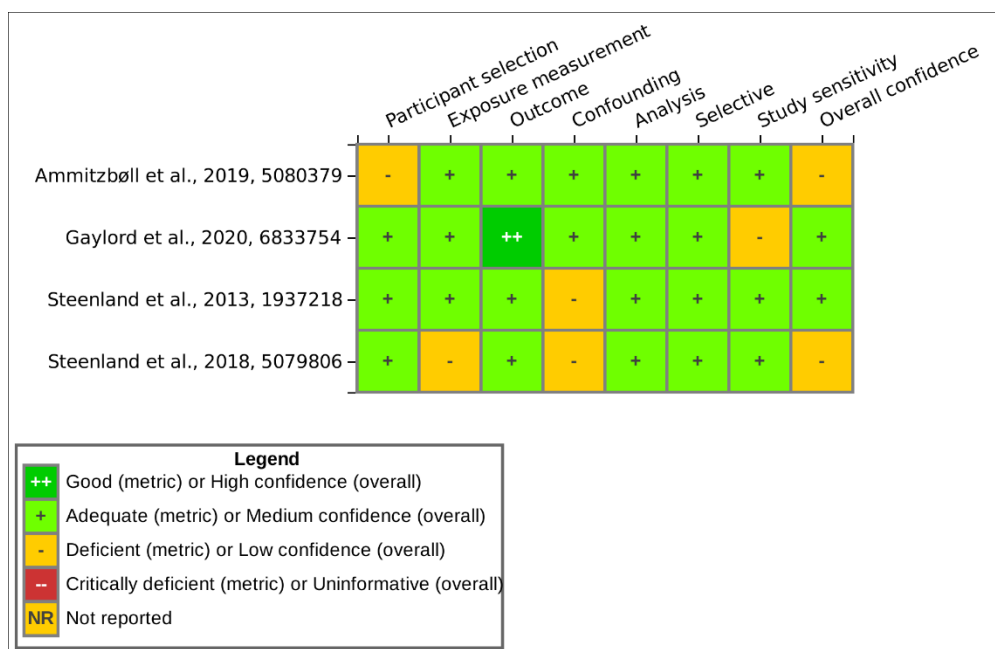


Figure 63. Summary of Study Evaluation for Epidemiology Studies of PFOA and Autoimmune Effects

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

In the C8 Health Project study {Steenland, 2013, 1937218}, associations for rheumatoid arthritis were generally consistent and positive across unlagged and 10-year lagged PFOA quartiles. The risk of rheumatoid arthritis was significantly elevated comparing those in the third quartile of 10-year lagged exposure compared to participants in the first quartile, but this was the only significant association. The risk of MS was non-significantly elevated in unlagged and 10-year lagged models {Steenland, 2013, 1937218}. Significant increased risk of ulcerative colitis among adults across increasing quartiles of PFOA exposure was also observed (p-trend <0.0001). Associations with lupus and Crohn's disease were non-significant and inconsistent in the direction of effect {Steenland, 2013, 1937218}.

Evidence from a case-control study suggested lower PFOA concentrations among healthy controls compared to those with MS {Ammitzbøll, 2019, 5080379}. Serum PFOA concentrations were 12% lower (95% CI: -24%, 2%; p = 0.099) in healthy controls compared to cases of relapsing remitting MS and clinically isolated MS. Restricting the analysis to men, serum PFOA levels were 28% lower (95% CI: -42%, -9%; p = 0.006) in healthy controls compared to cases, but this effect was not seen in women. Steenland, 2018, 5079806 detected significantly increased levels of PFOA in ulcerative colitis cases versus those with Crohn's disease or controls and observed statistically significantly increased odds of ulcerative colitis with increased PFOA exposure among combined children and adults; however, the trend was not consistent across increasing quintiles of PFOA exposure, with a peak in the third quintile. The risk of celiac disease was elevated among children and young adults (≤ 21 years old) in a case-control study {Gaylord, 2020, 6833754}, particularly in females (p <0.05), but the association did not reach significance among the whole population.

3.3.4.2 Animal Evidence

There are 4 studies from the most recent literature search conducted in 2020 and 5 key studies from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and immune effects. Study quality evaluations for these 9 studies are shown in Figure 64.

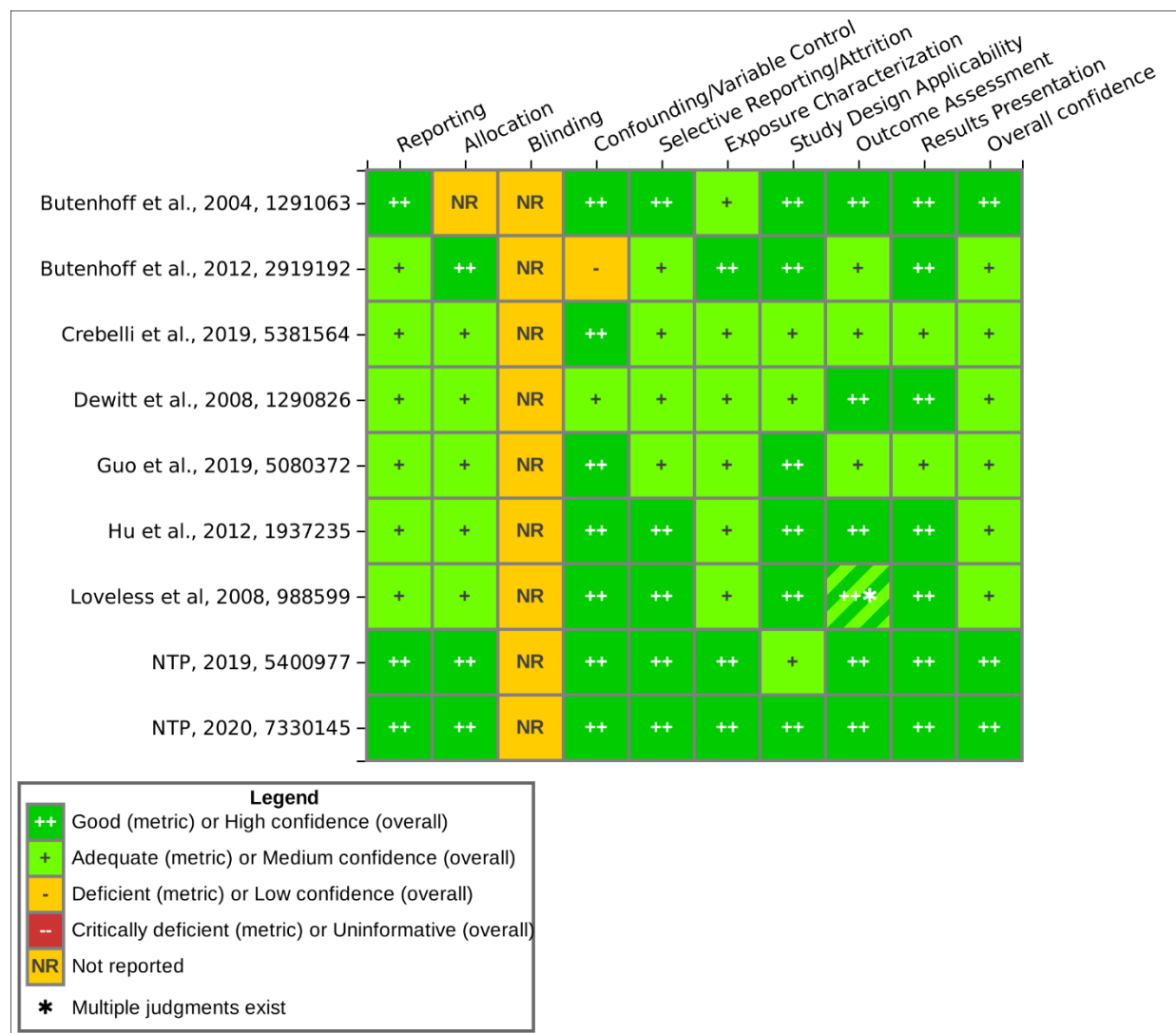


Figure 64. Summary of Study Evaluation for Toxicology Studies of PFOA and Immune Effects

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

The data available on immunological responses of animals following oral exposure to PFOA are extensive, especially as they apply to mice. A number of studies reported effects on spleen and thymus weights, immune system cellular composition, and the ability to generate an immune response following doses ranging from approximately 1–40 mg/kg/day.

3.3.4.2.1 Organ Weight/Histopathology

Short term exposure studies by Yang et al. (2000, 699394; 2001, 1014748) and Qazi et al. (2009, 1937259) using male C57BL/6 mice and by DeWitt et al. (2008, 1290826) using female C57BL/6 mice were conducted using relatively high PFOA doses (up to approximately 40 mg/kg/day). In each study, the PFOA-treated C57BL/6 mice exhibited significant reductions in spleen and thymus weights after 5–16 days of exposure. Because PFOA causes a decrease in body weight of treated animals, relative organ weights were also reported in two studies. Yang et al. (2000, 699394) and DeWitt et al. (2008, 1290826) observed an approximate 80% reduction in absolute and relative thymus weight and a 30–48% reduction in absolute and relative spleen weight. In male CD-1 mice exposed for 29 days via gavage to 1, 10, or 30 mg/kg/day PFOA, absolute and relative spleen weights were reduced to 89, 56, and 44% of controls, respectively. Absolute and relative thymus weights were decreased to 50% of controls in the 10 and 30 mg/kg/day groups {Loveless, 2008, 988599}. Spleen and thymus weights were only reduced by up to 9% (not statistically significant) in male ICR mice administered 47.21 mg/kg/day PFOA in drinking water for 21 days {Son, 2009, 1290821}. Male Sprague Dawley rats administered 2.5–10 mg/kg/day for 28 days displayed significantly lower absolute spleen weights that reached 75% of control at the highest dose. Absolute thymus weight was decreased to 74% of control in males administered 10 mg/kg/day compared to those of the vehicle group. Although females were assessed in the study, female spleen and thymus weights were not reported {NTP, 2019, 5400977}.

To investigate the possible involvement of PPAR α in the immunomodulation exerted by PFOA, C57BL/6Tac PPAR α knock-out mice and C57BL/6Tac wild-type mice were exposed to PFOA in drinking water for 14 days. Neither spleen nor thymus weights were affected in the knockout mice, whereas in wild-type mice, relative spleen weights were significantly reduced by 30% after exposure to 30 mg/kg/day and relative thymus weights were significantly reduced by 55.4% after exposure to 7.5 mg/kg/day {DeWitt, 2016, 2851016}, suggesting a role of PPAR α in PFOA-induced toxicity. Similarly, absolute spleen weights of Sv/129 PPAR α -null mice fed approximately 40 mg/kg/day for 7 days were unaffected by PFOA exposure. A significant decrease in absolute thymus weight was observed in PPAR α -null mice, that was not as dramatic as the reduction observed in PFOA-exposed wild-type mice (39% reduction in PPAR α -null mice and 79% reduction in wild-type mice), supporting a role of PPAR α in PFOA-induced toxicity {Yang, 2002, 1332453}.

A reduction in spleen and thymus weights have also been reported following developmental PFOA exposure. NTP (2020, 7330145) exposed pregnant rats administered PFOA beginning on GD6 and exposure was continued in offspring postweaning for a total of 107 weeks. Dose groups for this report are referred to as “[perinatal exposure level (ppm)]/[postweaning exposure level (ppm)]” (see further study design details in Section 3.3.1.2.1.2). Following perinatal and postweaning PFOS exposure (150/150 and 300/300 ppm), a significant reduction in absolute and relative spleen weight, and absolute thymus weight were observed at 16 weeks in male mice. Reduced absolute and relative spleen weights were also observed in mice following 300/20, 300/40, and 300/80 ppm PFOS exposure. Postweaning exposure alone (0/20, 0/40, 0/150, and 0/300 ppm) significantly reduced absolute and relative spleen weights. Absolute thymus weight was reduced following 0/150 and 0/300 ppm {NTP, 2020, 7330145}.

Two studies describing effects of subchronic and chronic PFOA exposure in adult mice did not report reduced spleen weight, and thymus weights were not examined. No changes to spleen weights were observed in C57BL/6 male mice administered 5 mg/kg/day and lower PFOA for 5 weeks {Crebelli, 2019, 5381564}. Similarly, spleen weight was not affected in male Sprague-Dawley rats chronically exposed to 30 or 300 ppm (1.3 or 14.2 mg/kg/day) for 1 or 2 years. An increase in absolute and relative spleen weight (40 and 30% increase, respectively) was observed only in female rats exposed to 30 ppm (1.6 mg/kg/day) for 2 years {Butenhoff, 2012, 2919192}.

3.3.4.2.2 Histopathology

A number of studies reported on histological evaluations of the spleen and thymus from rodents orally administered PFOA at varying doses and durations. In male Crl:CD-1 (ICR)BR mice administered PFOA for 29 days, decreased spleen weights at 10 and 30 mg/kg/day correlated with the gross observation of small spleens {Loveless, 2008, 988599}. The decreased thymus weights at these doses correlated with the microscopic finding of lymphoid depletion and with the gross observation of small thymuses {Loveless, 2008, 988599}.

Histological assessment of Sprague-Dawley rats exposed to PFOA for 28 days, revealed atrophy of the thymus that was characterized by decreased thickness of the thymic cortex, although the dose and severity of these observations were not specified {NTP, 2019, 5400977}. These findings were complemented by Son et al. (2009, 1290821), in the histological evaluation of male ICR mice administered PFOA (0–47.21 mg/kg/day) for 21 days. The thymus of mice exposed to 47.21 mg/kg/day PFOA revealed atrophy with decreased thickness of the cortex and medulla compared to control, but increased cellular density of lymphoid cells in the cortex was observed {Son et al. 2009; 1290821}. The authors also reported an enlargement of the spleen with marked hyperplasia of the white pulp in the 47.21 mg/kg/day PFOA-treated group, and an increased area of the lymphoid follicles in the spleen with increased cellular density {Son, 2009, 1290821}.

Loveless et al. (2008, 988599) observed increased extramedullary hematopoiesis in the spleen of male Crl:CD(SD)IGS BR rats and Crl:CD-1 (ICR)BR mice exposed to 30 mg/kg/day PFOA for 29 days. However, splenic hematopoiesis was not affected in male or female Sprague-Dawley rats administered 0 – 10 or 0 – 50 mg/kg/day PFOA, respectively {NTP, 2019, 5400977}.

Histological evaluation of the spleen and thymus following chronic PFOA exposure was only reported in 1 study, which administered 30 or 300 ppm PFOA to male and female Sprague-Dawley rats for 2 years. Hemosiderin, an iron rich pigment, was found in greater amounts in the spleens of males and females dosed with 300 ppm (approximately 15 mg/kg/day) but was reduced in the 30 ppm group (approximately 1.5 mg/kg/day) {Butenhoff, 2012, 2919192}.

3.3.4.2.3 Immune Cellularity

3.3.4.2.3.1 White Blood Cells and Differentials

Evidence supporting an effect of PFOA exposure on immune system-associated cellularity has been reported. A decrease in total serum white blood cells to 28% of control was observed in male C57BL/6 (H-2^b) mice fed 40 mg/kg/day for 10 days {Qazi, 2009, 1937259}. Total number of circulating neutrophils and lymphocytes (T and B cells) were decreased to 50 and 27% of control, respectively. The number of circulating monocytes, eosinophils and basophils were too small to be determined reliably, according to the study {Qazi, 2009, 1937259}.

In a similar study, male Crl:CD-1(ICR)BR mice were exposed to PFOA (10 and 30 mg/kg/day) by oral gavage for 29 days. At both doses tested, increases in total serum neutrophils and monocytes (reaching 296 and 285% of control, respectively, at the highest dose), and a decrease in total number of eosinophils (approximately 60% of control, data not statistically significant) were observed {Loveless, 2008, 988599}. Loveless et al. (2008, 988599) also reported a decrease in lymphocytes in male mice dosed with 30 mg/kg/day, but this data was not shown.

In male and female Sprague-Dawley rats chronically exposed to 30 or 300 ppm PFOA (approximately 1.5 or 15 mg/kg/day) for 2 years, PFOA did not affect total white blood cell count, blood lymphocytes, or neutrophils levels {Butenhoff, 2012, 2919192}. However, absolute mean leukocyte counts were increased in both male groups through the first year of the study. The authors suggest that these changes were due to increases in absolute counts of lymphocytes at 3 and 6 months and in neutrophils at 12 months {Butenhoff, 2012, 2919192}.

3.3.4.2.3.2 Spleen, Thymus, Lymph Nodes, and Bone Marrow Cellularity

Short-term PFOA exposure (10–40 mg/kg/day) significantly decreased splenocyte and thymocyte cell populations to approximately 30 and 15% of control, respectively, in male Crl:CD-1 (ICR)BR mice {Loveless, 2008, 988599} and male C57BL/6 mice {Yang, 2001, 1014748}. Similarly, in male C57BL/6 mice administered 40 mg/kg/day PFOA for 7 days, the number of thymocytes was decreased to 14% of control; immature thymocyte populations (CD4⁺CD8⁺) were the most affected {Yang, 2000, 699394}. In the spleen, both B and T cells were significantly reduced in these mice, and the number of total splenocytes was decreased to 20% of control {Yang, 2000, 699394}. Reduced splenocyte and thymocyte CD4⁺CD8⁺ cells were also observed in male ICR mice administered PFOA (0, 0.49, 2.64, 17.63, and 47.21 mg/kg/day) in drinking water for 21 days, reflecting an impairment in cell maturation {Son, 2009, 1290821}.

In Sprague-Dawley rats exposed to PFOA for 28 days, decreased number of lymphocytes within the thymus, spleen, and lymph nodes were observed, although the dose and severity were not specified {NTP, 2019, 5400977}. In the same study, a significant increase in bone marrow hypocellularity of minimal to mild severity was reported in male (6/10 compared to 1/10 in controls), but not female rats exposed to 10 mg/kg/day {NTP, 2019, 5400977}.

Developmental PFOA exposure may also impact cellularity of the spleen. In one study by Hu et al. (2012, 1937235), a 22% reduction in splenic regulatory T cells (CD4⁺CD25⁺Foxp3⁺ T) was observed in PND42 offspring from C57BL/6N dams exposed to 2 mg/kg/day PFOA from gestation through lactation. Thymic cellularity was not examined in this study {Hu, 2012, 1937235}.

3.3.4.2.4 Ability to Generate an Immune Response

The ability to generate an immune response following PFOA has been investigated in rodent models. Male Crl:CD-1 (ICR)BR mice were exposed to PFOA (0, 0.3, 1, 10, or 30 mg/kg/day) by oral gavage for 29 days and received an injection of serum sheep red blood cells (SRBC) on day 24. The induced immunoglobulin M (IgM) response was significantly reduced in mice exposed to 10 and 30 mg/kg/day to 80 and 72% of controls, respectively (Loveless et al. 2008; 988599). In a separate study, the ability to respond to an immunological challenge was reduced in female C57BL/6N mice exposed to 3.75 to 30 mg/kg/day PFOA in drinking water for 15 days.

The mice showed a dose-dependent reduction in IgM levels (between 11 and 29% decrease) after injection with SRBC to induce an immune response {DeWitt, 2008, 1290826}. The IgG response to SRBC significantly increased by approximately 13% following 3.75 and 7.5 mg/kg/day PFOA exposure, but no change was observed at higher doses {DeWitt, 2008, 1290826}. Similarly, male C57BL/6 mice were fed approximately 40 mg/kg/day PFOA for 10 days and then evaluated for their immune response to horse red blood cells. PFOA exposed mice were unable to induce an increase in plaque-forming cells in response to the immune challenge, compared to control mice, suggesting a suppression of the humoral immune response {Yang et al 2002b; 1332454}.

To investigate the role of PPAR α in the PFOA-induced immune response, female C57BL/6Tac PPAR α knock-out mice and C57BL/6Tac wild-type mice were exposed to 0, 7.5, or 30 mg/kg/day PFOA in drinking water for 14 days and then injected with SRBC on day 11. Exposure to 30 mg/kg/day PFOA for 15 days reduced SRBC-specific IgM antibody responses in both wild-type and PPAR α knock-out mice by 16 and 14%, respectively. There was no significant difference between genotypes, suggesting that PPAR α may not be responsible for the suppression of the immune system induced by PFOA exposure {DeWitt, 2016; 2851016}.

Alterations in the serum levels of globulin can be associated with decreases in antibody production {FDA, 2002, 88170}. PFOA exposure at 12.5 mg/kg/day and up to 100 mg/kg/day for 28 days decreased globulin concentrations in female Sprague-Dawley rats by up to 70% of control. In males, a decrease in globulin concentrations was observed at 0.625 mg/kg/day (74% of control) and up to 10 mg/kg/day (60% of control), highlighting greater PFOA tolerance in females compared to males (Figure 65) (NTP, 2019, 5400977). In contrast, an increase in globulin concentrations, by approximately 7%, was observed in male BALB/c mice exposed to 0.4 or 2 mg/kg/day PFOA (but not 10 mg/kg/day) for 4 weeks (Figure 65) {Guo, 2019, 5080372}.

Globulin levels were also decreased in pregnant ICR dams on GD18 following 5 or 10 mg/kg/day PFOA from GD0–18 {Yahia, 2010, 1332451}. Globulin levels were decreased to 78 and 68 % of control, respectively. Globulin levels in offspring were not measured. In a developmental study conducted by NTP (2020, 7330145), Sprague-Dawley rats were exposed perinatally and/or postweaning for a total of 107 weeks to varying doses of PFOA ([perinatal exposure level (ppm)]/[postweaning exposure level (ppm)]); see further study design details in Section 3.3.1.2.1.2). In male Sprague-Dawley rats at the 16-week interim timepoint, perinatal exposure to 300 ppm (300/0) and/or postweaning exposure to doses ranging from 20–300 ppm (0/150, 0/300, 150/150, 300/300, 0/20, 0/40, 0/80, 300/20, 300/40, 300/80) significantly decreased globulin levels. Female rats displayed decreased globulin levels following 0/300, 0/1,000, 150/300, 300/1,000 PFOA {NTP, 2020, 7330145} (Figure 65).

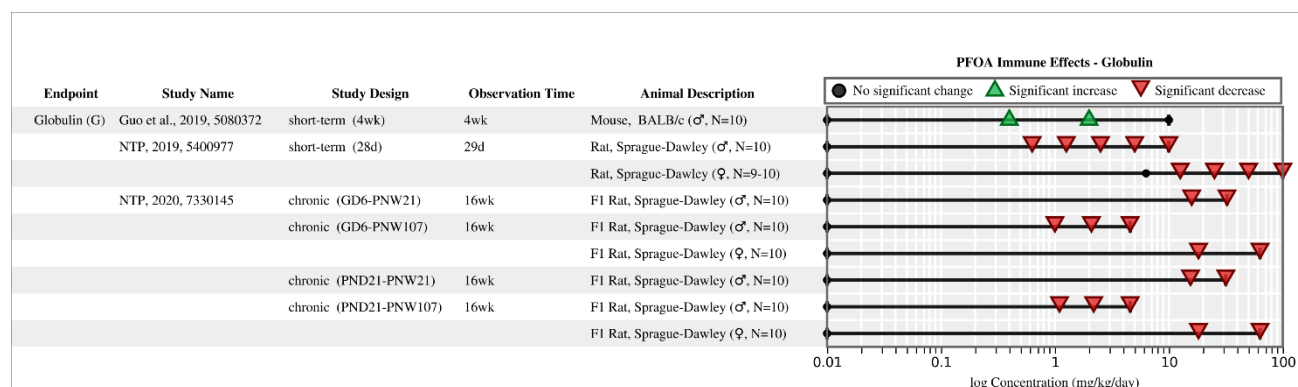


Figure 65. Globulin levels in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA 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; PNW = postnatal week; F1 = first generation; d = day; wk = week.

3.3.4.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse immune outcomes is discussed in Sections 3.3.2 and 3.4.1 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 23 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to immune effects. A summary of these studies is shown in Figure 66. Additional analysis on the mechanistic actions of PFOA on immune health outcomes is ongoing 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	3	0	3	6
Cell Signaling Or Signal Transduction	2	0	1	3
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	1	2
Inflammation And Immune Response	11	6	6	21
Oxidative Stress	1	0	2	3
Not Specified (Review Article)	1	0	0	1
Grand Total	12	6	8	23

Figure 66. Summary of Mechanistic Studies of PFOA and Immune Effects

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

3.3.4.4 Evidence Integration

Results from human epidemiological studies are most consistent for antibody response to vaccination in children, and multiple medium confidence studies report a positive association for

this outcome. The evidence of an association between PFOA exposure and immunosuppressive effects in human studies is moderate based on largely consistent decreases in antibody response following vaccination (against two different infectious agents: tetanus and diphtheria) in two medium confidence, overlapping birth cohorts. Reduced antibody response is an indication of immunosuppression and may result in increased susceptibility to infectious disease. The antibody results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFOA were associated with suppression of at least one measure of the anti-vaccine antibody response to common vaccines in two well-conducted (though overlapping) birth cohorts in the Faroe Islands, supported by a low confidence study in adults. Thus, antibody response to vaccination in children was considered for POD derivation.

Overall, associations in human epidemiological studies measuring PFOA concentrations and hypersensitivity were mixed. Studies that observed significant associations between PFOA and “ever” or “current” asthma were seen primarily in sex- or age-specific subgroups but were null or insignificant in whole study analyses. For allergy and eczema outcomes, results were inconsistent across studies.

The associations between PFOA exposure and human autoimmune disease were also mixed. Two studies {Steenland, 2013, 1937218; Steenland, 2018, 5079806} found significant associations indicating increased risk of autoimmune disease. PFOA levels were also lower in healthy controls compared to cases with MS {Ammitzbøll, 2019, 5080379}. Results were most consistent for ulcerative colitis, with significant associations indicating increased risk with increasing PFOA exposure in one *medium* confidence study {Steenland, 2013, 1937218} and one *low* confidence study {Steenland, 2018, 5079806}. Given the mixed results for hypersensitivity and autoimmune disease, these outcomes were not considered for derivation of PODs.

Evidence from available animal studies support the immune system as a target of PFOA toxicity. Short-term and developmental PFOA exposure in rodents resulted in reduced spleen and thymus weights, altered immune cell populations, and decreased splenic and thymic cellularity. In functional assessment of the immune response, PFOA exposure was associated with reduced globulin and immunoglobulin levels {Dewitt et al., 2008, 1290826; Loveless et al., 2008, 988599}. Suppression of the immunoglobulin response in these animals is consistent with decreased antibody response seen in human subpopulations. As such, EPA concluded the impaired IgM response reported in Dewitt et al. (2008, 1290826) and Loveless et al. (2008, 988599) supported the human results and this endpoint was considered for POD derivation.

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, 3982042} and HESD {U.S. EPA, 2016, 3603279} assessments did not identify strong evidence for an association between CVD and PFOA, based on five occupational studies. Several occupational studies examined cardiovascular-related cause of death among PFOA-exposed workers at the West Virginia Washington Works plant {Leonard, 2008, 1291100; Sakr, 2009, 2593135; Steenland and Woskie, 2012, 2919168} and the 3M Cottage Grove plant in Minnesota {Lundin, 2009, 1291108; Gilliland, 1993, 1290858}. This type of mortality is of interest because of the relation between lipid profiles (e.g., LDL) and the risk of CVD. A study in West Virginia did not find an association between cumulative PFOA levels and IHD mortality across four quartiles of cumulative exposure {Steenland, 2012, 2919168}. Based on these data from the worker cohorts, as part of the C8 Health Project, the C8 Science Panel (2012, 1430770) concluded that there is no probable link between PFOA and stroke and CAD. The analysis of the workers at the Minnesota plant also found no association between cumulative PFOA exposure and IHD risk, but an increased risk of cerebrovascular disease mortality was seen in the highest exposure category {Lundin, 2009, 1291108}. These studies are limited by the reliance on mortality (rather than incidence) data, which can result in a substantial degree of under ascertainment and misclassification.

For this updated review, 35 new epidemiological studies report on the association between PFOA and CVD, including outcomes such as hypertension, CAD, congestive heart failure (CHF), microvascular diseases, and mortality. Of these, 15 examined blood pressure or hypertension in adults. Pregnancy-related hypertension is discussed in Section 3.3.2.1.2. Two of the publications {Girardi, 2019, 6315730; Steenland, 2015, 2851015} were occupational studies and the remainder 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}, nine cohort studies {Fry, 2017, 4181820; Donat-Vargas, 2019, 5080588; Girardi, 2019, 6315730; Lin, 2020, 6311641; Manzano-Salgado, 2017, 4238509; Matilla-Santander, 2017, 4238432; Mitro, 2020, 6833625; Steenland, 2015, 2851015; Warembourg, 2019, 5881345}, one case-control study {Mattsson, 2015, 3859607}, and 23 cross-sectional studies {Bao, 2017, 3860099; Chen, 2019, 5387400; Christensen, 2016, 3858533; Christensen, 2019, 5080398; Graber, 2019, 5080653; He, 2018, 4238388; 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; Shankar, 2012, 2919176; 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 (DPP) and Outcomes Study (DPPOS) {Cardenas, 2019, 5381549} and weight loss in Prevention of Obesity Using Novel Dietary Strategies Lost (POUNDS-Lost) Study {Liu, 2018, 4238396}. Thus, these studies can 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; He, 2018, 4238388; 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, 4238514; Liu, 2018, 4238396; Ma, 2019, 5413104; Mi, 2020, 6833736; Mitro, 2020, 6833625; Shankar, 2012, 2919176; Steenland, 2015, 2851015 }. 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; Mobake, 2018, 4354163 }, Italy { Girardi, 2019, 6315730 }, and a single study conducted in several European countries { Warembourg, 2019, 5881345 }. All the studies measured PFOA 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 35 studies identified since the 2016 assessment (Figure 67, Figure 68), three studies were *high* confidence, 18 were *medium* confidence, 12 were considered *low* confidence, and two studies included an outcome considered *uninformative* { Jain, 2020, 6833623; Jain, 2020, 6311650 }. The main concerns with the *low* confidence studies included the possibility of outcome misclassification (e.g., reliance on self-reporting) in addition to 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 SES, which can be associated with both exposure and the cardiovascular outcome. Although PFOA has a long half-life in the blood, concurrent measurements may not be appropriate for cardiovascular effects with long latencies. Further, temporality of PFOA 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 PFOA levels were compared by cardiovascular disease and other characteristics (e.g., kidney function) with PFOA levels considered as the dependent variable in analysis, and there were concerns for residual confounding.

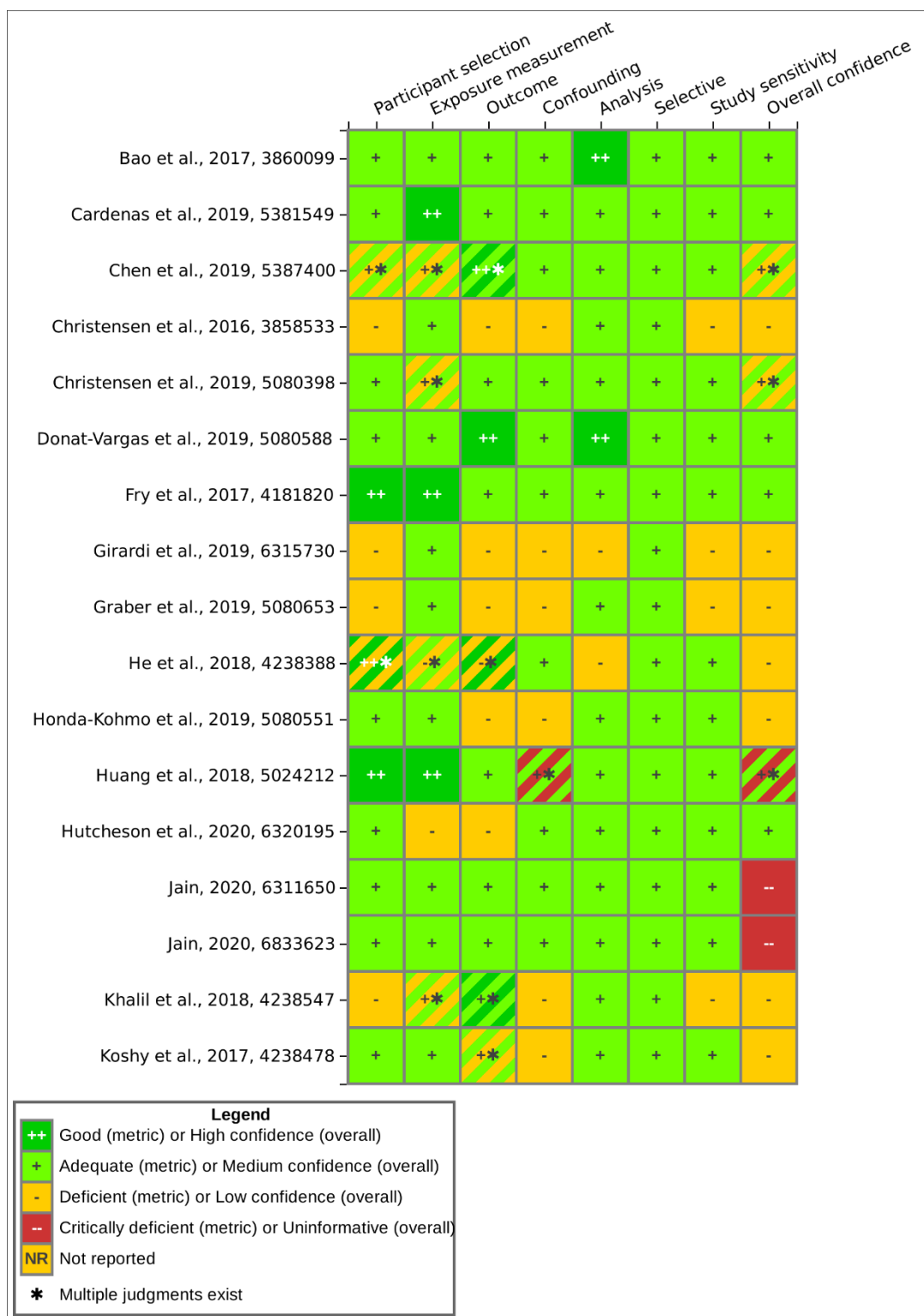


Figure 67. Summary of Study Evaluation for Epidemiology Studies of PFOA and Cardiovascular Effects

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

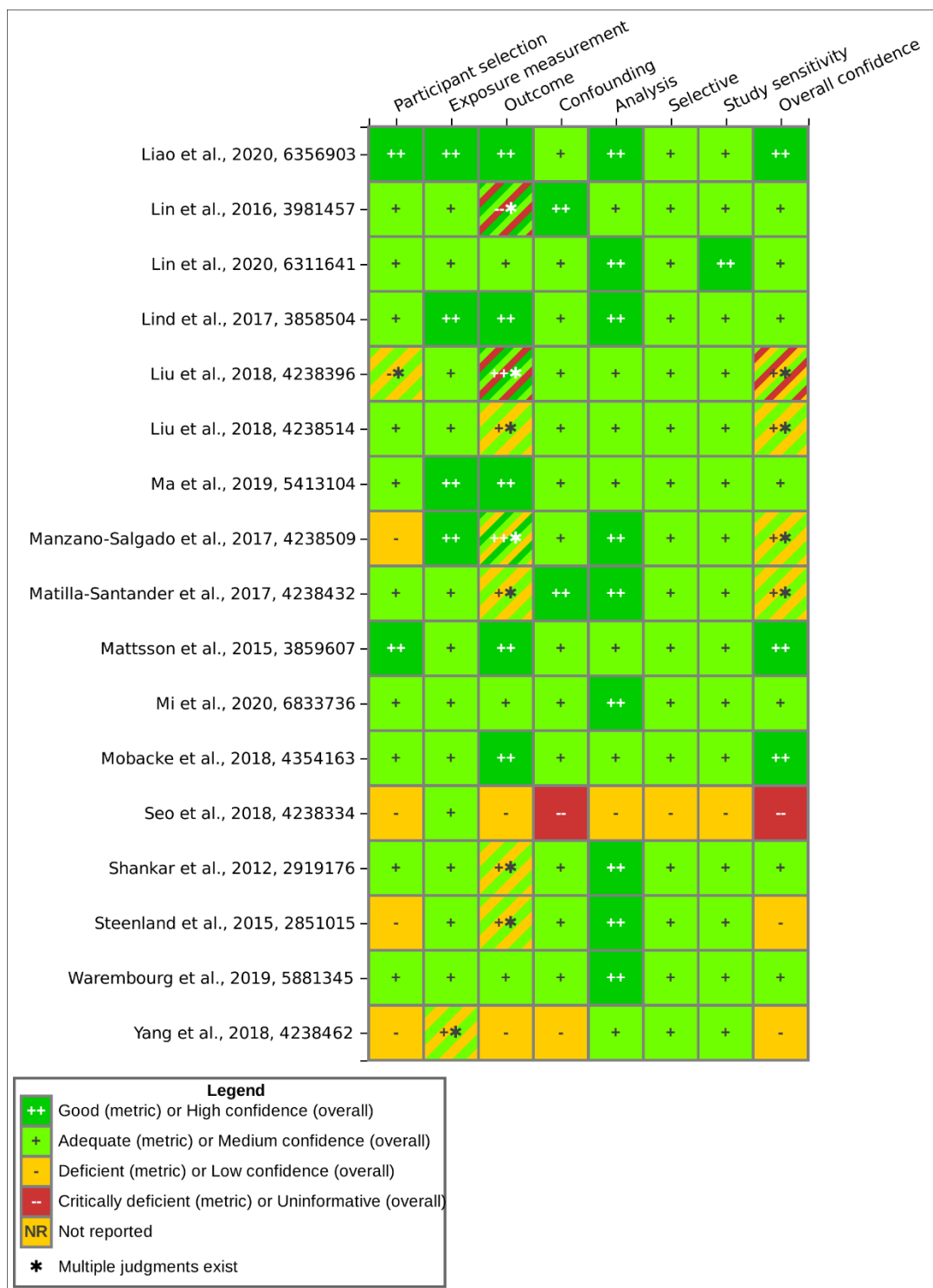


Figure 68. Summary of Study Evaluation for Epidemiology Studies of PFOA and Cardiovascular Effects (Continued)

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

3.3.5.1.1.3 Findings from Children and Adolescents

Three *medium* confidence studies examined blood pressure in children and adolescents {Ma, 2019, 5413104; Manzano-Salgado, 2017, 4238509; Warembourg, 2019, 5881345} and reported no associations (Table C-13). In a cross-sectional analysis, Ma et al., (2019, 5413104) did not observe an association between serum PFOA and blood pressure among 2,251 (NHANES (2003–2012)) participants (mean age 15.5 years). Similarly, Manzano-Salgado et al., (2017, 4238509) did not observe an association between maternal PFOA and 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) PFOA 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 {Warembourg, 2019, 5881345}. However, the association was significantly positive for SBP after co-adjustment for organochlorine compounds (i.e., dichlorodiphenyldichloroethane (DDE) and hexachlorobenzene (0.9; 95% CI: 0.1, 1.6; $p = 0.021$)).

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

Other cardiovascular conditions reported in children and adolescents include carotid intima-media thickness test (CIMT) and brachial artery distensibility. Two *medium* confidence studies that examined CIMT among adolescents and young adults from the Young Taiwanese Cohort Study {Lin, 2013, 2850967; Lin, 2016, 3981457} reported no associations. A *low* confidence study of children and adolescents from the World Trade Center (WTC) Health Registry reported PFOA was significantly associated with increased brachial artery distensibility (0.45; 95% CI: 0.04, 0.87; $p = 0.03$), but was not associated with pulse wave velocity {Koshy, 2017, 4238478}. However, concerns for residual confounding by age and SES contributed to the *low* confidence.

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 13 studies examined PFOA 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; He, 2018, 4238388; Mitro, 2020, 6833625; Liao, 2020, 6356903; Lin, 2020, 6311641; Liu, 2018, 4238514; Liu, 2018, 4238396; Mi, 2020, 6833736; Yang, 2018, 4238462}.

Of the nine studies that examined blood pressure as a continuous measure, six reported statistically significant positive associations {Liao, 2020, 6356903; Mi, 2020, 6833736; Bao, 2017, 3860099; Lin, 2020, 6311641; Liu, 2018, 4238396; Yang, 2018, 4238462}. However, the results were not always consistent between SBP and DBP.

A *high* confidence study in 6,967 National Health and Nutrition Examination Survey (NHANES) (2003–2012) participants 20 years and older reported a statistically significant positive association with SBP (per 10-fold change in PFOA: 1.83; 95% CI: 0.40, 3.25) in the fully adjusted model {Liao, 2020, 6356903}. No association was observed for DBP.

A *high* confidence study {Mitro, 2020, 6833625} conducted among 761 women that examined associations between PFOA concentrations measured during pregnancy and blood pressure assessed at 3 years post-partum reported a positive but non-significant association with SBP (beta per doubling of PFOA: 0.8 (95% CI: –0.3, 1.8). 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 1,612 participants with very high PFOA levels (median 6.19 ng/mL) Bao et al. (2017, 3860099) reported large increases in DBP (2.18; 95% CI: 1.38, 2.98) and SBP (1.69; 95% CI: 0.25, 3.13). After stratification by sex, a positive association was observed in men only for DBP (1.48; 95% CI: 0.58, 2.37) and in women only for SBP (6.65; 95% CI: 4.32, 8.99). In participants with high PFOA levels (median 4.8 ng/mL) Mi et al. (2020, 6833736) observed statistically significant increases in DBP (1.49; 95% CI: 0.34, 2.64). No association was observed for SBP.

Lin, 2020, 6311641, using data from the Diabetes Prevention Program, a randomized controlled health intervention trial, reported that an increase in baseline PFOA concentrations was significantly associated with higher SBP (1.49; 95% CI: 0.29, 2.70); no association was observed with DBP or pulse pressure. In a weight loss-controlled trial population (the POUNDS Lost Study) Liu, 2018, 4238396 observed that baseline PFOA was positively correlated with DBP ($p < 0.05$), but at 6- and 24-month follow-up assessments no associations were observed with SBP or DBP {Liu, 2018, 4238396}.

The findings from three *low* confidence studies {Chen, 2019, 5387400; He, 2018, 4238388; Yang, 2018, 4238462} of PFOA and blood pressure were mixed. Yang et al. (2018, 4238462) reported a statistically significant positive increased risk of high SBP (≥ 140 mmHg) for n-PFOA, but no association for SBP as a continuous measure. Two additional studies reported no associations for SBP {Chen, 2019, 5387400; He, 2018, 4238388}, and three studies reported no associations for DBP {Chen, 2019, 5387400; He, 2018, 4238388; Yang, 2018, 4238462}.

Of the eight studies that examined risk of elevated blood pressure (hypertension), four reported statistically significant associations {Liao, 2020, 6356903; Mi, 2020, 6833736; Bao, 2017, 3860099; Lin, 2020, 6311641}. Hypertension was defined as average SBP > 140 mmHg and average DBP > 90 mmHg, or self-reported use of prescribed anti-hypertensive medication. Using a generalized additive model and restricted cubic splines, Liao et al. (2020, 6356903) reported a non-linear (J-shaped) relationship with hypertension, with the inflection point of PFOA at 1.80 ng/mL. Each 10-fold increase in PFOA was associated with a 44% decrease (OR: 0.56; 95% CI: 0.32, 0.99) in the risk of hypertension on the left side of the inflection point, and an 85% increase (OR: 1.85; 95% CI: 1.34, 2.54) on the right side of the inflection point. A significant association with hypertension was observed for the highest (> 4.4 ng/mL) vs. lowest (≤ 2.5 ng/mL) tertile (OR: 1.32; 95% CI: 1.13, 1.54), and the test for trend was significant ($p < 0.001$). Additionally, positive associations were observed among women (OR: 1.42; 95% CI: 1.12, 1.79) and in participants 60 years and older (OR: 1.32; 95% CI: 1.03, 1.68). The studies {Mi, 2020, 6833736; Bao, 2017, 3860099} with overlapping data on high exposed “Isomers of C8 Health Project” participants reported significant associations. Mi et al. (2020, 6833736) reported higher risk of hypertension overall (OR: 1.72; 95% CI: 1.27, 2.31) and among women (OR: 2.32; 95% CI: 1.38, 3.91), but not in men. Bao et al. (2017, 3860099) did not observe an association between Total-PFOA and hypertension. However, in isomer-specific analysis a natural-log unit (ng/mL) increase of 6-m-PFOA was significantly associated with higher risk of hypertension (OR: 1.24; 95% CI: 1.05, 1.47) among all participants, and among women (OR: 1.86; 95% CI: 1.25, 2.78). These results suggest branched PFOA isomers have a stronger association with increased risk of hypertension compared to linear isomers (n-PFOA). A *medium* confidence study, Lin et al.

(2020, 6311641) reported in a cross-sectional analysis, that the association with hypertension was not statistically significant, but was modified by sex. Among males, a doubling of baseline plasma PFOA was associated with a significantly higher risk of hypertension (RR: 1.27; 95% CI: 1.06, 1.53); no association with hypertension was observed among females. In a prospective analysis, among participants who did not have hypertension at baseline, there was no association with hypertension at the approximately 15 years of follow-up {Lin, 2020; 6311641}. In addition, three *medium* confidence studies {Donat-Vargas, 2019, 5080588; Christensen, 2019, 5080398; Liu 2018, 4238514} and a *low* confidence study {Christensen, 2016, 3858533} did not observe associations with hypertension.

Ten studies examined other CVD-related outcomes including CHD, CVD, stroke, carotid artery atherosclerosis, angina pectoris, C-reactive protein, CHF, peripheral artery disease (PAD), microvascular disease, CIMT, and mortality.

Among the four studies that examined CHD, the findings were mixed. A *high* confidence study {Mattson, 2015, 3859607}, a *medium* confidence study of 10,850 NHANES participants from the 1999-2014 {Huang, 2018, 5024212}, and a *low* confidence study {Christensen, 2016, 3858533} all reported no associations with CHD. A *low* confidence study from the C8 Health Project {Honda-Kohmo, 2019, 5080551} reported a significantly inverse association between PFOA 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.

Among the two NHANES-based studies that examined CVD, the findings were mixed. Using data from NHANES 1999–2000 and 2003–2004 cycles, Shankar et al. (2012, 2919176) reported significant associations with CVD. The analysis by PFOA quartiles reported significantly higher odds for the presence of CVD in the third (OR: 1.77; 95% CI: 1.04-3.02) and the highest (OR: 2.01; 95% CI: 1.12-3.60) quartiles compared to the lowest quartile, with a significant trend ($p = 0.01$). In contrast, using a larger dataset from NHANES 1999–2014 cycles, Huang et al. (2018, 5024212) did not observe an association with total CVD by quartiles of exposure, nor a positive trend.

Shankar et al. (2012, 2919176) also observed a significant association with PAD. The analysis by PFOA quartiles reported significantly higher odds for the presence of PAD (OR: 1.78; 95% CI: 1.03-3.08) in the highest compared to the lowest quartile, with a significant trend ($p = 0.04$).

Among the two studies that examined stroke, the findings were also mixed. A borderline positive association ($p = 0.045$) was observed by {Huang, 2018, 5024212}. In contrast, Hutcheson, 2020, 6320195 observed a significant inverse association of history of stroke in adults with and without diabetes participating in the C8 Health Project (OR: 0.90; 95% CI: 0.82-0.98, $p=0.02$). However, a borderline-significant inverse association was observed among non-diabetics (OR: 0.94; 95% CI: 0.88, 1.00; $p = 0.04$), but not among those with diabetes, although the interaction was not significant.

In addition, a *low* confidence study of adults and children did not observe an association between serum PFOA and self-reported cardiovascular conditions, including high blood pressure, CAD, and stroke {Graber, 2019, 5080653}. 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.

Huang et al. (2018, 5024212) also reported a significantly higher odds of heart attack for the third quartile (OR: 1.62; 95% CI: 1.04, 2.53) and second quartile (OR: 1.57; 95% CI: 1.06, 2.34), compared to the first quartile. No associations were observed with CHF and angina pectoris.

No associations with microvascular diseases (defined as the presence of nephropathy, retinopathy, or neuropathy) were observed {Cardenas, 2019, 5381549}.

Two studies examined changes in heart structure {Mobacke, 2018, 4354163} and carotid atherosclerosis {Lind, 2017, 3858504} in participants 70 years and older. Mobacke et al. (2018, 4354163) examined alterations of left ventricular geometry, a risk factor for CVD and reported that serum PFOA was significantly associated with a decrease in relative wall thickness (-0.12 ; 95% CI: -0.22 , -0.001 ; $p = 0.03$), but PFOA was not associated with left ventricular mass or left ventricular end diastolic diameter. Lind et al. (2017, 3858504) examined 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 and observed no associations.

No association between PFOA 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 Spanish Environment and Childhood (Infancia y Medio Ambiente, INMA) study {Matilla-Santander, 2017, 4238432}.

One *medium* confidence study examined mortality due to heart/cerebrovascular diseases in 1,043 NHANES (2003-2006) participants 60 years and older and observed no associations {Fry, 2017, 4181820}.

Overall, the findings from one *high* confidence study and several *medium* confidence studies conducted among the general population did not provide consistent evidence for an association between PFOA and SBP and DBP. The evidence for an association between PFOA and increased risk of hypertension/elevated blood pressure, overall and in gender-stratified analyses was inconsistent. Evidence for other CVD-related outcomes was more limited, and similarly inconsistent.

3.3.5.1.1.5 Findings from Occupational Studies

Two *low* confidence studies examined occupational PFOA exposure and cardiovascular effects (Table C-13). Steenland et al. (2015, 2851015) examined 1,881 workers with high serum PFOA levels (median 113 ng/mL) from a subset of two prior studies conducted by the C8 Science Panel. No trend was observed in the exposure-response gradient for stroke, CHD, and hypertension and. In analysis of PFOA levels by quartiles, a significantly higher risk of stroke (no lag) was observed for the 2nd quartile vs. the 1st quartile (Rate Ratio [RR]: 2.63; 95% CI: 1.06, 6.56). No association was observed with 10-year lag stroke, CHD, and hypertension, respectively. For the assessment of stroke, this study had *low* confidence because of concerns for selection bias, specifically survival bias. For other chronic diseases examined, this study is of *low* confidence due to concerns for outcome misclassification, particularly for hypertension due to lack of medical record validation. In another occupational study of 120 male workers with

very high PFOA serum levels (geometric mean (GM): 4,048 ng/mL), Girardi et al. (2019, 6315730) reported no association with increased risk of mortality due to cardiovascular causes, including hypertensive disease, ischemic heart disease, stroke, and circulatory diseases. However, the potential for selection bias, outcome misclassification, and limited control for confounding may have influenced the reported results.

Overall, the limited evidence available from occupational studies was inconsistent for an association with risk of stroke and indicated PFOA is not associated with an increased risk of CHD, hypertension, and mortality due to cardiovascular causes. However, the findings based on two *low* confidence studies should be interpreted with caution due to potential biases arising from the selection of participants and outcome misclassification.

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 LDL and high-density-lipoprotein cholesterol (HDL). Elevated levels of 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 PFOA, there was relatively consistent and robust evidence of positive associations between PFOA and TC and LDL in occupational and high-exposure community settings. Positive associations between PFOA and HDL were also observed in most studies in the general population.

For this updated review, 43 new epidemiologic studies (42 publications)¹¹ report on the association between PFOA exposure and serum lipids. 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 PFOA using biomarkers in blood, and measured serum lipids with standard clinical biochemistry methods. Serum lipids were frequently analyzed as continuous outcomes, but a few studies examined the prevalence or incidence of hypercholesterolemia, hypertriglyceridemia, and low HDL based on 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 assumed 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 triglyceride levels are sensitive to recent food intake {Mora, 2016, 9564968}, outcome measurement error is likely substantial when triglyceride 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

¹¹ Dong 2019, 5080195 counted as two studies, one in adolescents and one in adults.

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 PFOA and serum lipids concurrently was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOA (median half-life = 2.7 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 not yet clear evidence to support these reverse causal pathways.

Based on these considerations, 16 studies were classified *medium confidence* for all lipid outcomes, four studies were rated *medium confidence* for TC or HDL, but *low confidence* for triglycerides or LDL, 19 studies were rated *low confidence* for all lipid outcomes, and 4 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 69 and Figure 70. 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, 2020, 6315756; Fassler, 2019, 6315820; 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; Convertino, 2018, 5080324; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Olsen, 2012, 2919185; Lin, 2013, 2850967; Lin, 2020, 6315756; Fassler, 2019, 6315820; 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}, or selective reporting {Dong, 2019, 5080195, adolescent portion}.

The most common reason *w* confidence rating was potential for selection bias, including 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; Liu, 2018, 4238396}, potential for self-selection {Li, 2020, 6315681; Christensen, 2016, 3858533; Graber, 2019, 5080653; Rotander, 2015, 3859842}, highly unequal recruitment efforts in sampling frames with potentially different joint distributions of PFOA and lipids {Lin, 2013, 2850967}, and missing key information on the recruitment process {Khalil, 2018, 4238547; Fassler, 2019, 6315820; 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; Fassler, 2019, 6315820; 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 PFOA 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.

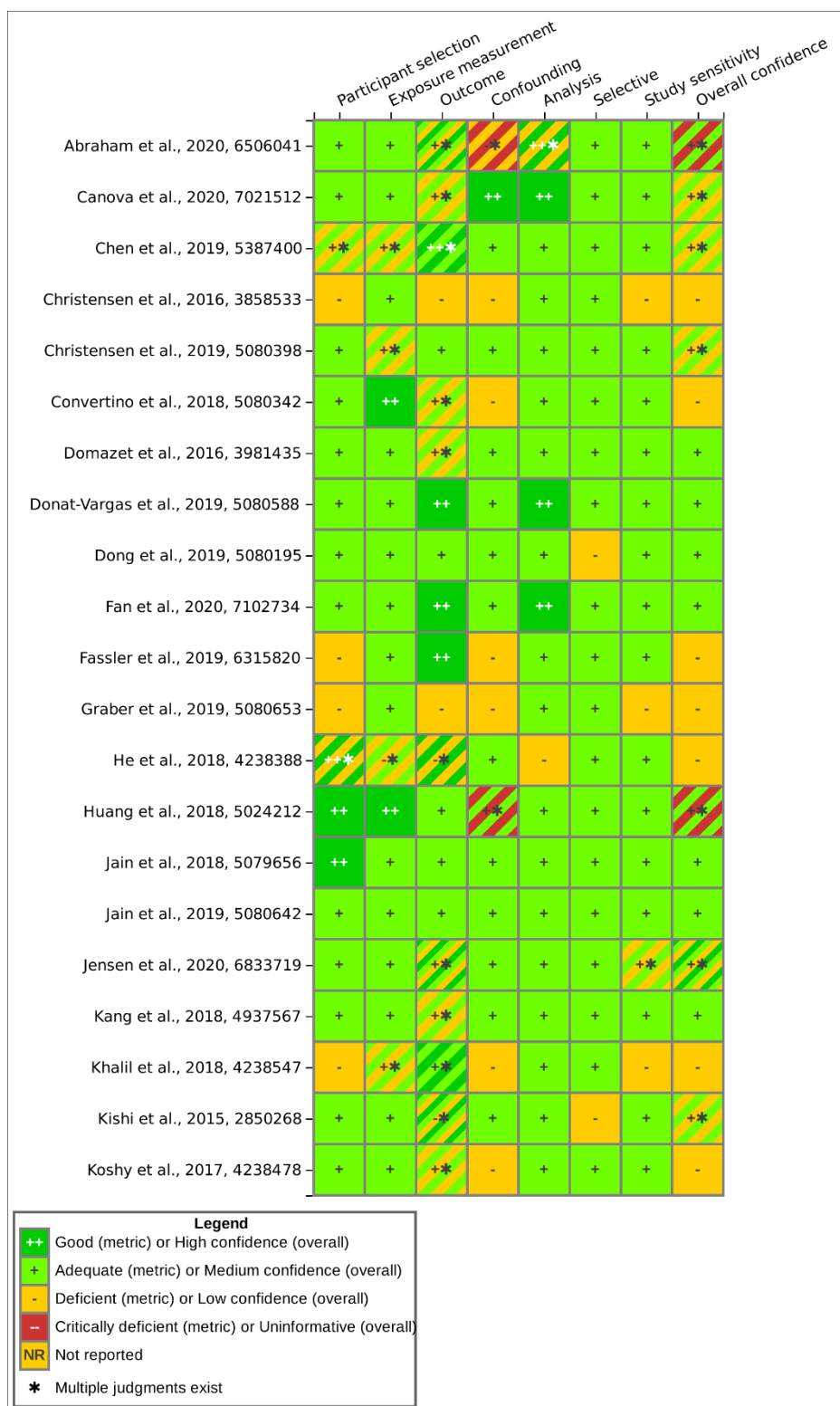


Figure 69. Summary of Study Evaluation for Epidemiology Studies of PFOA and Serum Lipids

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

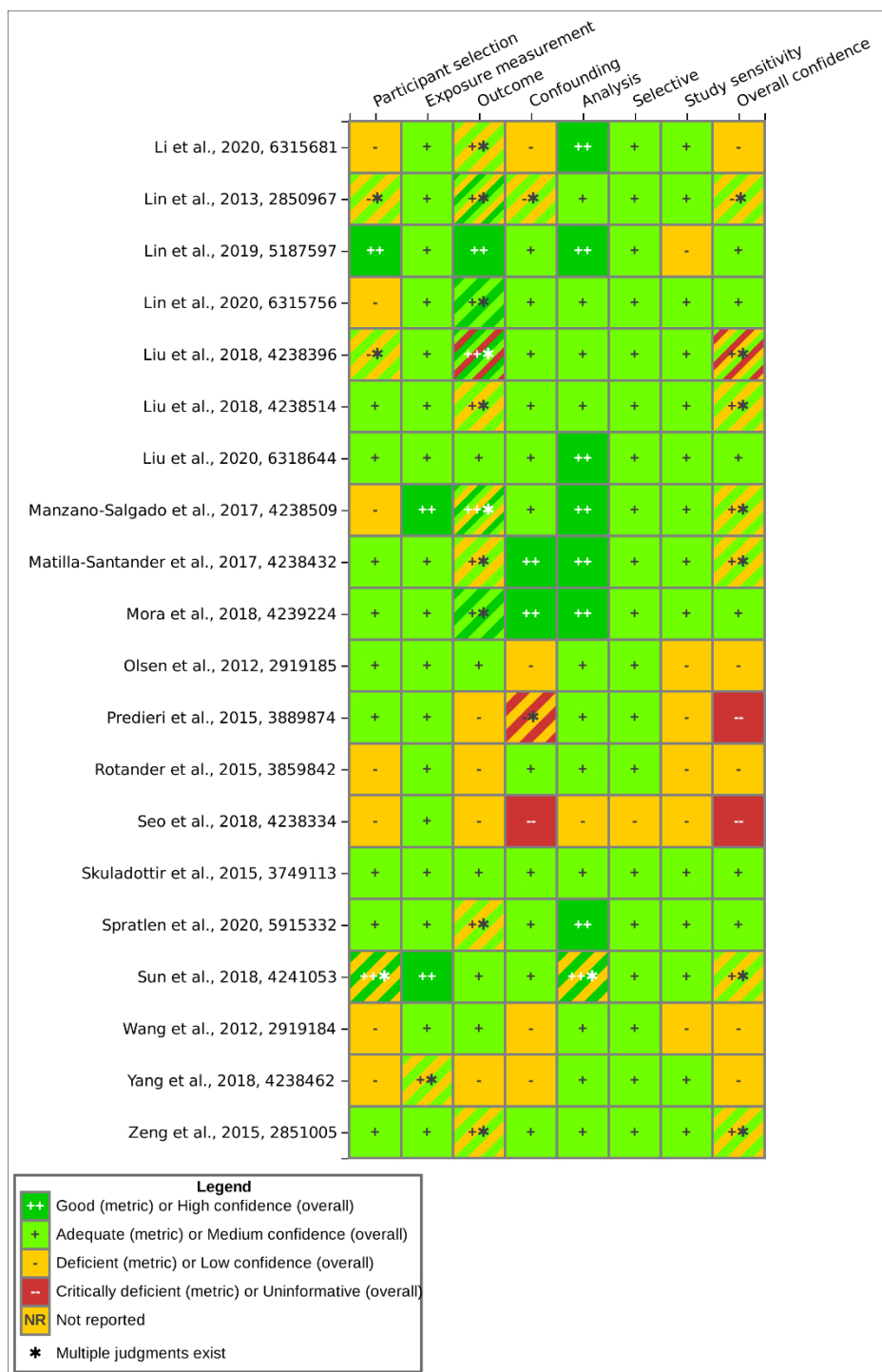


Figure 70. Summary of Study Evaluation for Epidemiology Studies of PFOA and Serum Lipids (Continued)

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 four *low* confidence studies examined the association between PFOA and TC in children. Of these, four studies examined the association between prenatal PFOA exposure and TC in childhood {Spratlen, 2020, 5915332; Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2017, 4239224} and eight examined the association between childhood PFOA exposure and concurrent TC {Mora, 2017, 4239224; Jain, 2018, 5079656; Zeng, 2015, 2851005; Kang, 2018, 4937567; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Fassler, 2019, 6315820; Dong, 2019, 5080195}. Positive associations between PFOA and TC were reported in five *medium* confidence studies {Zeng, 2015, 2851005, Spratlen, 2020, 5915332; Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2017, 4239224}, but the direction of association sometimes differed by age and sex {Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509}. Except in Zeng 2015, 2851005 and among girls in mid-childhood in Mora 2017, 4239224, none of the associations in *medium* confidence studies were statistically significant. In three out of four *low* confidence studies, PFOA was positively associated with TC {Khalil, 2018, 4238547; Koshy, 2017, 4238478; Fassler, 2019, 6315820}. However, residual confounding by SES may have positively biased these findings. Taken together, these studies suggest a positive association between PFOA and TC in children. However, the true association between PFOA and TC remains uncertain given the heterogeneity by age and sex and the imprecise findings in most *medium* confidence studies. Three *medium* confidence and five *low* confidence studies examined the association between PFOA and LDL in children. Of these, three examined prenatal exposure {Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2017, 4239224} and six examined childhood exposure {Mora, 2017, 4239224; Zeng, 2015, 2851005; Kang, 2018, 4937567; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Dong, 2019, 5080195, adolescent portion}. The *medium* studies generally reported small, positive associations between PFOA and LDL, but none of the associations were statistically significant (Table C-14) {Jensen, 2020, 6833719; Mora, 2017, 4239224; Kang, 2018, 4937567}. In one *medium* study, the association was inverse among 3-month-old infants and 18-month-old boys {Jensen, 2020, 6833719}. Most *low* confidence studies reported a positive association between PFOA and LDL {Khalil, 2018, 4238547; Koshy, 2017, 4238478; Zeng, 2015, 2851005; Manzano-Salgado, 2017, 4238509}, but 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 PFOA were observed in children, though less consistently.

Five *medium* confidence and four *low* confidence studies examined the association between PFOA and HDL in children. Of these, three examined prenatal exposure {Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2017, 4239224} and seven examined childhood exposure {Mora, 2017, 4239224; Jain, 2018, 5079656; Zeng, 2015, 2851005; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Fassler, 2019, 6315820; Dong, 2019, 5080195, adolescent portion}. Prenatal PFOA exposure was inversely associated with HDL, but most associations were not statistically significant {Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2017, 4239224} (Table C-14). Sex-stratified analyses showed that the inverse association occurred mainly in boys {Manzano-Salgado, 2017, 4238509; Mora, 2017, 4239224}. Results on childhood exposure were less consistent (Table C-14). One *medium* study reported a statistically significant, positive association between PFOA and HDL in mid-childhood {Mora, 2017, 4239224}, but another *medium* study reported an inverse, though statistically non-

significant association {Zeng, 2015, 2851005}. Most *low* confidence studies reported a positive association between childhood PFOA exposure and HDL {Khalil, 2018, 4238547; Koshy, 2017, 4238478; Fassler, 2019, 6315820}. In summary, PFOA was not consistently associated with lower HDL in children. Effect modification by exposure window may explain this inconsistency.

Five *medium* confidence and five *low* confidence studies examined the association between PFOA and triglycerides in children. Of these, four examined prenatal exposure {Spratlen, 2020, 5915332; Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2017, 4239224} and seven examined childhood exposure {Domazet, 2016, 3981435; Mora, 2017, 4239224; Zeng, 2015, 2851005; Kang, 2018, 4937567; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Fassler, 2019, 6315820}. PFOA was significantly associated with increased triglycerides in newborns in one *medium* study {Spratlen, 2020, 5915332} (Table C-14). Most other *medium* studies also reported positive associations, but they were not statistically significant {Jensen, 2020, 6833719; Mora, 2017, 4239224; Kang, 2018, 4937567}. In one *medium* study that examined the association between PFOA and triglycerides longitudinally, PFOA at age 9 years was associated with lower triglycerides at age 15 years and 21 years, while PFOA at age 15 years was associated with higher triglycerides at age 21 years {Domazet, 2016, 3981435}. None of the associations were statistically significant. In most *low* confidence studies, PFOA was positively associated with triglycerides {Manzano-Salgado, 2017, 4238509; Zeng, 2015, 2851005; Khalil, 2018, 4238547; Koshy, 2017, 4238478}, but the use of non-fasting samples and residual confounding by SES may have biased these results upwards. Overall, increased triglycerides with increasing PFOA were observed in children, but results were less consistent and not always statistically significant.

In summary, the association between PFOA and serum lipids in children remains inconclusive. For TC, LDL, and triglycerides, positive associations were generally observed, but few were statistically significant. Differences in the direction of association by age or sex further contributed to inconsistency in findings; it is difficult to determine if the differences were due to effect modification or random error. For HDL, prenatal exposure appeared to be associated with lower HDL, especially in boys, although childhood exposure was associated with higher HDL. Few findings were statistically significant, however, suggesting caution in interpreting these results.

3.3.5.1.2.4 Findings from Pregnant Women

Two *medium* confidence studies examined the association between PFOA and TC in pregnant women and reported significantly positive associations between PFOA and TC, suggesting a consistent relationship between PFOA and elevated TC (Table C-14) {Mattila-Santander, 2017, 4238432; Skuladottir, 2015, 3749113}.

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

One *medium* confidence and two *low* confidence studies examined the association between PFOA and triglycerides in pregnant women. The *medium* confidence study reported an inverse association between PFOA and triglycerides, but the association was small and not statistically significant {Starling, 2017, 3858473}. The *low* confidence studies each reported inverse {Mattila-Santander, 2017, 4238432} or positive associations {Kishi, 2015, 2850268} that were

not statistically significant. Both studies were limited by their use of non-fasting blood samples. Kishi et al. (2015, 2850268) additionally examined the association between PFOA and select fatty acids in serum. PFOA was not significantly associated with any fatty acids, but the associations were generally positive except for arachidonic acid, docosahexaenoic acid, and omega 3. Together, these studies suggest PFOA was not associated with triglycerides or fatty acids in pregnancy.

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

3.3.5.1.2.5 Findings from the General Adult Population

Eight *medium* confidence and nine *low* confidence studies examined PFOA and TC or hypercholesterolemia in adults (Figure 69, Figure 70). All studies examined cross-sectional associations {Dong, 2019, 5080195; Jain, 2019, 5080642; Liu, 2018, 4238514; Liu, 2020, 6318644; Lin, 2019, 5187597; Donat-Vargas, 2019, 5080588; Wang, 2012, 2919184; Convertino, 2018, 5080342; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Christensen, 2016, 3858533; Graber, 2019, 5080653; Sun, 2018, 4241053; Canova, 2020, 7021512; Fan, 2020, 7102734; Liu, 2018, 4238396}; two studies additionally examined the association between baseline PFOA and changes in TC or incident hypercholesterolemia {Liu, 2020, 6318644; Lin, 2019, 5187597}.

Of the eight *medium* confidence studies, six 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 significant, positive associations with TC. Canova 2020, 7021512 also reported a concentration-response curve when PFOA was categorized in deciles, with a higher slope at lower PFOA concentrations, which tended to flatten above around 20/30 ng/mL.

The four *medium* studies using overlapping data from NHANES 2003-2014 reported significantly positive association between PFOA and TC in adults {Dong, 2019, 5080195; Jain, 2019, 5080642; Liu, 2018, 4238514; Fan, 2020, 7102734} (Table C-14). Stratified analyses in Jain 2019 (5080642) suggest that the positive association occurred mainly in obese men. Significantly positive association between PFOA and TC also was observed at baseline in the DPPOS {Lin, 2019, 5187597}. This study reported positive associations between PFOA and prevalent, as well as incident, hypercholesterolemia. However, the HR for incident hypercholesterolemia was relatively small and not statistically significant (HR = 1.06, 95% CI: 0.94, 1.19). In contrast to these findings, Liu (2020, 6318644) reported no association between PFOA and TC. Further, Donat-Vargas (2019, 5080588) reported generally inverse association between PFOA and TC, regardless of whether PFOA was measured concurrently or averaged between baseline and follow-up. 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 PFOA and TC or hypercholesterolemia were reported in seven of nine 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 PFOA 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. Contrary to these findings, in one *low* confidence study, participants dosed with extremely high levels of ammonium perfluorooctanoate (APFO), a PFOA precursor, in an open-label, nonrandomized, phase 1 trial, were found to have reduced levels of TC with increasing plasma PFOA concentrations {Convertino, 2018, 5080342}. This study differed from the other studies in several ways. First, all participants were solid-tumor cancer patients who failed standard therapy. Second, participants ingested APFO rather than being exposed to PFOA. Third, participants' plasma PFOA concentrations were several orders of magnitude higher than those reported in the general population. It is unclear if these factors explained the inverse association between PFOA and TC. Considering *medium* and *low* confidence studies together, increased TC with increasing PFOA was observed in some adults. Inconsistencies in the direction of association across studies were found. Further studies are needed to determine if these inconsistencies reflect effect modification by subject characteristics or PFOA dose levels.

Five *medium* confidence studies examined PFOA and LDL in adults, and all reported positive associations (Figure 69, Figure 70). Higher PFOA was significantly associated with higher LDL at baseline in the DPPOS {Lin, 2019, 5187597} (Table C-14). This study also reported statistically significant, positive associations between PFOA and cholesterol in non-HDL and VLDL, which are lipoprotein fractions related to LDL and associated with increased cardiovascular risks {Lin, 2019, 5187597}. Positive associations between PFOA and LDL were also reported in the four NHANES studies {Dong, 2019, 5080195; Jain, 2019, 5080642; Liu, 2018, 4238514; Fan, 2020, 7102734}, but statistical significance was observed in obese men only {Jain, 2019, 5080642} and in NHANES cycle 2011-2012 {Dong, 2019, 5080195; Fan, 2020, 7102734}. Liu (2020, 6318644) report that PFOA was positively associated with cholesterol and apolipoprotein C-III (ApoC-III) in combined fractions of intermediate-density (IDL) and LDL that contained ApoC-III; the association with ApoC-III was statistically significant. IDL and LDL containing ApoC-III and ApoC-III itself are strongly associated with increased cardiovascular risks. Thus, the positive associations with cholesterol and ApoC-III in ApoC-III-containing fractions of IDL and LDL were consistent with the positive associations reported for LDL. Consistent with these findings, six of the eight *low* confidence studies report positive associations between PFOA and LDL {Lin, 2020, 6315756; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Canova, 2020, 7021512; Liu, 2018, 4238396}. Altogether, the available evidence supports a relatively consistent positive association between PFOA and LDL in adults, especially those who are obese or prediabetic. Associations with other lipoprotein cholesterol known to increase cardiovascular risks were also positive, which increased confidence in the findings for LDL.

Eight *medium* confidence and eight *low* confidence studies examined PFOA and HDL or clinically defined low HDL in adults (Figure 69, Figure 70). All studies examined cross-sectional associations {Dong, 2019, 5080195; Jain, 2019, 5080642; Christensen, 2019, 5080398; Fan, 2020, 7102734; Liu, 2018, 4238514; Liu, 2020, 6318644; Lin, 2019 5187597; Wang, 2012, 2919184; Convertino, 2018, 5080342; Lin, 2020, 6315756; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Canova, 2020, 7021512; Liu, 2018,

4238396}. Two studies also examined the association between baseline PFOA 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 significant, positive associations with HDL. Canova (2020, 7021512) also reported a concentration-response curve when PFOA was categorized in deciles. PFOA was inversely associated with HDL at baseline in the DPPOS, but the association was not statistically significant {Lin, 2019, 5187597} (Table C-14). Four studies used overlapping data from NHANES 2003-2014 and reported associations with HDL that were sometimes positive {Liu, 2018, 4238514; Christensen, 2019, 5080398; Fan, 2020; 7102734} and sometimes inverse {Dong, 2019, 5080195}. The direction of association differed by survey cycles {Dong, 2019, 5080195}. Few associations in this set of NHANES analyses were statistically significant. In an additional *medium* confidence study, PFOA was not associated with HDL at baseline or changes in HDL over two years {Liu, 2020, 6318644}. Similarly, *low* confidence studies also reported a mix of positive {Lin, 2020, 6315756; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Liu, 2018, 4238396} association with changes in HDL in the 6-24 months of the study), inverse (Chen 2019, 5387400; Liu 2018, 4238396, association with concurrent HDL or changes in HDL in the first 6 months of the study), or essentially null {Wang, 2012, 2919184; Convertino, 2018, 5080342} associations, with few being statistically significant. Given the inconsistent findings in both *medium* and *low* confidence studies, the available evidence suggests PFOA is not associated with HDL in adults.

Seven *medium* confidence and eleven *low* confidence studies examined the association between PFOA and triglycerides 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; Convertino, 2018, 5080342; 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 PFOA and changes in triglycerides or incident hypertriglyceridemia {Liu, 2020, 6318644; Lin, 2019, 5187597; Liu, 2018, 4238396}. Higher PFOA was significantly associated with higher levels of triglycerides in the DPPOS {Lin, 2019, 5187597} (Table C-14). This study also reported that PFOA was significantly associated with higher odds of hypertriglyceridemia at baseline and higher incidence of hypertriglyceridemia prospectively {Lin, 2019, 5187597}. Similarly, PFOA was associated with slightly higher levels of triglycerides in Liu 2020, 6318644. The association was stronger and statistically significant for triglycerides in the apoC-III-containing combined fractions of IDL and LDL and apoC-III-negative HDL {Liu, 2020, 6318644}. In contrast, the four *medium* studies using overlapping data from NHANES 2005-2014 reported positive {Jain, 2019, 5080642; Christensen, 2019, 5080398} or inverse associations {Jain, 2019, 5080642; Liu, 2018, 4238514; Fan, 2020; 7102734} between PFOA and triglycerides/hypertriglyceridemia. The direction of association appeared to differ by survey cycle, sex and obesity status {Jain 2019, 5080642}. No associations in these NHANES analyses were statistically significant. In an additional *medium* confidence study, PFOA was inversely associated with triglycerides, regardless of whether PFOA was measured concurrently or averaged between baseline and follow-up {Donat-Vargas, 2019, 5080588}. All participants in this study were free of diabetes for over 10 years, as opposed to the obese or prediabetic adults in Liu (2020, 6318644) and Lin (2019, 5187597). It is unclear if participants' different health status explained differences in the findings across *medium* studies. In *low* confidence studies, a mix of

positive {Lin, 2020 6315756; Chen, 2019, 5387400; He, 2018, 4238388; Yang, 2018, 4238462; Sun, 2018, 4241053; Canova, 2020, 7021512; Liu, 2018, 4238396}, association with concurrent triglycerides or changes in triglycerides in the first 6 months of the study), inverse {Lin, 2013, 2850967; Li, 2020, 6315681; Liu, 2018, 4238396}, association with changes in triglycerides in the 6-24 months of the study), and essentially null {Wang, 2012, 2919184; Convertino, 2018, 5080342} associations with triglycerides or hypertriglyceridemia were reported. Some associations were statistically significant. Overall, the available evidence suggests that PFOA was associated with elevated triglycerides in some adults. Whether PFOA increases triglycerides in all adults is unclear given inconsistency in reported associations.

In summary, in the general adult population, a relatively consistent, positive association was observed between PFOA and LDL. Increased TC and triglycerides with increasing PFOA exposure were also observed, but less consistently. HDL was not associated with PFOA.

3.3.5.1.2.6 Findings from Occupational Studies

Workers are usually exposed to higher levels of PFOA, in a more regular manner (sometimes daily), 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 {Shar 2009; PMID: 20386623}. 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 PFOA and TC or hypercholesterolemia in workers. Two of these studies examined the cross-sectional association between PFOA 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 PFOA and changes in TC over the course of a fluorochemical plant demolition project {Olsen, 2012, 2919185}. The cross-sectional studies reported positive {Wang 2012, 2919184} or inverse {Rotander, 2015, 3859842} associations between PFOA and TC; neither association was statistically significant. Olsen et al. (2012, 2919185) reported that over the course of the demolition project, changes in PFOA were inversely associated with changes in TC; this association was not statistically significant {Olsen, 2012, 2919185}. Taken together, these studies suggest no association between PFOA and TC in workers.

Two studies examined PFOA and LDL in workers. One study examined PFOA and non-HDL, of which LDL is a major component. All studies were considered *low* confidence. The two studies on LDL reported positive {Wang, 2012, 2919184} or inverse {Rotander, 2015, 3859842} association between PFOA and concurrent LDL; neither association was statistically significant. The study examining non-HDL reported that changes in PFOA 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 association between PFOA and LDL in workers.

The studies that examined LDL or non-HDL also examined the association between PFOA and HDL {Wang, 2012, 2919184; Rotander, 2015, 3859842; Olsen, 2012, 2919185}. The two cross-sectional studies in this set of studies reported inverse association between PFOA and HDL, including a statistically significant finding in Wang 2012, 2919184 {Wang, 2012, 2919184; Rotander, 2015, 3859842}. Contrary to these findings, Olsen (2012, 2919185) reported that

changes in PFOA over the demolition project was positively associated with changes in HDL {Olsen, 2012, 2919185}. This association was not statistically significant. When changes in TC to HDL ratio was examined as an outcome, however, a statistically significant, inverse association was observed. This suggests that increasing PFOA exposure was associated with decreases in TC/HDL over time, potentially partly due to a positive association between changes in PFOA and changes in HDL. Together, the occupational studies reported a consistently inverse association between PFOA and concurrent HDL, but this cross-sectional association was not coherent with longitudinal findings.

Two *low* confidence cross-sectional studies examined PFOA and triglycerides in workers and reported inverse associations between PFOA and triglycerides {Wang, 2012, 2919184; Rotander, 2015, 3859842}. Neither association was statistically significant.

In summary, among workers, the available evidence suggests no association between PFOA and TC or LDL. Inverse, cross-sectional associations between PFOA and HDL and triglycerides were found, but these associations were small, often not statistically significant, and were not coherent with longitudinal findings. Overall, the associations between PFOA and serum lipids among workers are different than those in the general adult population. It is unclear if well-known biases in occupational studies such as “healthy worker effect” may have attenuated the association between PFOA and an unfavorable serum lipid profile. More higher quality occupational studies are needed to improve hazard identification among workers.

3.3.5.2 *Animal Evidence*

There are 5 studies from the most recent literature search conducted in 2020 and 1 key study from the 2016 PFOA HESD {EPA, 2016, 3603279} that investigated the association between PFOA and cardiovascular effects. Study quality evaluations for these 6 studies are shown in Figure 71.

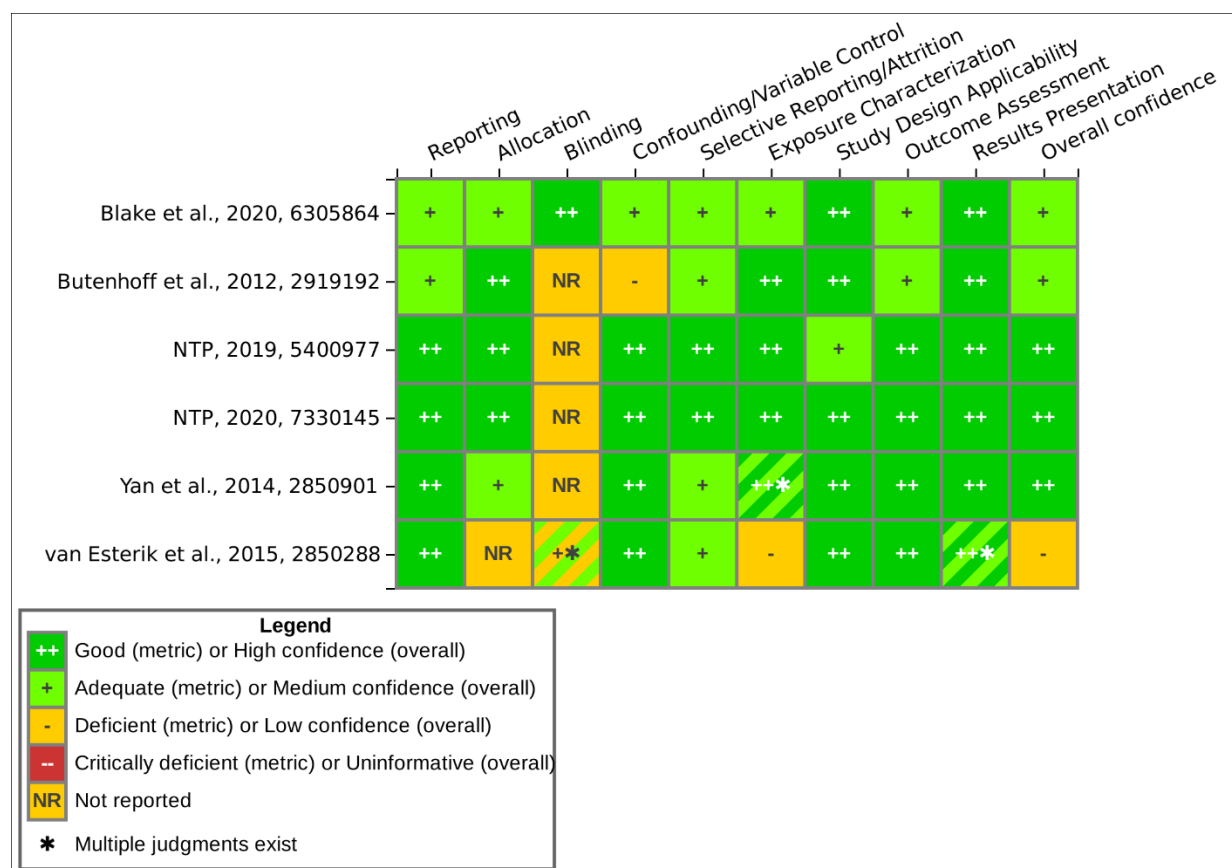


Figure 71. Summary of Study Evaluation for Toxicology Studies of PFOA and Cardiovascular Effects

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

Cardiovascular effects following exposure to PFOA were minimal according to two chronic studies with doses between 1.1–14.2 mg/kg/day {Butenhoff, 2012, 2919192; NTP, 2020, 7330145} and one short-term 28-day study with doses between 0.312–5 mg/kg/day {NTP, 2019, 5400977}. No changes were observed for heart weight {Butenhoff, 2012, 2919192; NTP, 2019, 5400977; NTP, 2020, 7330145}, heart histopathology {Butenhoff, 2012, 2919192; NTP, 2019, 5400977; NTP, 2020, 7330145}, or aorta histopathology {Butenhoff, 2012, 2919192; NTP, 2019, 5400977} following exposure to PFOA in male and female Sprague-Dawley rats.

PFOA 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 mice and rats in subchronic, chronic, and developmental studies of oral exposure to PFOA (Figure 72). Overall, studies have generally reported consistent decreases in serum lipids including TC, triglycerides, LDL cholesterol, HDL cholesterol, and/or non-HDL cholesterol in rats {Martin et al., 2007, 758419; Loveless et al., 2008, 988599; Elcombe et al., 2010, 2850034; NTP, 2019, 5400977; NTP, 2020, 7330145} and mice {Loveless et al., 2008, 988599; De Witt et al., 2009, 1937261; Minata et al., 2010, 1937251; Yahia 2010, 1332451 ; Yan, 2014, 2850901; Quist et al., 2015, 6570066; Blake et al., 2020, 6305864}. In a developmental study of female CD-1 P₀ mice exposed to PFOA (0, 1, and 5 mg/kg/day) by oral gavage from either GD1.5–11.5 or GD 1.5–

17.5, authors reported maximum decreases in serum triglyceride levels of 58% and 66%, respectively, at the highest dose of 5 mg/kg/day. No changes were observed for serum TC, HDL cholesterol, or LDL cholesterol {Blake, 2020, 6305864}. Male BALB/c mice exposed to PFOA by gavage for 28 days had significant decreases in serum TC and HDL levels at concentrations as low as 1.25 mg/kg/day {Yan et al., 2014, 2850901}. For serum triglyceride levels, significant increases were observed at lower exposure concentrations of PFOA (0.31 and 1.25 mg/kg/day) while significant decreases were seen following exposure to higher PFOA concentrations (5 and 10 mg/kg/day); no changes were observed in serum LDL cholesterol levels. In a study conducted by NTP, sex differences were observed in Sprague-Dawley rats exposed to PFOA by gavage for 28 days {NTP, 2019, 5400977}. Males had significantly decreased serum TC and triglyceride levels at exposure concentrations as low as 0.625 mg/kg/day. Female rats in the same study were exposed to 10-fold higher doses than their male counterparts due to sex differences in PFOA excretion (Section D.4). Females had significant increases in both serum TC and triglyceride levels at the two highest doses (50 and 100 mg/kg/day). In the available chronic study {NTP, 2020, 7330145}, F₁ male Sprague-Dawley rats were exposed during gestation and lactation (perinatal exposure) with postweaning exposure or postweaning exposure only until animals were 19 weeks of age (e.g., 16-week interim time point; see further study design details in Section 3.3.1.2.1.2). Serum TC levels were significantly decreased only in animals exposed during both the perinatal and postweaning phases (at postweaning doses of approximately 1 and 4.6 mg/kg/day); serum triglyceride levels were decreased in all exposure groups. Serum lipid levels were not altered in F₁ females from the same study. 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.

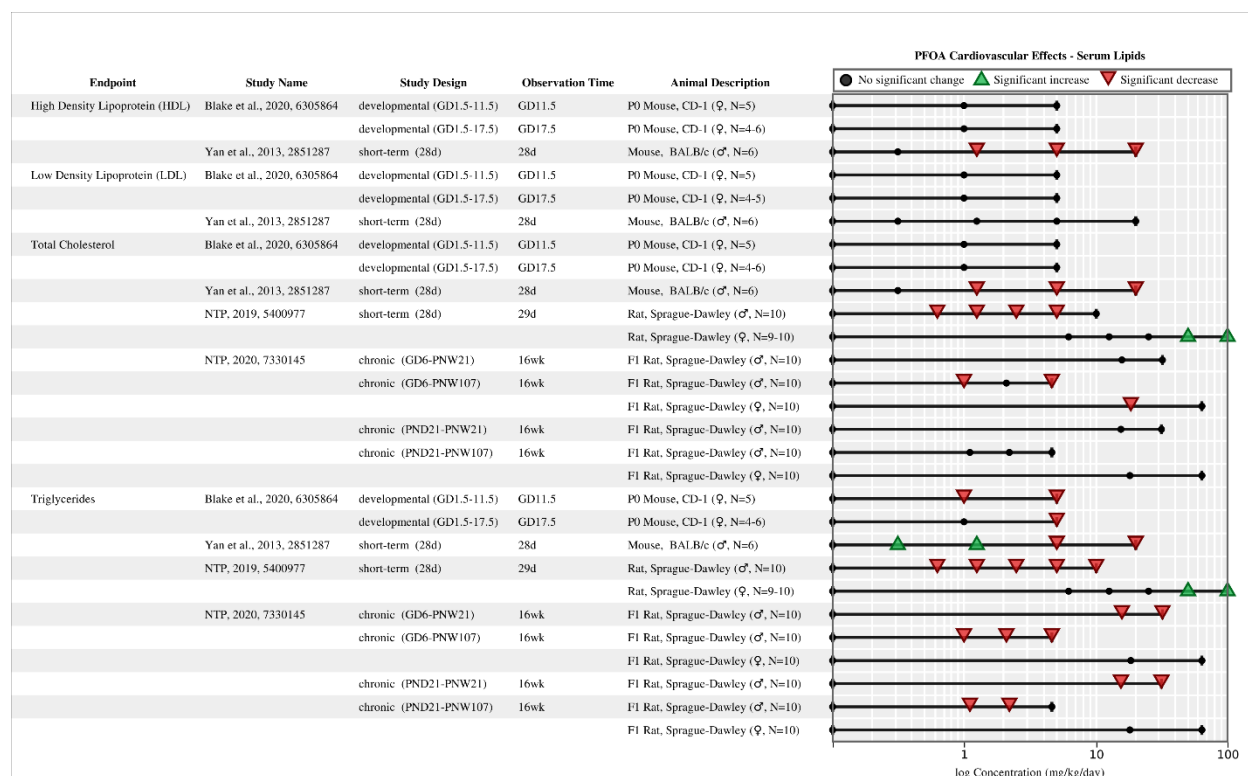


Figure 72. Serum Lipid Levels in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA 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; PNW = postnatal week; F1 = first generation; PND = postnatal day; d = day; wk = week.

3.3.5.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse cardiovascular outcomes is discussed in Sections 3.1.1.1 and 3.4.1 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 6 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to cardiovascular effects. A summary of these studies is shown in Figure 73. Additional analysis on the mechanistic actions of PFOA on cardiovascular health outcomes is ongoing 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	2	2
Big Data, Non-Targeted Analysis	1	0	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	0	0	1	1
Cell Signaling Or Signal Transduction	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	0	1
Oxidative Stress	0	2	0	2
Grand Total	1	2	3	6

Figure 73. Summary of Mechanistic Studies of PFOA and Cardiovascular Effects

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

3.3.5.4 Evidence Integration

In summary, the studies identified since the 2016 assessments do not provide additional clarity on the association between PFOA and CVD. Most of the CVD evidence identified focused on blood pressure in the general adult population (13 studies). The findings from a single *high* confidence study and five *medium* confidence studies conducted in the general adult population did not provide consistent evidence for an association between PFOA and blood pressure. The evidence for an association between PFOA and increased risk of hypertension overall and in gender-stratified analysis was inconsistent. Evidence in children and adolescents also is less consistent. Five studies in children and adolescents, and one study in pregnant women suggest no associations with elevated blood pressure in these populations. Evidence for other CVD-related outcomes across all study populations was more limited, and similarly inconsistent. Consequently, the evidence for these CVD outcomes is broadly consistent with the conclusions of the C8 Science Panel and in the 2016 PFOA assessment, which found no probable link between PFOA exposure and multiple other conditions, including high blood pressure and CAD. It is challenging to compare findings on CVD related mortality in the current assessment to the prior assessment due to differences in how this outcome was defined. Findings from the prior assessment were mixed, with one study reporting an increased risk of cerebrovascular disease mortality observed in the highest PFOA exposure category among occupationally exposed subjects. However, no association was reported with IHD mortality. The current evidence from a single study indicated PFOA was not associated with an increased risk of mortality due to cardiovascular causes, including hypertensive disease, IHD, stroke, and circulatory diseases. Future analyses of cause-specific CVD mortality could help elucidate whether there is a consistent association between PFOA and cerebrovascular disease mortality. No studies or endpoints were considered for the derivation of PODs since findings for an association between PFOA and CVD outcomes are mixed.

However, based on this systematic review of 43 epidemiologic studies, the available evidence supports positive associations between PFOA and TC, LDL, and triglycerides in some human populations. For TC, the association was consistently positive in pregnant women, positive but less consistently so in adults and children, and generally null in workers. For LDL, the association was generally positive among adults, positive but less consistently so in children, and generally null in workers. Data were not available for PFOA and LDL in pregnant women. For triglycerides, positive associations were observed in some adults and children, but not pregnant women and workers. Except for workers, these results are consistent with findings from the 2016 Health Assessment. Differences in findings from occupational studies between the 2016 Health Assessment and this review may be attributable to limitations of occupational studies in this review. Similar to the 2016 Health Assessment, the available evidence in this review does not support an inverse association between PFOA and HDL in any populations. Based on this evidence, alterations in the serum lipids TC, LDL, and triglycerides were considered for the derivation of PODs.

In animal studies, no effects or minimal alterations were noted for heart weight and histopathology in the heart and aorta. 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 encompasses conditions such as hypothyroidism and hyperthyroidism, and it is more common in females than in males. Hypothyroidism is characterized by elevated thyroid stimulating hormone (TSH) and concurrently low 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, 3982042} and HESD {U.S. EPA, 2016, 3603279} identified limited evidence of endocrine effects of PFOA for thyroid disease, hypothyroidism, and hypothyroxinemia. Evidence from occupational cohorts and from general population studies was mixed. An analysis of an occupational cohort in Minnesota (Olsen, 1998, 1290857) showed elevated TSH ($p = 0.002$) levels in a single exposure group (10–30 $\mu\text{g/mL}$ serum PFOA); however, this increase was not observed for those with greater exposure ($>30 \mu\text{g/mL}$ serum PFOA). Pooled occupational analyses, combining the Minnesota cohort with cohorts from Belgium and Alabama {Olsen, 2003, 1290020; Olsen, 2007, 1290836}, showed a negative association for free T4, and a positive association was found for T3. Two studies on participants from the C8 Health Project showed positive associations between estimated PFOA exposure (cumulative and yearly) and all incident self-reported thyroid disease in women {Winquist, 2014, 2337818}, and thyroid disease in children examining modeled in utero PFOA exposure and concurrent PFOA serum concentrations {Lopez-Espinosa, 2012, 1291122}. As a result of these

findings, the C8 Science Panel concluded that a probable link exists between PFOA and thyroid disease {C8 Science Panel, 2012, 1430770}. In general population studies, positive associations were found with T4 in older adults {Shrestha, 2015, 2851052}, with T3 (free and total) in females {Wen, 2013, 2850943}, and between prenatal PFOA (cord blood) and T4 concentrations in thyroid disease-free girls {de Cock, 2014, 2718059}. Other studies did not observe significant associations in adults and children {Bloom, 2010, 757875; Lin, 2013, 1332458}. Most results in studies on pregnant women were not significant except for small positive associations with TSH {Berg, 2015, 2851002}, especially in pregnant women with elevated TPOAb {Webster, 2014, 2850208}.

For this updated review, 32 studies (32 publications) report on the association between PFOA exposure and endocrine effects. Five publications were studies in pregnant women {Aimuzi, 2020, 6512125; Inoue, 2019, 5918599; Itoh, 2019, 5915990; Reardon, 2019, 5412435; Shah-Kulkarni, 2016, 3859821}, and the remainder of the publications were on the general population. One study was a controlled trial {Convertino, 2018, 5080342}, six were cohort studies {Blake, 2018, 5080657; Crawford, 2017, 3859813; Lebeaux, 2020, 6356361; Liu, 2018, 4238396; Preston, 2018, 4241056; Reardon, 2019, 5412435}, five were cohort and cross-sectional studies {Itoh, 2019, 5915990; Kim, 2020, 6833758; Kato, 2016, 3981723; Wang, 2014, 2850394; Xiao, 2019, 5918609} two case-control studies {Kim, 2016, 3351917; Predieri, 2015, 3889874}, one case-control and cross-sectional study {Zhang, 2018, 5079665}, and 18 cross-sectional studies {Abraham, 2020, 6506041; Aimuzi, 2019, 5387078; Aimuzi, 2020, 6512125; Byrne, 2018, 5079678; Caron-Beaudoin, 2019, 5097914; Christensen, 2016, 3350721; 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; Shah-Kulkarni, 2016, 3859821; Tsai, 2017, 3860107; Yang, 2016, 3858535}. All observational studies measured PFOA in blood components (i.e., blood, plasma, or serum). 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 PFOA and thyroid hormone concentrations, other endocrine outcomes examined included: thyroid disease, thyroid antibodies (thyroglobulin antibodies [TgAb] and thyroid peroxidase antibody [TPOAb]), and thyroid hormone-associated proteins (e.g., thyroglobulin, T4-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 PFOA 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 PFOA concentrations measured during pregnancy and thyroid hormone levels in cord blood or the child's blood attenuate any concerns for potential reverse causality. Measuring PFOA and thyroid hormone concentrations concurrently in maternal serum was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOA (median half-life = 2.7 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 31 studies identified since the 2016 assessment, four studies were classified as *high* confidence, 16 as *medium* confidence, 9 as *low* confidence, and 4 were considered *uninformative* {Abraham, 2020, 6506041; Kim, 2016, 3351917; Predieri, 2015, 3889874; Seo, 2018, 4238334} (Figure 74, Figure 75).

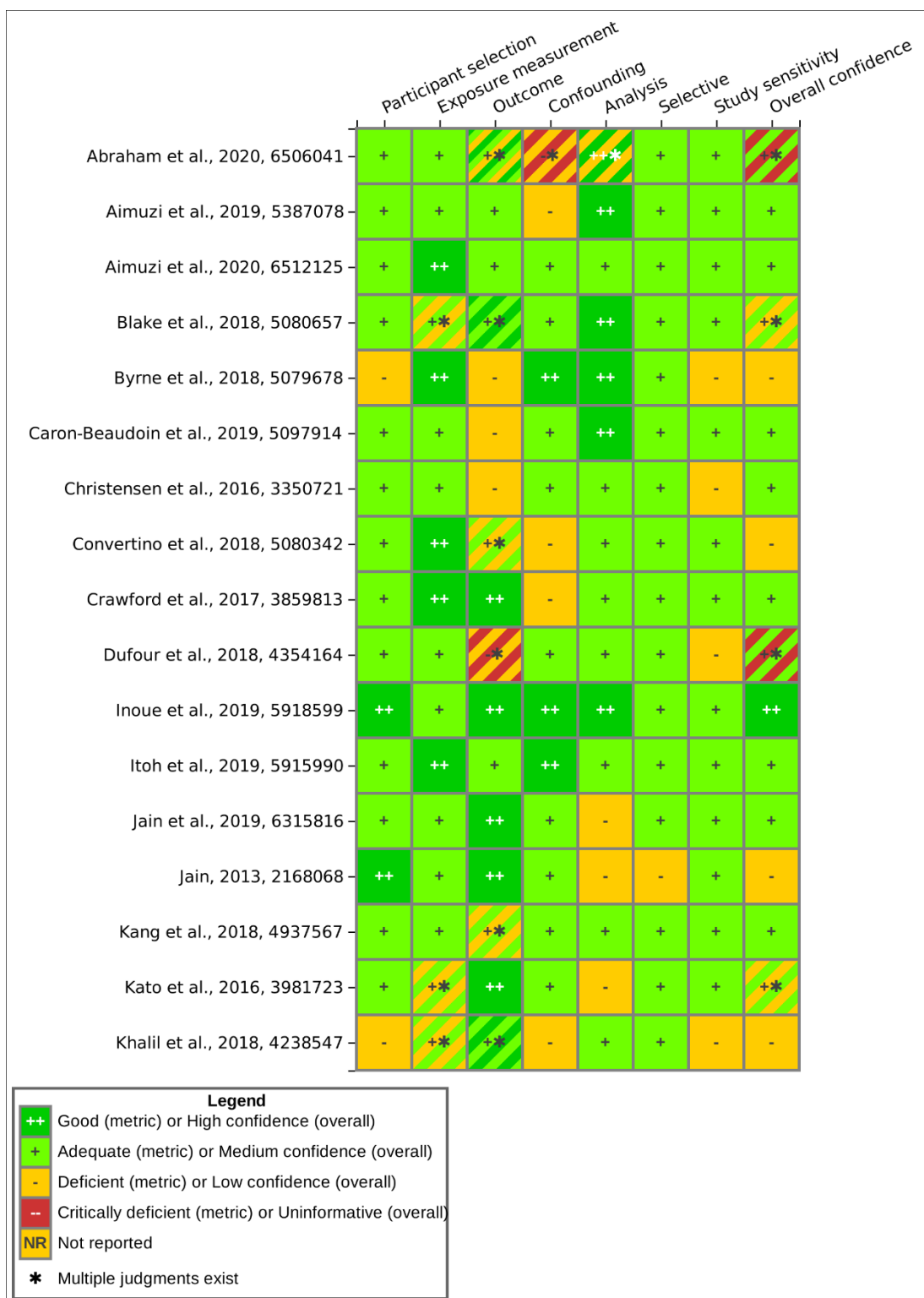


Figure 74. Summary of Study Evaluation for Epidemiology Studies of PFOA and Endocrine Effects

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

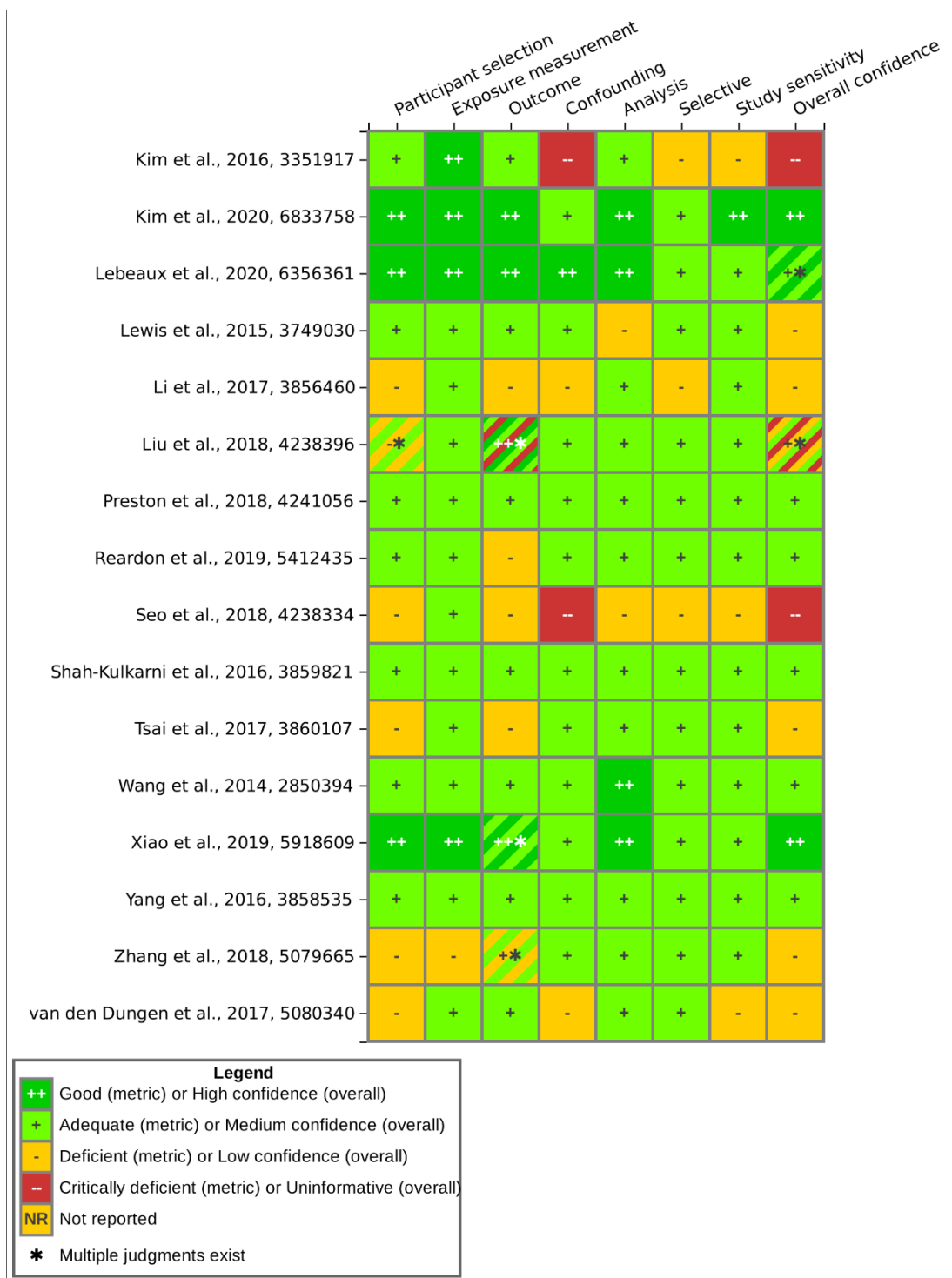


Figure 75. Summary of Study Evaluation for Epidemiology Studies of PFOA and Endocrine Effects (Continued)

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

These differences resulted in *high* confidence {Lebeaux, 2020, 6356361} and *medium* confidence {Dufour, 2018, 4354164; Kato, 2016, 3981723} for infant or child analyses. For maternal analyses which tend to be cross-sectional in nature, the uncertainty regarding temporality resulted in *medium* confidence {Lebeaux, 2020, 6356361}, *low* confidence {Kato, 2016, 3981723}, or *uninformative* {Dufour, 2018, 4354164} ratings.

Studies rated as *low* confidence or *uninformative* had deficiencies including lack of accounting for population sampling methods {Lewis, 2015, 3749030}, or residual confounding {Abraham, 2020, 6506041; Convertino, 2018, 5080342; Kim, 2016, 3351917; Predieri, 2015, 3889874}, or lack of information on allocation of participants to treatment levels {Convertino, 2018, 5080342}, participant recruitment and case definitions (studies {Kim, 2016, 3351917; Predieri, 2015, 3889874}) or small sample sizes {Kim, 2016, 3351917; Predieri, 2015, 3889874}.

3.3.6.1.3 Findings from Children

One *high* confidence study {Kim, 2020, 6833758} observed no association with subclinical hypothyroidism in children six years of age. Congenital hypothyroidism (CH) was assessed in South Korean infants in a very small case-control study {Kim, 2016, 3351917}. PFOA concentrations were significantly higher in infants with CH compared to controls (means 5.4 and 2.12 ng/mL, respectively, p -value<0.01) (Table C-15). However, the study was considered *uninformative* because of potential key confounding factors were not controlled for in the analysis, and the small sample size.

Thyroid hormone levels were examined in 19 studies {Abraham, 2020, 6506041; Aimuzi, 2019, 5387078; Caron-Beaudoin, 2019, 5097914; Dufour, 2018, 4354164; Itoh, 2019, 5915990; Kang, 2018, 4937567; Kato, 2016, 3981723; Khalil, 2018, 4238547; Kim, 2016, 3351917; Kim, 2020, 6833758; Lebeaux, 2020, 6356361; Predieri, 2015, 3889874; Preston, 2018, 4241056; Shah-Kulkarni, 2016, 3859821; Tsai, 2017, 3860107; Wang, 2014, 2850394; Xiao, 2019, 5918609; Yang, 2016, 3858535} and four observed significant effects (Table C-15). One *high* confidence study {Xiao, 2019, 5918609} observed a large positive association between maternal third trimester PFOA and cord serum TSH. The effect size for TSH was similar after stratification by infant sex, but no longer significant. Additionally, sex-stratified analyses showed positive associations between maternal PFOA and measures of T4 (total T4 and free T4 index [FTI]) in cord blood from female infants. No other significant associations were observed for TSH among other studies on children. Another *high* confidence study {Kim, 2020, 6833758} showed positive associations between serum PFOA concentrations and free T4 levels at age 6. After stratifying by child sex, the association remained among boys but was not observed in girls. This effect was also observed in a *medium* confidence cross-sectional study in newborns {Aimuzi, 2019, 5387078}, which reported significant positive associations with free T4 in cord blood. When stratified by sex, the effect persisted in male newborns, but was not seen in female newborns. These three studies report consistent, significant positive associations with T4 in children; however, the effect was not consistent between boys and girls in different populations. Similarly, a *medium* confidence cross-sectional study {Kang, 2018, 4937567} showed a borderline significant positive association between serum PFOA and free T4 ($p = 0.075$). Analyses of children from the Hokkaido Study {Itoh, 2019, 5915990; Kato, 2016, 3981723} did not observe significant associations with thyroid hormones.

Thyroid antibody (TA) levels were examined in one study {Itoh, 2019, 5915990} which found significant effects (Table C-15). A *medium* confidence study on children from the Hokkaido Study on the Environment and Children's Health {Itoh, 2019, 5915990} showed mixed associations between maternal PFOA concentrations and thyroglobulin antibody levels. An inverse association was found for TgAb levels among boys born to TA-negative mothers; no effects were seen among all boys or boys born to TA-positive mothers. The opposite trend was seen in girls; a positive association for TgAb levels was observed for girls born to TA-positive mothers. No effects were observed in all girls or girls born to TA-negative mothers.

3.3.6.1.4 Findings from Pregnant Women

Thyroid hormone levels were examined in five studies {Aimuzi, 2020, 6512125; Inoue, 2019, 5918599; Itoh, 2019, 5915990; Reardon, 2019, 5412435; Shah-Kulkarni, 2016, 3859821} and two observed significant effects (Table C-15). A *medium* confidence study {Preston, 2018, 4241056} in pregnant women showed a significant decrease in the FTI with increasing first trimester serum PFOA concentrations. Associations with other thyroid hormones were not observed among the whole study sample. However, analyses stratified by TPOAb status showed a borderline significant ($p = 0.08$) inverse effect of PFOA on TSH among TPOAb-positive women; no effects were seen in TPOAb-negative women. Another *medium* confidence study {Aimuzi, 2020, 6512125} observed a positive association between serum PFOA and early pregnancy free T4, but this effect was not seen when stratified by TPOAb-status.

Thyroid hormone antibodies were examined in one study {Itoh, 2019, 5915990} which found a significant effect. A negative association was observed for TPOAb levels in first trimester serum among mothers in the Hokkaido Study. One cross-sectional study {Dufour, 2018, 4354164} on mother-child dyads showed evidence of a large increased risk of hypothyroidism in mothers (OR Q4 vs Q1 [95% CI]: 5.62 [1.64-26.11]), however, there was a great deal of uncertainty in regard to 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

One study examined thyroid disease among male anglers (age >50 years) and observed a non-significant increase in odds of self-reported thyroid disease with increasing serum PFOA concentrations {Christensen, 2016, 3350721}.

Thyroid function was examined in 11 studies {Blake, 2018, 5080657; Byrne, 2018, 5079678; Convertino, 2018, 5080342; Crawford, 2017, 3859813; Jain, 2013, 2168068; Jain, 2019, 6315816; Lewis, 2015, 3749030; Li, 2017, 3856460; Liu, 2018, 4238396; Seo, 2018, 4238334; Zhang, 2018, 5079665} and seven observed significant effects (Table C-15). A *low* confidence case-control study {Zhang, 2018, 5079665} examined women with and without POI found a positive association among controls (i.e., women without POI) for TSH concentrations with increasing plasma PFOA concentrations. Similarly, TSH levels were elevated in women with POI which was accompanied by a concomitant negative association with free T4 concentrations. The thyroid hormone concentrations were within normal ranges in both cases and controls. Another *low* confidence case-control study {Heffernan, 2018, 5079713} on women with and without PCOS found a similar increase in TSH among cases. However, findings need to be interpreted with caution, since both studies were considered *low* confidence due to a lack of information on the control recruitment and selection process.

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 including an inverse association with total T4 in men aged 40 to 60 years, increased total T4 and decreased TSH in women aged 12 to 20 years, increased free T3 in women aged 20 to 40 years, and concurrent increases in free and total T3 among women aged 60 years or older. However, there is no evidence NHANES complex sampling design was accounted for in the analysis which contributed to a *low* confidence rating. Jain, 2013, 2168068, another *low* confidence study, found a significant increase in TSH levels among those NHANES (2007–2008) participants in the highest tertile (≥ 5.1 ng/mL) of PFOA exposure compared to the lowest (≤ 3.3 ng/mL). A *medium* confidence follow-up study {Jain, 2019, 6315816} on NHANES (2007–2012) participants investigated associations with serum PFOA and thyroid hormone concentrations, stratified by glomerular function (GF) status (GF1, GF-2, GF-3A, and GF-3B/4). Few significant and borderline significant results were observed; however, the direction of association was inconsistent across increasing glomerular filtration groups and did not suggest an interaction with glomerular filtration status. Associations between PFOA and thyroid hormones were inconsistent across NHANES studies. Lewis, 2015, 3749030 and Jain, 2013, 2168068 found significant effects in opposite directions for TSH, however, these effects were observed in different NHANES cycles and among different subpopulations. In the 2011–2012 NHANES participants, Lewis, 2015, 3749030 found consistent effects for T3 in women of different ages, but other results were inconsistent between age and sex groupings.

Inverse associations with TSH and T4 were also observed in 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). One additional *low* confidence, cross-sectional study {Byrne, 2018, 5079678} on Alaska natives found a significant positive association for TSH among all participants and an inverse association with total T3 in men; however, this population was relatively small (total $n = 85$; male $n = 38$) with low exposure levels (median: 1.01 ng/mL [25th–75th percentile: 0.753–1.44 ng/mL]).

In a controlled trial {Convertino, 2018, 5080342} in which subjects were administered APFO doses ranging 50–1200 mg for six weeks, {Convertino, 2018, 5080342} report an increase in the average rate of change in free T4. A dose-dependent increase was also demonstrated by grouping subjects into three treatment bins and showing increasing mean and median free T4 concentrations. This study, however, was rated as *low* confidence because potential confounders were not considered during participant allocation to treatment groups or in the statistical analysis.

3.3.6.2 Animal Evidence

There are 4 studies from the most recent literature search conducted in 2020 and 3 key study from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and endocrine effects. Study quality evaluations for these 7 studies are shown in Figure 76.

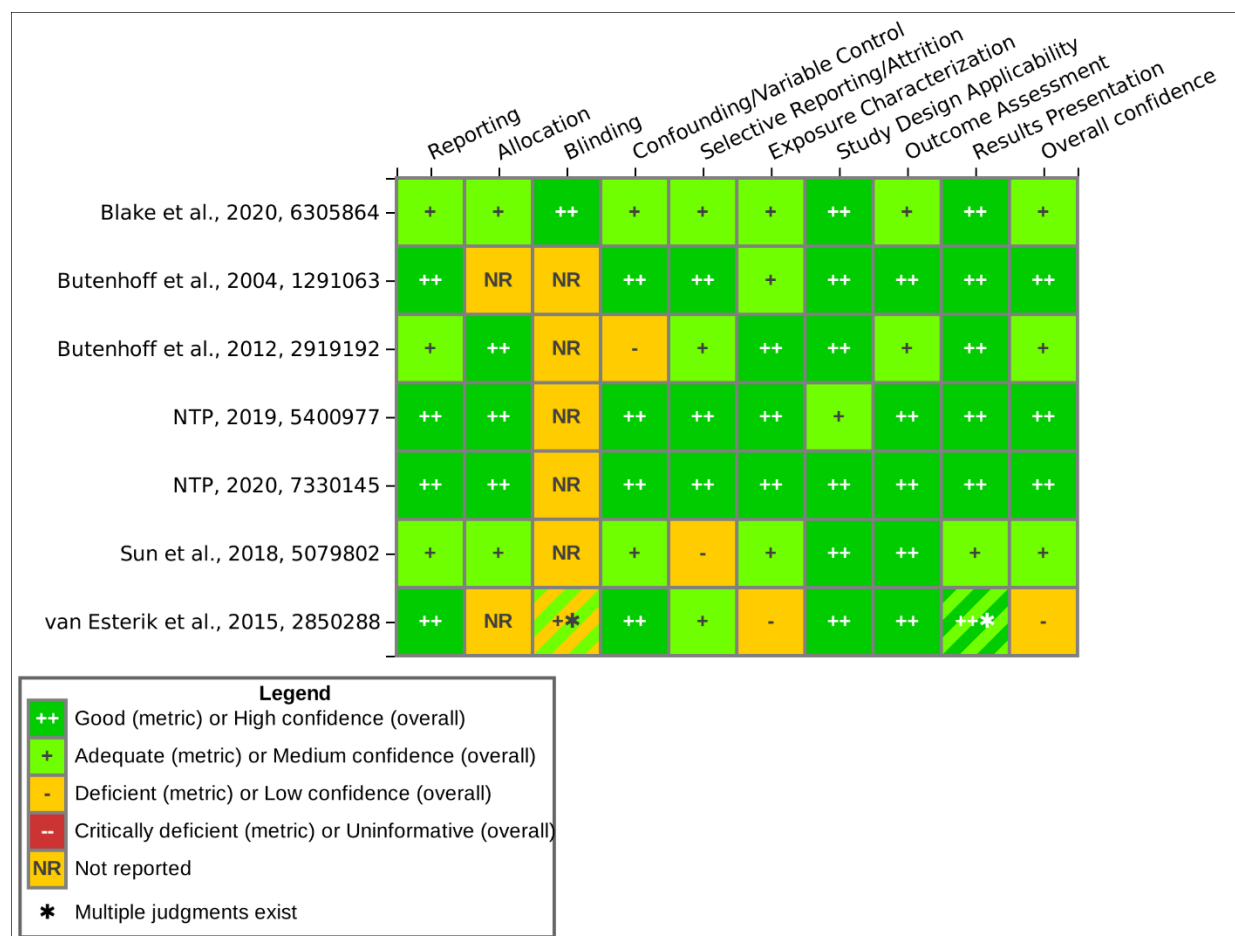


Figure 76. Summary of Study Evaluation for Toxicology Studies of PFOA and Endocrine Effects

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

Available animal toxicity data suggest that PFOA exposure can interfere with male and female endocrine systems. Overall, studies have reported endocrine organ weight changes, hormone fluctuations, and organ histopathology across studies of varying durations of oral exposure to PFOA. Effects typically exhibit a sex-bias depending on the species, endpoint, and exposure paradigm, likely due to known toxicokinetic differences (Section 3.2). The thyroid gland and thyroid hormones appear to be affected by PFOA exposure. Effects of PFOA on gonads and placenta and on reproductive hormones are described in detail in Section 3.3.2.2.

3.3.6.2.1 Organ Weight Changes

Significant changes in absolute and relative endocrine organ weights have been observed in monkeys {Goldenthal, 1978, 1291068} and rats {Butenhoff, 2012, 2919192; Butenhoff, 2004, 1291063; NTP, 2019, 5400977} following oral exposure to PFOA, often with a male-bias in response (Figure 77).

Absolute and relative thyroid gland weight was quantified as part of a short-term exposure study conducted by NTP (2019, 5400977). In that study, male Sprague-Dawley rats received 0, 0.625,

1.25, 2.5, 5, or 10 mg/kg/day PFOA and females received 0, 6.25, 12.5, 25, 50, or 100 mg/kg/day via gavage for 28 days. Absolute thyroid weight was only significantly increased in males of the 2.5 mg/kg/day exposure group. Thyroid gland weight relative to body weight was elevated in males administered ≥ 1.25 mg/kg/day PFOA by the end of the study, which may be related to reductions in mean body weights that were observed in males but not females (Section 3.3.7.2.2), though body weight in males of the 1.25 mg/kg/day dose group was only modestly reduced by 4.6% compared to controls. No statistically significant effects were observed in females at any dose and no effects were observed on absolute or relative adrenal gland weight in either sex {NTP, 2019, 5400977}.

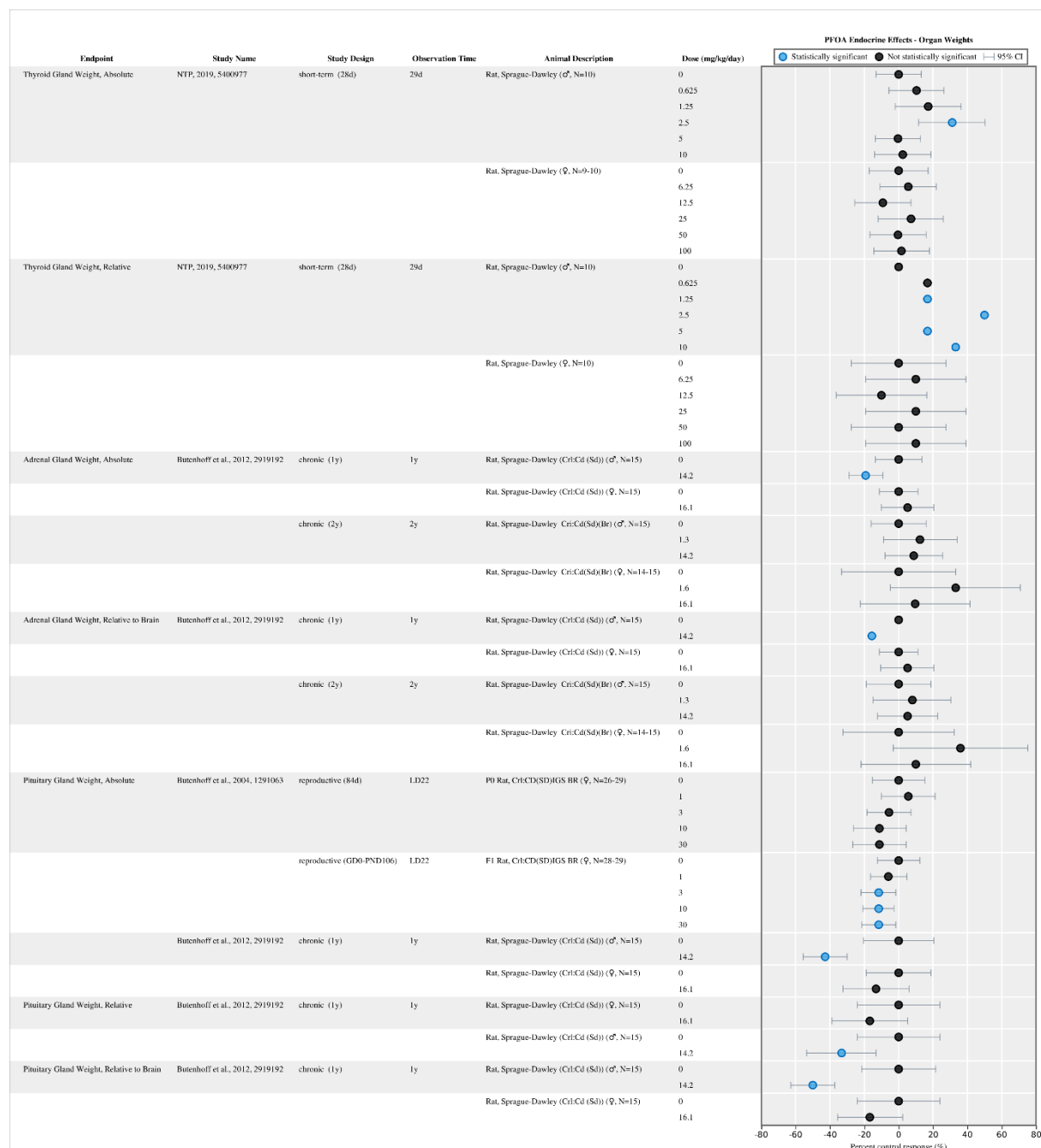


Figure 77. Percent Change in Endocrine Organ Weights Relative to Controls in Rodents Following Exposure to PFOA^a

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

GD = gestation day; PND = postnatal day; LD= Lactational Day; P0 = parental generation; F1 = first generation.

^aConfidence intervals (CI) for some studies may be too narrow to view at this scale; d = day; y = year.

Relative pituitary gland weight was elevated in male rhesus monkeys exposed to 3 mg/kg/day via gavage for 90 days. Changes in body weight were similar to controls for these animals {Goldenthal, 1978, 1291068}. In male Sprague-Dawley (CrI:COBS@CD(SD)BR) rats, pituitary weights (absolute and relative to brain or body weight) were reduced following a year-long

dietary exposure to 300 ppm PFOA, which is equivalent to 14.2 mg/kg/day {Butenhoff, 2012, 2919192}. The decrease was consistent across all measures despite slight (i.e., <10%) non-significant decreases in both body weight and absolute brain weight. Decrements in pituitary gland weight were not observed in female rats given the same 300 ppm exposure for one year (16.1 mg/kg/day equivalent) {Butenhoff, 2012, 2919192}. Another study by Butenhoff et al. in Sprague-Dawley rats described female-specific reductions in pituitary gland weight following a multi-lifestage PFOA exposure paradigm {Butenhoff, 2004, 1291063}. In this study, absolute pituitary gland weights were reduced in adult F₁ females (on lactational day 22 of the F₂ generation) following oral exposure to 3, 10, or 30 mg/kg/day PFOA from GD0–PND127 {Butenhoff, 2004, 1291063}. Although relative pituitary weights were not provided, there were not significant changes in body weights at sacrifice nor absolute brain weights (Section 3.3.8.2), which implies the reduction in absolute pituitary weight may reflect a specific effect on the pituitary gland. F₁ pup weight was only reduced in the 30 mg/kg/day group during development, indicating that slower pup growth is not an explanation for the reduced absolute pituitary weights.

Male-specific reductions in absolute adrenal gland weight and relative to brain weight were observed by Butenhoff et al. (2012, 919192) after one year of exposure to 300 ppm PFOA (equivalent to 14.2 mg/kg/day), but was not observed after two years {Butenhoff, 2012, 2919192}.

3.3.6.2.2 Hormone fluctuations

Several studies have described fluctuations in the levels of hormones secreted from the adrenal, pituitary, and thyroid glands following short term exposure to rats and mice {NTP, 2019, 5400977; Sun, 2018, 5079802} and chronic exposure to non-human primates {Butenhoff, 2002, 1276161}.

In the aforementioned 28-day rat study conducted by NTP (2019, 5400977), male-specific reductions in T₄, FT₄, and T₃ were observed in almost all exposure groups (Table 8; Figure 78); T₃ was not significantly affected in the 10 mg/kg/day group, though statistically significant reductions were observed in all lower dose groups. Notably, these effects in males occurred at doses lower than those that resulted in decreased body weight, which may be confounding with hormone responses, as shown in dietary restriction studies in rats {Laws et al., 2007, 1411456}. T₄ and FT₄ were significantly reduced in females from the 100 mg/kg/day exposure group {NTP, 2019, 5400977}. Opposing effects of TSH were observed between the sexes. Although female TSH concentrations were increased in all exposure groups (6.25–100 mg/kg/day), male TSH was reduced in the 5 and 10 mg/kg/day exposure groups when compared to controls {NTP, 2019, 5400977}.

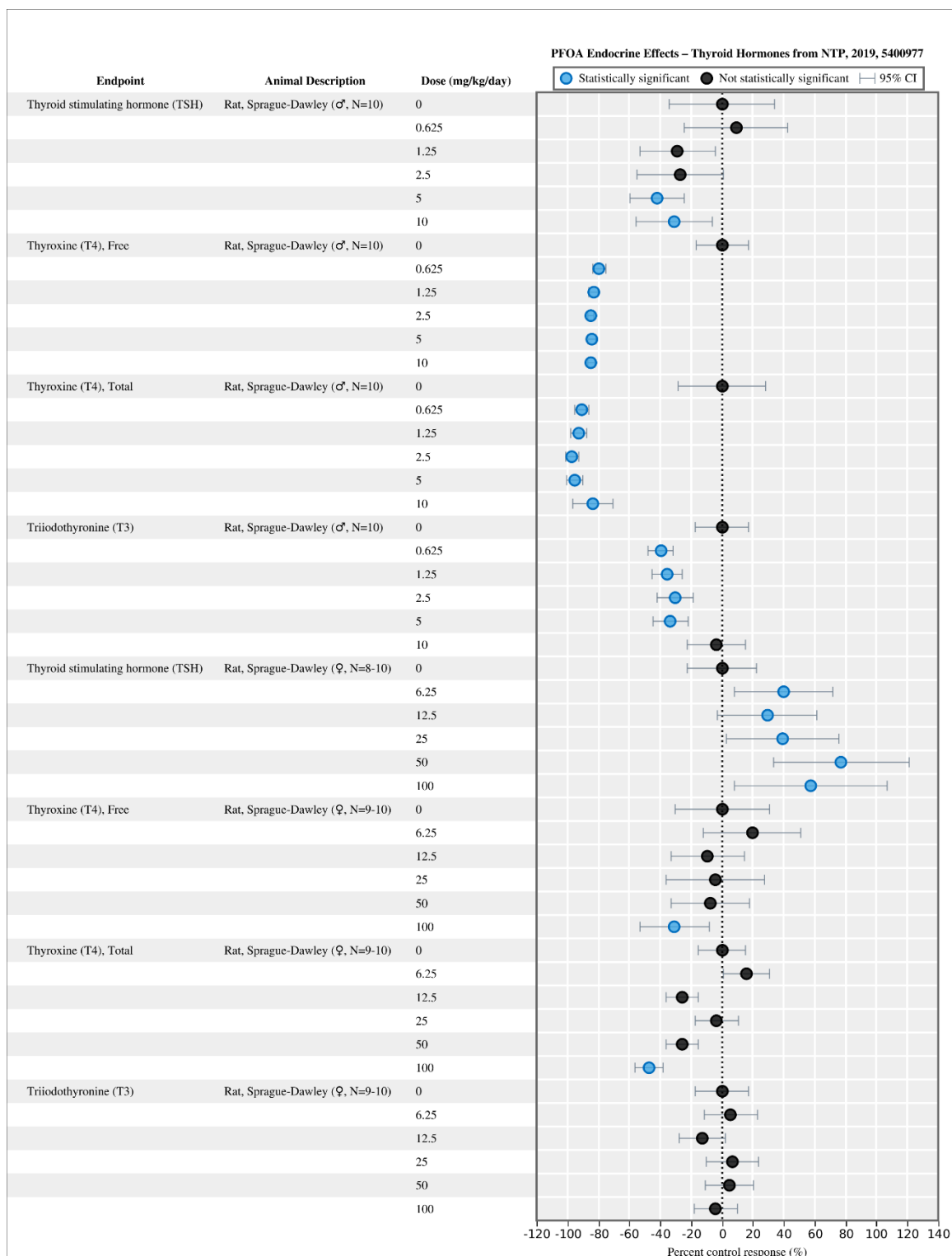


Figure 78. Percent Change in Thyroid Hormone Levels of Male and Female Rats Exposed to PFOA for 28 Days as Reported by NTP, 2019, 5400977^a

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

TSH = thyroid stimulating hormone; T3 = triiodothyronine; T4 = thyroxine; CI = confidence interval.

^aSome hormone measurements in male rats were below or approaching the limit of quantifications for FT4 (0.3 ng/dL), T4 (0.5 µg/dL), and T3 (50 ng/dL).

In a chronic exposure study by Butenhoff et al. (2002, 1276161), male cynomolgus monkeys were given 0, 3, 10, or 30/20 mg/kg PFOA per day for 26 weeks. The “30/20” notation reflects a reduction from 30 to 20 mg/kg/day at day 22 of the study due to toxicity in this exposure group. Only 2 animals from the 30/20 mg/kg/day group survived until sacrifice, which introduces uncertainty to the results of this dose group, though they are discussed here. Although no change in TSH was noted in the highest-dose group, it was significantly elevated in both the 3 and 10 mg/kg/day exposure groups by the end of the study, at day 182 (increases of 63% and 118% changes, respectively). In the lowest exposure group (3 mg/kg/day), T4 was reduced across multiple timepoints and decreases reached significance in all three dose groups (33%, 29%, and 32% decreases, respectively) at the conclusion of the study. A dose-dependent decrease in FT4 was also observed across multiple time points, with decreases at day 182 of 33%, 38%, and 42% in the 3, 10, and 30/20 mg/kg/day dose groups, respectively, compared to control levels. Similar trends were seen in T3 and free triiodothyronine (FT3) levels throughout the study. By day 182, total and free T3 levels were decreased by 15%, 14%, and 34% and 13%, 17%, and 40%, respectively, with increasing dose levels.

Prior to this updated assessment, the available literature measuring thyroid hormones was limited and acute studies were discussed in the 2016 HESD {U.S. EPA, 2016, 3603279}. One such study in adult male Sprague-Dawley rats given a single oral exposure of PFOA (20 mg/kg) reported an 80% reduction in T4 and FT4, and a 25% reduction in serum T3 {Martin et al., 2007, 758419}. This single-dose study supports the thyroid hormone level perturbations, specifically, the sensitivity of T4 and FT4, that are observed in the current literature update.

Table 8. Associations Between PFOA Exposure and Thyroid Hormone Effects in Rodents and Non-human Primates

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
TSH	NTP, 2019, 5400977	Sprague-Dawley rat	28 day	0, 0.625, 1.25, 2.5, 5.0, 10 mg/kg/day	M	↓ 5–10 mg/mg/day
				0, 0.625, 12.5, 25, 50, 100 mg/kg/day	F	↑ 6.25–100 mg/kg/day
	Butenhoff et al., 2002 1276161	Cynomolgus monkeys	26 weeks	0, 3, 10, or 30/20 mg/kg	M	↑ 3–10 mg/kg/day
T3 (Total)	Martin et al., 2007 758419	Sprague-Dawley rat	single dose	20 mg/kg/day	M	↓ 20 mg/kg/day
	NTP, 2019, 5400977	Sprague-Dawley rat	28 day	0, 0.625, 1.25, 2.5, 5.0, 10 mg/kg/day	M	↓ 0.625–5.0 mg/kg/day
				0, 0.625, 12.5, 25, 50, 100 mg/kg/day	F	n.s.
	Butenhoff et al., 2002 1276161	Cynomolgus monkeys	26 weeks	0, 3, 10, 30/20 mg/kg/day	M	↓ 30/20 mg/kg/day
FT3	Butenhoff et al., 2002 1276161	Cynomolgus monkeys	26 weeks	0, 3, 10, 30/20 mg/kg/day	M	↓ 30/20 mg/kg/day
T4 (Total)	Martin et al., 2007 758419	Sprague-Dawley rat	single dose	20 mg/kg/day	M	↓ 20 mg/kg/day

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
FT4	NTP, 2019, 5400977	Sprague-Dawley rat	28 day	0, 0.625, 1.25, 2.5, 5.0, 10 mg/kg/day	M	↓ 0.625–10 mg/kg/day
				0, 0.625, 12.5, 25, 50, 100 mg/kg/day	F	↓ 100 mg/kg/day
	Butenhoff et al., 2002 1276161	Cynomolgus monkeys	26 weeks	0, 3, 10, or 30/20 mg/kg	M	↓ 3–30/20 mg/kg/day
	Martin et al., 2007 758419	Sprague-Dawley rat	single dose	20 mg/kg/day	M	↓ 20 mg/kg/day
	NTP, 2019, 5400977	Sprague-Dawley rat	28 day	0, 0.625, 1.25, 2.5, 5.0, 10 mg/kg/day	M	↓ 0.625–10 mg/kg/day
				0, 0.625, 12.5, 25, 50, 100 mg/kg/day	F	↓ 100 mg/kg/day
	Butenhoff et al., 2002 1276161	Cynomolgus monkeys	26 weeks	0, 3, 10, or 30/20 mg/kg	M	↓ 10–30/20 mg/kg/day

TSH = thyroid stimulating hormone; T3 = triiodothyronine; T4 = thyroxine; M = male; F = female; n.s. = nonsignificant.

Perturbations in adrenal and pituitary hormone levels have been described primarily in rodent studies (Table 9). Loveless et al. (2008, 988599) reported elevations in serum corticosterone in male Crl:CD(SD)IGS BR rats and male Crl:CD-1(ICR)BR mice exposed to 10 or 30 mg/kg/day PFOA for 29 days, although statistically significant effects were only noted at the 10 mg/kg/day dose in mice. Increases in rats of the 10 and 30 mg/kg/day groups were 36% and 98% changes, respectively and in mice were 130% and 133% changes, respectively {Loveless et al., 2008, 988599}. Likewise, Sun et al. (2018, 50798020 found that serum corticosterone was elevated in male BALB/c mice exposed to 5 or 20 mg/kg/day PFOA for 28 days (146 and 175% changes, respectively). This study also quantified adrenocorticotrophic hormone (ACTH). A dose-dependent reduction in ACTH was observed, however significant effects were only observed at the 20 mg/mg/day dose (–26 and –58% changes in the 5 and 20 mg/kg/day groups, respectively) {Sun, 2018, 5079802}.

Table 9. Associations Between PFOA Exposure and Adrenocortical Hormone Effects in Rodents

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
CORT	Sun et al., 2018	BALB/c	28 days	0, 1.25, 5, 20 mg/kg/day	M	↑ 5 and 20 mg/kg/day
	Loveless et al., 2008	Sprague-Dawley rat	29 days	0, 0.3, 1, 10, 30, mg/kg/day	M	n.s.
		CD-1(ICR)BR mice	29 days	0, 0.3, 1, 10, 30, mg/kg/day	M	↑ 10 mg/kg/day

Endpoint	Study Name	Species	Exposure Length	Dose (mg/kg/day)	Sex	Change
ACTH	Sun et al., 2018	BALB/c	28 days	0, 1.25, 5, 20 mg/kg/day	M	↓ 20 mg/kg/day

ACTH = Adrenocorticotrophic Hormone; CORT = serum corticosterone; M = male; F = female; n.s. = nonsignificant.

3.3.6.2.3 Histopathology

In addition to the neoplastic lesions described in Section 3.3.17.2, several non-neoplastic lesions have been observed in the thyroid gland and adrenal glands (Figure 79).

3.3.6.2.3.1 Thyroid

In the 28-day exposure study, NTP (2019, 5400977) found higher incidences (8/10, minimal severity) of thyroid follicular cell hypertrophy in female rats following exposure to 100 mg/kg/day PFOA. Three of 10 high-dose males (10 mg/kg/day) also exhibited these abnormalities. No such lesions were observed in any of the other groups. Although statistical significance was not achieved, the presence of thyroid follicular cell hypertrophy in both males and females supports that it is likely an exposure-related effect {NTP, 2019, 5400977}.

In two chronic exposure studies {Butenhoff, 2012, 2919192; NTP, 2020, 7330145}, male and female Sprague-Dawley rats were fed diets containing PFOA for approximately two years. NTP (2020, 7330145) used a matrix-type exposure paradigm whereby pregnant rats were administered PFOA on GD6 and exposure was continued in offspring postweaning for a total of 107 weeks. Tissue sections from endocrine organs, including the thyroid gland, were analyzed for histology in both male and female offspring. Dose groups for this report are referred to as “[perinatal exposure level (ppm)]/[postweaning exposure level (ppm)]” (e.g., 300/1,000; see further study design details in Section 3.3.1.2.1.2).

In the thyroid gland, NTP (2020, 7330145) reported higher incidences of follicular cell hypertrophy in males from the 0/300 ppm group at the 16-week interim evaluation as well as the terminal evaluation. In females, higher incidences were noted in the 300/1,000 ppm group at the 16-week interim. No differences were observed between groups with combined perinatal and postweaning exposure compared to groups with postweaning exposure only {NTP, 2020, 7330145}. NTP (2020, 7330145) suggested the elevated incidence of follicular cell hypertrophy in males could be related to lower concentrations of circulating total T4 and T3, a result that was observed in the aforementioned NTP 28-day toxicity study {NTP, 2019, 5400977} but were not assessed in the chronic study. Similarly, Butenhoff et al. (2012, 2919192) observed increased incidences (13%, n = 49; compared to 2% in controls, n = 50) of thyroid c-cell hypertrophy in male rats exposed to 30 ppm PFOA for two years (equivalent to 1.3 mg/kg/day), although the effects did not reach statistical significance nor was there an increase in the 300 ppm males. Females had an apparent dose-dependent increase in follicular cell hypertrophy with an incidence of 0/50, 1/49, and 3/49 in the control, 30 ppm, and 300 ppm, respectively; however, the results were not statistically significant. Although there were sporadic occurrences of follicular cell hyperplasia in the males, there were no apparent treatment-related effects {Butenhoff, 2012, 2919192}.

3.3.6.2.3.2 Adrenal

In a chronic dietary study in rats, the incidence of adrenal gland hyperplasia was 18% (n = 50) in males exposed to 300 ppm PFOA compared to 4% in controls (n = 49), but the effect did not reach statistical significance {Butenhoff, 2012, 2919192}. A rat reproductive study by Butenhoff et al. (2004, 1291063) observed treatment-related microscopic changes in the adrenal glands of high-dose F₁ animals including cytoplasmic hypertrophy and vacuolation of the cells of the adrenal cortex following exposure to 3, 10, or 30 mg/kg/day {Butenhoff, 2004, 1291063}. In males, the cells of the adrenal glands were thicker, the zona glomerulosa was more prominent, and adrenal cortex cells were more vacuolized in 2/10 males from the 10 mg/kg/day exposure group and 7/10 males from the 30 mg/kg/day group. No effects were observed in females {Butenhoff, 2004, 1291063}. The adrenal glands appeared normal, and no histopathology was observed in a study of male cynomolgus monkeys administered up to 30 mg/kg/day PFOA for 6 months by oral tablet {Butenhoff, 2002, 1276161}, or the 28-day and chronic rat studies conducted by NTP (2019, 5400977; 2020, 7330145).

Non-neoplastic lesions in the pancreas are described in Section 3.3.17.2.

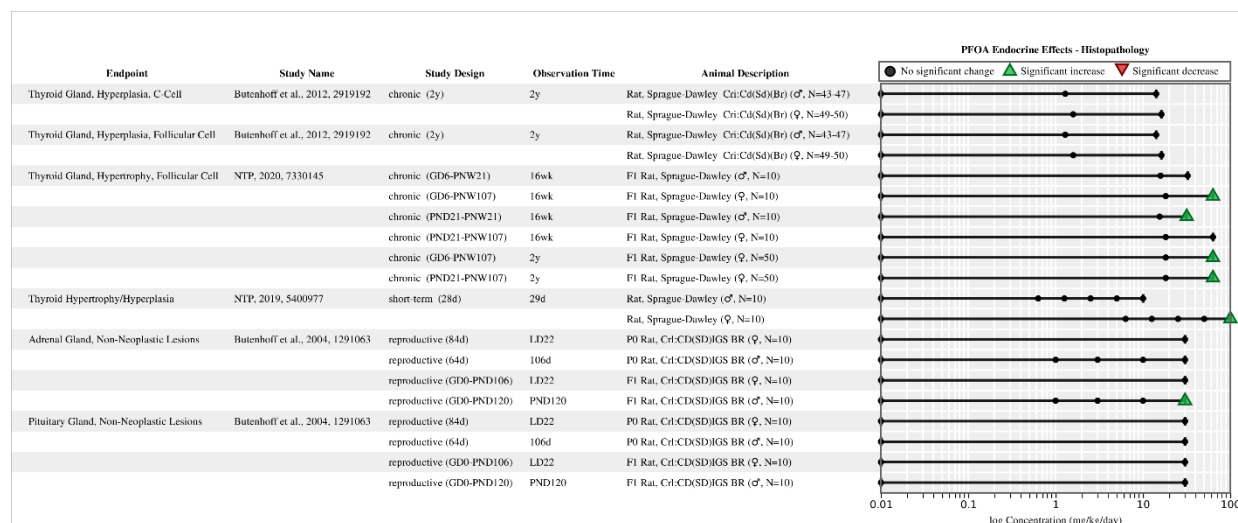


Figure 79. Endocrine Organ Histopathology in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA 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= Lactational Day; P₀ = parental generation; F₁ = first generation; d = day, wk = week; yr = year.

3.3.6.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse endocrine outcomes is discussed in Sections 3.3.2, 3.3.3, 3.3.4, and 3.4.1 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 29 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to endocrine effects. A summary of these studies is shown in Figure 80. **Error! Reference source not found..** Additional analysis on the mechanistic actions of PFOA on endocrine health outcomes is ongoing and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	0	1	1
Cell Growth, Differentiation, Proliferation, Or Viability	1	12	13
Cell Signaling Or Signal Transduction	2	15	15
Extracellular Matrix Or Molecules	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	3	4
Hormone Function	4	15	18
Xenobiotic Metabolism	1	4	5
Other	0	1	1
Not Specified (Review Article)	1	0	1
Grand Total	5	26	29

Figure 80. Summary of Mechanistic Studies of PFOA 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 PFOA exposure and endocrine outcomes, but the results are suggestive of positive associations for TSH, especially in adults, and for T4, especially in children. The 2016 Health Assessment found two studies supporting positive associations with thyroid disease and one study with negative associations. Similar to the 2016 Health Assessment, this updated review supports positive associations with thyroid disease (hypothyroidism). The most consistent effects were for T4 seen in children. One study reporting significant effects on TSH in children {Aimuzi, 2019, 5387078} conducted multi-pollutant models including other measured PFAS (i.e., PFOS, PFNA, PFDA, PFUA, PFHxS, PFDoA, and perfluorobutane sulfonate (PFBS)). PFOA was moderately correlated with other PFAS ($r = 0.23$ – 0.56) in cord blood, and estimates were found to be largely unchanged in multipollutant models. Most results in general population studies indicated positive associations for TSH. Many *high* and *medium* confidence studies generally did not observe significant associations with endocrine outcomes. Several *low* confidence studies found significant associations, but the interpretation of these results is limited by several factors related to study quality. Additional uncertainty exists due to the potential for confounding by other PFAS.

Findings on associations between PFOA and endocrine outcomes are inconsistent among *high* and *medium* confidence studies. As a result, no studies or endpoints identified in the epidemiological literature were considered for the derivation of PODs.

However, the available evidence from animal studies supports that the endocrine system is a target of PFOA toxicity. The strongest evidence of endocrine effects is from perturbations in

hormones related to the thyroid gland. Thyroid hormones appear to be sensitive to PFOA exposure but exhibit highly complex responses depending on sex, species, and exposure duration. Perturbations were observed in both sexes, sometimes with opposite effects between the sexes (in the case of TSH). Reductions in free and total T4 as well as total T3 were noted in both rodents and chronically exposed non-human primates that in some cases (female rats, male non-human primates) coincided with compensatory increases in TSH, indicative of classical hypothyroidism. Reductions in free and total T4, as well as declines in TSH in male rats may suggest hypothyroxinemia. Elevations in thyroid gland weight were also noted {Butenhoff, 2012, 2919192} in males, as well as increases in thyroid gland follicular cell hypertrophy in male and female rats {NTP, 2019, 5400977; NTP, 2020, 7330145}, however, the hormones released from the respective organs (i.e., T4 and FT4) may be more sensitive and direct indicators of toxicity. Thyroid hormones influence numerous other body systems, notably the nervous system via the hypothalamic-pituitary-thyroid (HPT) axis, thus effects on other systems may stem from thyroid-specific targets and vice versa. The available animal evidence supports evidence from human epidemiological studies indicating that PFOA exposure may affect T4 in children. Therefore, EPA concluded that changes in thyroid hormone levels in animals indicate toxicity of relevance to humans exposed to PFOA. Changes in thyroid hormones in animal models were considered for the derivation of PODs.

Elevations in corticosterone were noted across two animal studies {Sun et al., 2018, 5079802; Loveless et al., 2008, 988599} using male rodents, which coincided with a reduction in ACTH in one study {Sun et al., 2018, 5079802}. Such effects may indicate adrenocortical toxicity, which can involve increased secretion of endogenous glucocorticoids and long-loop feedback on the hypothalamic-pituitary-adrenal (HPA) axis to reduce ACTH levels {Harvey, 2016, 1201708}. However, 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 PFOA on adrenocortical hormone levels. Given the perturbations of adrenocortical hormones and thyroid hormones, it is crucial to interrogate the interaction of multiple systems in order to evaluate potential dysregulation of the HPA axis and/or HPT axis. Although changes in corticosterone may indicate toxicity of relevance to humans exposed to PFOA, EPA is not considering these endpoints for derivation of PODs since the relevance of an effect of PFOA on adrenocortical hormone levels is not well understood.

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 ≥ 200 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 PFOA concluded that there is no evidence of an association between PFOA and diabetes, metabolic syndrome, or related outcomes. No associations were observed between mean serum PFOA up to 91.3–113.0 ng/mL and type 2 diabetes incidence in high-exposure (C8 Health Project) {MacNeil et al., 2009 2919319} or occupational populations {Steenland et al., 2015 2851015}. Additionally, the C8 Science Panel (2012), based on combined data from high-exposure and worker cohorts, concluded that there was no probable link between PFOA and type II diabetes. One general population study observed an increased risk of gestational diabetes in women with a mean pre-pregnancy serum PFOA level of 39.4 ng/mL {Zhang et al. 2015 2857764}. Serum PFOA was significantly positively associated with beta-cell function, but not associated with metabolic syndrome, metabolic syndrome waist circumference, glucose concentration, homeostasis model of insulin resistance, or insulin levels in adults or adolescents from NHANES {Lin et al., 2009, 1290820}. No association was observed between serum PFOA concentrations {Nelson et al., 2010, 1291110} and insulin resistance. Another study reported no association between PFOA and metabolic syndrome in adolescents or adults {Lin et al., 2009 1290820}. Overall, these studies show a lack of association of PFOA with diabetes, metabolic syndrome, and related outcomes.

For this updated review, 72 new epidemiologic studies examined the association between PFOA and metabolic outcomes. Of these, 35 were cohort studies, 6 were case-control studies, 26 were cross-sectional studies, 2 were nested case-control studies, and 3 were controlled trials. Most studies measured exposure to PFOA using biomarkers in blood. One study measured exposure to PFOA using biomarkers in blood and in semen {Di Nisio, 2019, 5080655}. Biomarkers in maternal blood were used in 16 studies and cord blood was used in two studies. Shapiro et al. (2016, 3201206) measured exposure to PFOA in urine and Mancini et al. (2018, 5079710) estimated dietary exposure to PFOA. 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-four studies examined diabetes (1 in children, 9 in pregnant women), and four studies 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 for 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, Homeostatic Model Assessment of Beta-Cell Function (HOMA-B), or Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) without consideration of diabetes status, since the treatment of diabetes, particularly in those being treated with hypoglycemic medications, influences insulin production and secretion.

Based on these considerations, 9 studies were classified as *high* confidence for all metabolic outcomes, 40 as *medium* confidence for all metabolic outcomes, two as *medium* confidence for one outcome (anthropometric measurements or diabetes) and *low* confidence for multiple other outcomes, two as *medium* confidence for one outcome (metabolic syndrome or metabolic function) and *low* confidence for one other (adiposity or insulin resistance), 15 as *low* confidence for all metabolic outcomes, and 4 were considered *uninformative* for all outcomes. (Figure 81, Figure 82, Figure 83). One study (Liu et al., 2018, 4238396) was considered *uninformative* for insulin resistance, and medium confidence for other metabolic outcomes.

Uninformative studies had critical deficiencies in at least one domain. These deficiencies included a lack of control for confounding {Predieri 2015 3889874; Huang, 2018, 5024212; Jiang, 2014, 2850910}, lack of fasting measures for glucose measurements {Jiang, 2014, 2850910}, and treating PFOA 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 et al., 2015 3889874}.

The most common reason provided for a *low* confidence rating was potential for residual confounding, particularly by SES {Christensen et al. 2016 3858533; Fassler et al., 2019 6315820; Heffernan et al., 2018 5079713; Koshy et al., 2017 423878; Lin et al., 2013 2850967; Convertino et al., 2018 5080342; Khalil et al. 2018 4238547}, by adiposity {Lin, 2013, 2850967}, by age {Koshy, 2017 4238478}, or by diabetes status {Lind et al., 2014 2215376}. *Low* confidence studies presented concerns with the outcome measures including potential for outcome misclassification {Christensen et al. 2016 3858533; He et al., 2018 4238388; Steenland, Zhao, and Winquist 2015 2851015; Zong, 2016, 3350666}, failing to account for diabetes status {Lind et al., 2014 2215376} or use of medications that would impact insulin levels or beta-cell function {He et al., 2018 4238388; Fleisch et al., 2017 3858513}, analytical methods {Koshy et al. 2017 423878}, and failure to establish temporality between PFOA exposure and diabetes {Lind et al., 2014 2215376}. Other concerns included selection bias {Fassler et al., 2017 6315820}, which resulted from self-selection {Christensen et al., 2016 3858533}, failure to provide information on control group selection {Heffernan et al., 2018 5079713}, differential recruitment for cases and controls {Lin et al., 2013 2850967}, or survival bias {Steenland, Zhao, and Winquist 2015 2851015}. Small sample size was also a concern in some studies {Christensen et al., 2016 3858533; Heffernan et al., 2018 5079713; Khalil et al., 2018 4238547}. 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.

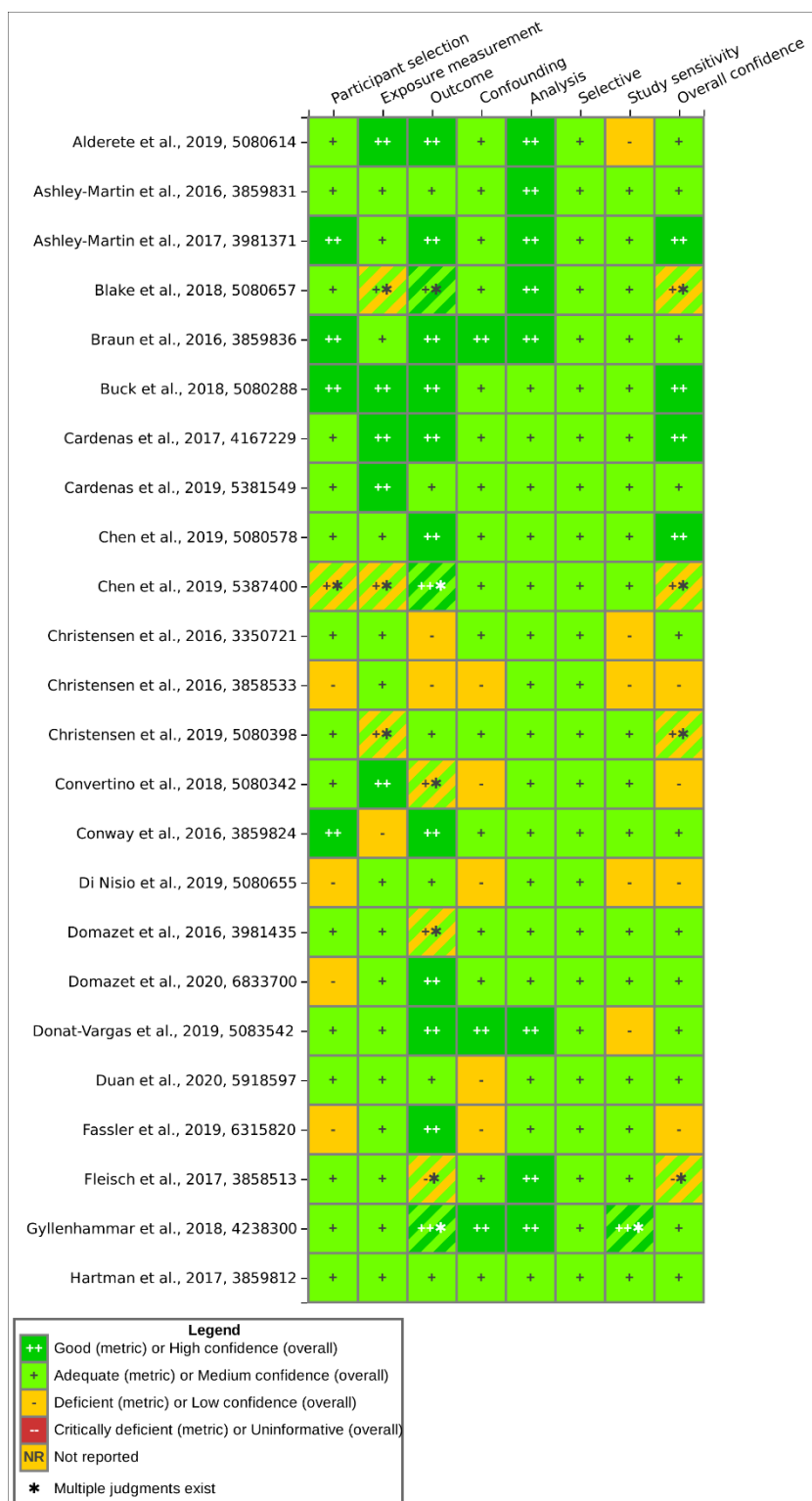


Figure 81. Summary of Study Evaluation for Epidemiology Studies of PFOA and Metabolic Effects

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

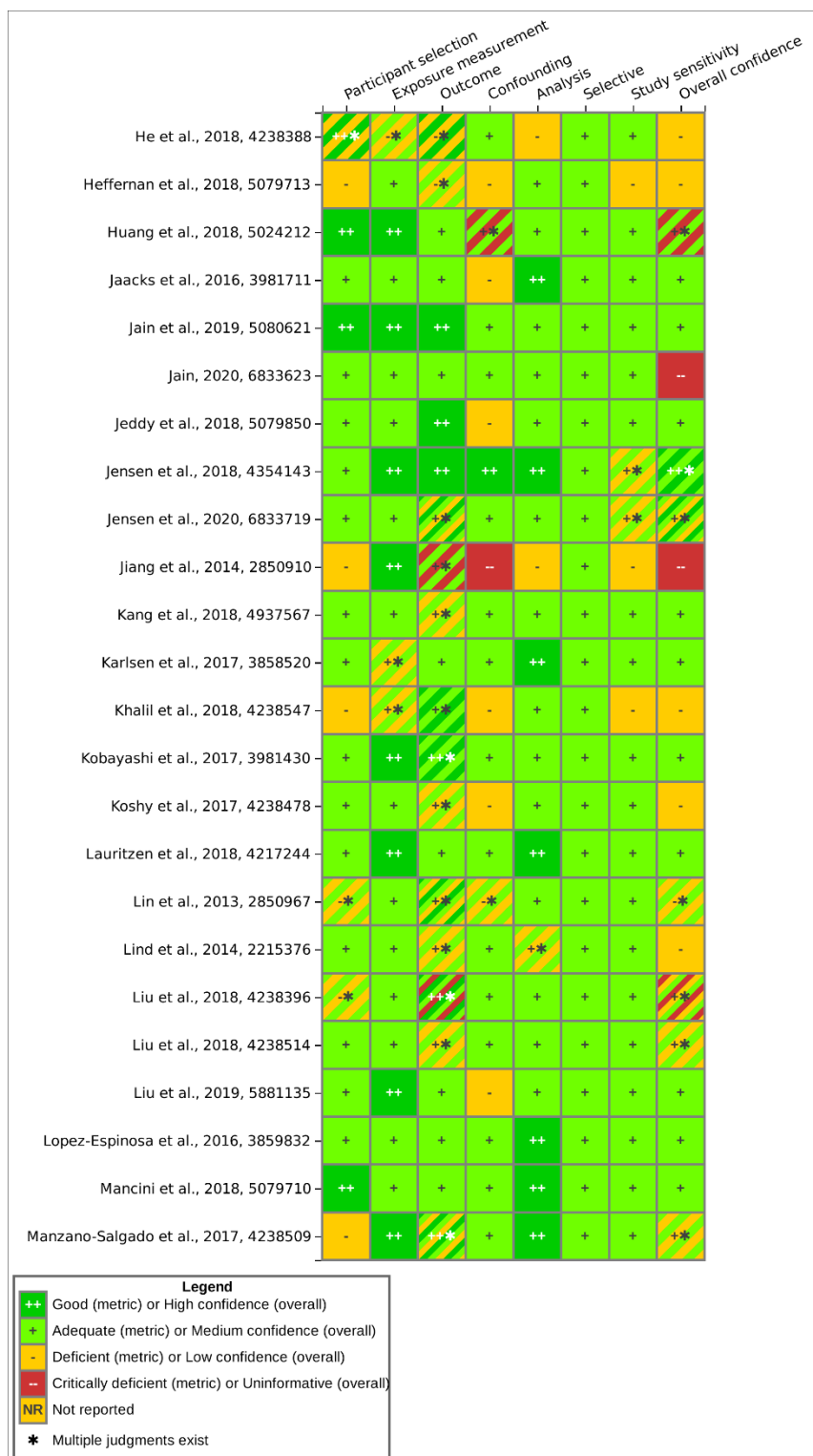


Figure 82. Summary of Study Evaluation for Epidemiology Studies of PFOA and Metabolic Effects (Continued)

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



Figure 83. Summary of Study Evaluation for Epidemiology Studies of PFOA and Metabolic Effects (Continued)

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

3.3.7.1.3 Findings from Children and Adolescents

Two *medium* and two *low* confidence studies examined blood glucose in children, and only one reported a positive association with 2-hour glucose. No associations were observed for fasting glucose. Alderete et al. (2019 5080614) examined a cohort of obese Hispanic children aged 8–14, from the SOLAR Project and observed a significant association with 2-hour glucose, but no association with fasting glucose. Two cross-sectional studies reported positive non-significant associations with fasting glucose, one *medium* confidence study in 3–18-year-old Koreans {Kang et al., 2018 4937567}, and one *low* confidence study in American obese 8–12 years {Khalil et al., 2018 4238547}. Another cross-sectional study in girls ages 6–8 years from the Breast Cancer and Environment Research Program reported a negative, non-significant association with glucose levels {Fassler et al., 2019 6315820}.

One *medium* confidence study observed positive, non-significant associations with blood glucose levels at age 15, using PFOA measured at ages 9 and at age 15 {Domazet et al., 2016 3983465}. A non-significant negative association was observed between PFOA measured at age 15 and blood glucose measured at age 21 {Domazet et al., 2016 3983465}.

Three studies examined the association between PFOA and insulin levels and reported no associations. One *medium* confidence study reported a positive, non-significant association with fasting insulin in obese Hispanic children aged 8–14 {Alderete et al., 2019 5080614}. In contrast, two *low* confidence studies reported negative non-significant associations between PFOA and fasting insulin {Fassler et al., 2019 6315820; Khalil et al., 2018 4238547}.

Insulin resistance, as described by the HOMA-IR, was examined in five studies with mixed results. Alderete et al. (2019 5080614) observed a positive, non-significant association, while four *low* confidence studies reported non-significant negative associations (i.e., decreasing insulin resistance with increasing serum PFOA) {Khalil et al., 2018; Fassler et al., 2019 6315820; Koshy et al., 2017 4238478; Fleisch et al., 2017 3858513}.

A positive, but non-significant association was observed between PFOA and 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 {Fassler et al., 2019 6315820}.

One *medium* confidence study reported negative associations with insulin-like growth factor 1 (IGF-1) in 6–9-year-old children in the C8 Health Project {Lopez-Espinosa et al., 2016 3859832}. There was a significant negative association with IGF-1 in girls, and a significant negative association with IGF-1 in the second quartile of PFOA exposure among boys {Lopez-Espinosa et al., 2016 3859832}.

Adiponectin and leptin were both examined in a *medium* confidence study from the European Youth Study, and non-significant associations were observed with adiponectin (positive), and leptin (negative) {Domazet et al., 2020 6833700}. Similarly, Fleisch et al., (2017, 3858513) reported a non-significant negative association with leptin in both early- and mid-childhood. Positive, non-significant association was observed between maternal blood PFOA and cord blood adiponectin {Ashley-Martin et al., 2017 3981371; Minatoya et al., 2017 3981691}.

Three studies examined adiposity, and one reported a significant negative association with fat mass. One *low* confidence study observed a significant negative association with log fat mass and fat mass percentage in girls ages 6–8 years {Fassler et al., 2019 6315820}. However, concerns about selection bias and residual confounding by SES limit confidence in these results.

Chen et al., {2019, 5080578} observed a positive, non-significant association with children's body fat percentage; non-significance persisted after stratification by child sex. Non-significant negative associations were observed in the third tertile of PFOA exposure for girls and in the second tertile of PFOA exposure for boys {Chen et al., 2019 5080578}. Similarly, a positive, non-significant association was observed with children's body fat mass, and non-significance persisted after stratifying by child sex {Chen et al., 2019 5080578}. In a tertile analysis, positive, non-significant associations were observed in the third tertile of PFOA exposure for all children and in the third tertile of PFOA exposure for boys; negative, non-significant associations between PFOA and body fat mass were observed in the second and third tertiles of PFOA exposure among girls {Chen et al., 2019 5080578}. A *medium* confidence cross-sectional study of 9-year-old children in the European Youth Heart Study reported a negative non-significant association with fat mass {Domazet et al., 2020 6833700}.

Seven studies examined BMI measures, with mixed results. Four studies observed no associations with BMI, and two observed associations with BMI-z-score.

One *high* confidence study examined the association between cord blood PFOA and age 5 BMI in the Shanghai Prenatal Cohort {Chen, 2019 5080578}. There was a negative but non-significant association between PFOA and BMI (i.e., decreased BMI with higher PFOA exposure levels). The effect was larger in females (beta = 0.07, 95% CI: -0.4, 0.53) than for males (beta = 0.2, 95% CI: -0.3, 0.69). Results from a tertile analysis were also non-significant, even after stratification by sex. For females, BMI increased with increasing tertiles of PFOA, while BMI decreased with increasing tertiles of PFOA in males. {Chen, 2019, 5080578}. Two *medium* confidence studies observed positive, non-significant associations with BMI {Manzano-Salgado et al., 2017 4238509; Braun et al. 2016 3859836}. In a sex-stratified analysis, the association between maternal blood PFOA and BMI at age 7 remained positive among boys but became negative among girls {Manzano-Salgado et al., 42358509}.

Of the three *low* confidence studies examining BMI, two reported positive, non-significant associations {Di Nisio et al., 2019 5080655; Koshy et al., 2017 4238478}, and one reported a negative non-significant association with BMI {Khalil et al., 2018 4238547}.

Six studies examined BMI z-score, two of which reported significant negative associations. Two studies from the Breast Cancer and the Environment Research Program (one *medium*, one *low* confidence) observed significant negative associations with BMI z-score in girls ages 6–8 {Pinney et al., 2019 6315819; Fassler et al., 2019 6315820}. Pinney et al. (2019 6315819) observed a significant negative association with BMI z-score in girls living in the Greater Cincinnati and the San Francisco Areas. Karlsen et al., (2017,3858520) observed a non-significant negative association with BMI z-score at 18 months and age 5. 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 and 3 and 4-years old children; the association with BMI z-score among 5-year-old children was positive, but not significant.

Additionally, a non-significant association was observed with BMI z-score in early- and mid-childhood {Mora et al., 2017 3859823}. Another *low* confidence study reported a negative, non-significant association with BMI z-score {Koshy et al., 2017 4238478}.

A *medium* confidence study reported a weak non-significant negative association between serum PFOA levels and ponderal index at birth in infants from the Hokkaido Study on Environment and Children's Health {Kobayashi et al., 2017 3981430}.

No associations were observed in two *low* confidence studies examining body weight {Fassler et al., 2019 6315820} or being overweight {Koshy et al., 2017 4238478}.

Four studies examined waist measurements, and two reported associations. Two studies (one *medium*, one *low* confidence) observed a significant negative associations with waist-to-height ratio (i.e., increased waist-to-height ratio as a continuous measure with higher serum PFOA exposure levels) in girls ages 6–8 {Pinney et al., 2019 6315819; Fassler et al., 2019 6315820}. However, one *high* confidence study observed a positive non-significant association between cord blood PFOA and waist-to-height ratio in girls ages 5 years {Chen et al., 2019 5080578}. Negative non-significant associations were observed for all children combined, and in boys, and a non-significant decreasing trend was observed {Chen et al., 2019 5080578}.

Two studies (one *medium* and one *low* confidence) examined waist-to-hip ratio. The *medium* confidence study observed a non-significant negative association {Pinney et al., 2019 6315819} between PFOA and waist-to-hip ratio, while the *low* confidence study reported a non-significant positive association between PFOA and waist-to-hip ratio {Fassler et al., 2019 6315820}. One *medium* confidence study reported a positive, non-significant association with waist-to-hip circumference. After stratification by sex in the early childhood analysis, a non-significant negative association was observed among girls. In the mid-childhood analysis, the increase in waist-to-hip circumference ratio was greater for girls than for boys {Mora et al., 2017 3859823}.

One *high*, two *medium*, and one *low* confidence study examined waist circumference and reported one association {Hartman et al. 2017 3859812}. The *medium* confidence study, from the ALSPAC, assessed data from mother-daughter pairs and observed a significant decrease in female children's waist circumference {Hartman et al. 2017 3859812}. Two *medium* confidence studies {Chen et al. 2019 5080578; Mora et al., 2017 3859823} reported a positive, non-significant association between PFOA and waist circumference. After stratification by sex, non-statistical significance persisted; associations remained negative for males but were positive for females {Chen et al. 2019 5080578}. A cohort study of maternal-child pairs from the European Youth Heart Study reported a non-significant percent decrease in waist circumference at 21 years old with PFOA exposure at age 9 and age 15, and a significant percent decrease in waist circumference at 21 years old with concurrent PFOA exposure, and a non-significant percent increase in waist circumference at age 15 with age 9 PFOA exposure {Domazet et al., 2016 3981435}.

In the *low* confidence study, Di Nisio et al. (2019 5080655) reported a significant difference between mean waist circumference of Italian male high school students exposed to PFOA pollution compared to those who were not exposed {Di Nisio et al., 2019 5080655}.

There were three studies, each of *medium* confidence, measuring the association between PFOA and skinfold thickness. A *medium* confidence study from the SGA Study reported a non-significant positive association with tricep skinfold z-score, and a non-significant negative association with subscapular skinfold thickness z-score among 412 children {Lauritzen et al., 2018 4217244}.

Another cohort study, which used a subset of data on children from the European Youth Heart Study, observed a non-significant percent increase in skinfold thickness at age 15 for increases in PFOA exposure at 9 years old, as well as a non-significant percent increase in skinfold thickness at age 21 for increases in PFOA exposure at 9 years old. However, there was a non-significant percent decrease in skinfold thickness at 21 years old with increase in PFOA exposure from 15 years old {Domazet et al., 2016 3981435}.

A cohort study of mother-child pairs was used to assess the association between maternal PFOA and skinfold thickness {Mora et al., 2017 3859823} There was a positive, non-significant association between PFOA and subscapular-to-triceps skinfold thickness ratio measured in both early childhood and mid-childhood. After stratification by sex, the effect increased for females, but decreased non-significantly for males during both early- and mid-childhood. Similarly, the association between PFOA and the sum of subscapular and tricep skinfold thickness during mid-childhood decreased for males but increased for females when stratified by sex, but the sum of subscapular and tricep skinfold thickness during early childhood decreased for females and increased for males when stratified by sex {Mora et al., 2017 3859823}.

3.3.7.1.4 Findings from Pregnant Women

Eleven studies examined gestational diabetes, and one reported a negative association between PFOA and gestational diabetes.

A *medium* confidence study of adults aged 20–60 living in Taiwan reported a significant negative association with gestational diabetes {Su et al., 2016 3860116}.

In a *high* confidence cohort study from Project Viva of pregnant women, Preston et al., 2020 (6833657) reported a non-significant, null association with gestational diabetes (OR = 1.0; 95% CI = 0.6, 1.6), but non-significant increased odds of gestational diabetes with increasing quartiles of PFOA {Preston et al., 2020 6833657}.

Two *medium* confidence case-control studies reported increased, non-significant odds of gestational diabetes {Wang et al., 2018 5079666; Xu et al., 2020 6833677}. In pregnant women with no family history of diabetes Liu et al., 2019 588135 reported a non-significant, positive association between m-PFOA or L-PFOA and odds of gestational diabetes {Liu et al., 2019 588135}. Increased, non-significant odds of gestational diabetes were observed in the second and third tertiles of L-PFOA exposure, and in the third tertile of m-PFOA exposure; decreased, non-significant odds of gestational diabetes were observed in the second tertile of m-PFOA exposure {Liu et al., 2019 588135}.

A study from the NICHD Fetal Growth Study reported a non-significant increased risk of gestational diabetes among all women, women with a family history of type 2 diabetes, and women with an overweight pre-pregnancy BMI {Rahman et al., 2019 5024206}. A non-significant decreased risk of gestational diabetes was observed among pregnant women without a

family history of type 2 diabetes and among women who did not have an overweight pre-pregnancy BMI {Rahman et al., 2019 5024206}.

Three *medium* and one *low* confidence studies reported negative, non-significant associations with gestational diabetes {Shapiro et al., 2016 3201206; Wang et al., 2018 5080352; Valvi et al., 2017 3983872; Zong et al., 2016 3350666}.

Seven studies evaluated blood glucose and related measures, with mixed results. Two studies reported an association with oral glucose tolerance test results; no associations were reported for fasting glucose, impaired glucose tolerance, or hyperglycemia.

A medium confidence study of pregnant women with and without gestational diabetes reported increased, but non-significant odds of increased fasting blood glucose with increasing tertiles of n-PFOA {Wang et al., 2018 5079666}. Liu et al. (2019 5881135) observed a positive, non-significant associations between both sum m-PFOA and L-PFOA and fasting glucose. Three *medium* confidence cohort studies observed negative, non-significant associations with fasting blood glucose {Wang et al., 2018 5080352; Jensen et al., 2018 4354143; Starling et al., 2017 3858473}.

Overall oral glucose tolerance test results were evaluated in one study {Wang et al., 2018 5080352}. When modeled continuously, there was a positive, non-significant association between PFOA and OGTT glucose. No significant difference was observed in mean oral glucose tolerance test results between tertiles of PFOA {Wang et al., 2018 5080352}.

Two *medium* confidence studies examined 1-hour blood glucose, and both reported positive significant associations. Ren et al. (2020 6833646) observed a significant increase in 1-hour plasma glucose levels and Liu et al. (2019, 5881135) reported a significant positive association between serum L-PFOA and glucose homeostasis at 1-hour, and a negative, non-significant association between sum m-PFOA and 1-hour glucose.

Two *medium* confidence studies examined 2-hour blood glucose. A significant positive association was observed between L-PFOA and 2-hour glucose, but the positive association between sum m-PFOA and 2-hour glucose was not significant {Liu et al., 2019 5881135}. A *medium* confidence study from the Odense Child Cohort reported a negative non-significant association between serum PFOA and 2-hour glucose among 158 women at high risk for gestational diabetes {Jensen et al., 2018 4354143}.

Three studies examined impaired glucose tolerance. In a subset of women from Project Viva Preston et al., 2020 6833657 observed decreased, non-significant odds of impaired glucose tolerance. This was also observed in a tertile analysis, but the odds of impaired glucose tolerance were greater with increasing tertiles of PFOA {Preston et al., 2020 6833657}. A medium confidence study also reported decreased odds of impaired glucose tolerance {Shapiro et al., 2016 3201206}.

The single low confidence study observed non-significant increased odds of impaired glucose tolerance with PFOA increasing continuously, but non-significant decreased odds of impaired glucose tolerance with increasing quartiles of PFOA {Matilla-Sandtander et al., 2017 4238432}.

One *high* confidence study examined isolated hyperglycemia in pregnant women from the Project Viva cohort {Preston et al., 2020 6833657}. When analyzed continuously, increasing PFOA did not affect the odds of hyperglycemia. A quartile analysis showed non-significant decreased odds of hyperglycemia with increasing quartiles of PFOA {Preston et al., 2020 6833657}.

Two studies (one *high* confidence and one *medium* confidence) evaluated blood glucose levels {Preston et al., 2020 6833657; Ren et al., 2020 6833646}. Both studies reported a non-significant positive association with blood glucose levels. After stratifying by age, Preston et al. (2020 6833657) reported a non-significant negative association with blood glucose among women aged 35 and older. In the *medium* confidence study, results from an age-stratified analysis showed non-significant decreased odds of high plasma glucose for women at 20–23 gestational weeks {Ren et al., 2020 6833646}.

Two studies evaluated insulin resistance measures; neither reported any associations.

There were two studies of *medium* confidence evaluating insulin levels {Jensen et al., 2018 4354143; Wang et al., 2018 5080352}. One of these studies reported a non-significant negative association with fasting insulin levels {Jensen et al., 2018 4354143}, while the other observed a non-significant positive association with fasting insulin levels {Wang et al., 2018 5080352}.

Two *medium* confidence studies assessed insulin resistance. One reported a non-significant negative association {Jensen et al., 2018 4354143}, while the other observed a non-significant positive association {Wang et al., 2018 5080352} with insulin resistance. Wang et al. (2018 5080352) reported no significant difference in mean insulin resistance between tertiles of PFOA.

One *medium* confidence study evaluated insulin sensitivity (measured using the Matsuda index) and observed a positive, non-significant association {Jensen et al., 2018 4354143}.

A non-significant percent decrease in beta-cell function was observed {Jensen et al., 2018 4354143}.

Adiponectin and leptin were both examined in a *high* confidence study from Project Viva, and no significant associations were observed. A non-significant negative association with adiponectin and a non-significant positive association with leptin were reported {Mitro et al., 2020 6833625}. After stratification by age during pregnancy, non-significant positive associations with leptin persisted; a positive, non-significant association with adiponectin was observed among women under age 35 during pregnancy {Mitro et al., 2020 6833625}.

Three *medium* confidence cohort studies examined gestational weight gain, with one reporting an association. Ashley-Martin et al., 2016 3859831 used data from mother-infant pairs from the Maternal-Infant Research on Environmental Chemicals (MIREC) to estimate the odds of having high cord blood PFOA (>0.39 ng/mL) per increase in gestational weight gain. ORs were significant for both 1 kg increase in gestational weight gain and IQR increase in gestational weight gain {Ashley-Martin et al., 2016 3859831}.

Jaacks et al. (2016 3981711) observed a positive, non-significant association with gestational weight gain among 218 mothers, mothers with a BMI <25 ; a negative association was reported among mothers with a BMI ≥ 25 . Increased, non-significant odds of excessive gestational weight

gain were observed with increasing PFOA and decreased, non-significant odds of inadequate weight gain were reported {Jaacks et al., 2016 3981711}.

Another study reported a positive, non-significant association with gestational weight gain among all women who were underweight or of normal weight and among under- or normal-weight mothers of daughters. Negative, non-significant associations with gestational weight gain were observed among overweight or obese mothers of all children, of boys, and of girls, and among normal or underweight mothers of sons {Marks et al., 2019 5381534}.

One study evaluated anthropometric measurements and PFOA from the Project Viva cohort study and followed 801 pregnant women to 3 years postpartum {Mitro et al., 2020 6833625}. Positive, non-significant associations were reported with 3-year postpartum arm circumference, subscapular skinfold thickness, tricep skinfold thickness, and 3-year postpartum waist circumference. After stratification by age during pregnancy, there was a significant increase in waist circumference measured at 3 years postpartum among women who were 35 or older during pregnancy {Mitro et al., 2020 6833625}.

One *high* confidence cohort study evaluated BMI. A significant positive association with BMI among 786 pregnant women was reported {Mitro et al., 2020 6833625}. Statistical significance did not persist after stratification by age (under 35/age 35 and older) {Mitro et al., 2020 6833625}.

3.3.7.1.5 Findings from the General Adult Population

Eight studies investigated the relationship between PFOA and diabetes in the general population, and three reported a positive association.

A *medium* confidence study from the E3N cohort reported a non-significant increased risk of type 2 diabetes in the 7th and 8th deciles of PFOA exposure, and increased risk of type 2 diabetes was observed in the 4th–6th deciles of PFOA exposure. {Mancini et al., 2018 5079710}. Another *medium* confidence study, from the Nurses' Health Study II, reported a significant association with type 2 diabetes among female nurses {Sun et al., 2018 4241053}.

One *high* confidence cohort study from the Diabetes Prevention Program followed adults at increased risk of type 2 diabetes and observed an increased, but non-significant risk of diabetes per doubling of PFOA {Cardenas et al., 2017 4167229; Cardenas et al., 2019, 5381549}. After stratification by sex, a non-significant negative association was observed among men {Cardenas et al., 2017 4167229}. Non-significant negative associations were also observed in analyses by tertiles {Cardenas et al., 2019, 5381549}.

Another *medium* confidence study reported non-significant increased odds of type 2 diabetes were observed in the 2nd tertile of PFOA exposure, while non-significant decreased odds were observed in the 3rd tertile of PFOA exposure {Donat-Vargas et al., 2019 598342}.

Significant decreased odds of type 1, type 2, and uncategorized diabetes were observed in participants in the C8 Health Project {Conway et al., 2016 3859824}. After stratifying by age, significant decreased odds of type 1, type 2, and uncategorized diabetes were observed among adults. Significant decreased odds of type 1 diabetes were observed for children with type 1

diabetes, but non-significant increased odds of type 2 and uncategorized diabetes were observed among children {Conway et al., 2016 3859824}.

Among the three *low* confidence studies, one reported a non-significant negative association with diabetes {Lind et al., 2014 2215376}, while two overlapping NHANES studies reported non-significant positive associations with diabetes {He et al., 2018 4238388} and prediabetes {Christensen et al., 2016 3858533}. Significantly increased odds of diabetes were observed for males, non-significant increased odds were observed for females {Christensen et al., 2016 3858533}. *Low* confidence ratings resulted from concerns with potential for outcome misclassification {Christensen et al., 2016 3858533; He et al., 2018 4238388}, self-selection into the study, residual confounding by SES {Christensen et al., 2016 3858533}, and failure to establish temporality between exposure and outcome {He et al., 2018 4238388}.

Four studies (three *medium* confidence and one *low* confidence) evaluated metabolic syndrome; 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 MetS as defined by the Adult Treatment Panel III criteria (OR = 1.89, 95% CI: 0.93, 3.86). Two *medium* confidence studies used overlapping data from NHANES and reported non-significant negative associations with metabolic syndrome. Liu et al., (2018 4238514) 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.

A *low* confidence study observed significant increased odds of metabolic syndrome for participants with serum n-PFOA >1.90 ng/mL compared to those with serum PFOA ≤1.90 ng/mL {Yang et al., 2018 4238462}. However, concerns for selection bias, outcome misclassification, and residual confounding by SES diminish confidence in the study results.

There were five studies examining the association between PFOA and glucose, and three reported associations with fasting blood glucose, and one reported an association with 2-hour glucose.

A *medium* confidence study of adults aged 19-87 years from China reported a significant positive association with fasting blood glucose {Duan et al., 2020 5918597}. Similarly, a study using NHANES data on adults from 1999-2014 observed a significant positive correlation between fasting glucose and serum PFOA {Huang et al., 2018 5024212}. Su et al. (2017 38606116) reported a statistically significant decrease in fasting blood glucose for both increasing quartiles of PFOA and per doubling of PFOA among Taiwanese adults aged 20-60.

Another cohort study, which followed adults at high risk of type 2 diabetes, observed a positive, non-significant increase in 30-minute glucose per doubling of PFOA, while a negative, non-significant association was observed between with 2-hour glucose {Cardenas et al., 2017 4167229}. A non-significant negative association with 2-hour glucose was reported per doubling in PFOA among Taiwanese adults aged 20-60, but a significant decrease in 2-hour glucose was observed for increasing quartiles of PFOA {Su et al., 201738606116}.

One study reported non-significant decreased odds of elevated glucose with increasing tertiles of PFOA {Christensen et al., 2019 5080398}. Odds were adjusted for PFDA, PFOS, PFHxS, 2-(N-methyl-PFOSA) acetate (MPAH), PFNA, perfluoroundecanoic acid (PFUnDA) simultaneously.

The association between PFOA and resting metabolic rate was assessed in the POUNDS LOST trial, a clinical trial of overweight and obese adults aged 30–70. A non-significant positive correlation between PFOA and resting metabolic rate was observed {Liu et al., 2018 4238396}. In the first 6 months of the trial, resting metabolic rate decreased non-significantly across all tertiles of PFOA exposure for both men and women. Neither the trend across tertiles nor the interaction between PFOA and sex were significant {Liu et al., 2018 4238396}. In months 6–24 of the trial, resting metabolic rate decreased significantly for males, and non-significantly for females. No statistical significance was observed for the interaction between PFOA and sex {Liu et al., 2018 4238396}.

Twelve studies examined insulin resistance measures; of these studies, one found reported significant associations with fasting insulin, insulin resistance, insulinogenic index 1, fasting plasma insulin, 30-minute insulin, fasting proinsulin, and insulin (corrected response), and one reporting associations with the ratio of proinsulin to insulin.

The single *high* confidence study used a subset of data on adults at high risk of type 2 diabetes from the Diabetes Prevention Program {Cardenas et al., 2017 416722}. A positive, significant association was observed between PFOA and fasting insulin {Cardenas et al., 2017 416722}. Two *low* confidence studies examined fasting insulin, and both reported non-significant negative associations with fasting insulin {Chen et al., 2019 5387400; He et al., 2018 4238388}.

Two *medium* confidence studies reported negative, non-significant associations with insulin levels {Sun et al., 2018 4241053; Domazet et al., 2016 3981435}. In contrast, another *medium* confidence observed a positive, non-significant association with insulin levels {Liu et al., 2018 4238514}.

Nine studies examined insulin resistance, 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 statistically significant, positive association with insulin resistance {Cardenas et al., 2017 4167229}. A *medium* confidence study of adults in NHANES observed a non-significant increase in insulin resistance with increase in PFOA {Liu et al., 4238514}. However, Donat-Vargas et al., 2019 50803542 reported a non-significant negative association with insulin resistance in both continuous and tertile analyses. In a sensitivity analysis, a non-significant negative association was observed between insulin resistance and baseline PFOA second tertile, and between insulin resistance and PFOA measured at the end of follow-up for both the second and third tertile of PFOA exposure. A non-significant positive association with insulin resistance was reported in the third tertile of baseline PFOA exposure {Donat-Vargas et al., 2019 5083542}.

In a *medium confidence* study, a non-significant decrease in insulin resistance (measured as HOMA-IR) was observed at age 15 and 21 years old per increase in PFOA exposure from 9 years old {Domazet et al., 2016 3981435}. At age 21, there was a non-significant increase in HOMA-IR per increase in PFOA measured at age 15 {Domazet et al., 2016 3981435}.

Three *low* confidence studies that examined the association between PFOA and insulin resistance. Non-significant negative associations between PFOA and insulin resistance were observed in continuous analyses {Lind et al., 2014 2215376; Chen et al., 2019 5387400}. In a sex-stratified tertile analysis, a non-significant negative association was observed with log-

HOMA-IR among males, with non-significant increasing HOMA-IR observed with increasing quartiles of PFOA {He et al., 2018 4238388}. HOMA-IR decreased non-significantly with increasing quartiles of PFOA among females {He et al., 2018 4238388}. These studies were given *low* confidence ratings due to failure to account for diabetes status {Lind et al., 2014 2215376}, or use of medications that impact insulin levels in HOMA-IR analyses {Chen et al., 2019 5387400}, and failure to account for the complex sampling design of NHANES in statistical analyses {He et al., 2018 4238388}.

The association between plasma PFOA and insulinogenic index 1 was investigated in a *high* confidence study from the Diabetes Prevention Program. A significant positive association was observed with insulinogenic index among adults at high risk for type 2 diabetes {Cardenas et al., 2017 4167229}.

In a *high* confidence study, Cardenas et al. (2017 4167229) reported significant associations were observed between PFOA and fasting plasma insulin, 30-minute insulin, fasting proinsulin, and insulin (corrected response).

In a *low* confidence study, a significant positive association was reported for the ratio of proinsulin to insulin and PFOA {Lind et al., 2014 2215376}.

Five studies examined beta cell function and two reported a significant association. A *high* confidence study from the Diabetes Prevention Program reported a significant positive association with beta cell function (measured as HOMA-B) among adults at high risk for type 2 diabetes {Cardenas et al., 2017 4167229}. A significant positive association with beta-cell function was reported in a *medium* confidence study of adults from NHANES {Liu et al., 2018 4238514}. Two *medium* confidence studies reported negative, non-significant associations with HOMA-B {Donat-Vargas et al., 2019 5083542; Domazet et al., 2016 3981435}.

One *low* confidence study reported a positive, non-significant association with HOMA-B {Chen et al., 2019 5387400}. This study was given a *low* confidence rating due to failure to exclude participants using medications that could impact beta-cell function.

Five studies examined adiponectin, and one observed an association. A *high* confidence study from the HOME study reported non-significant positive association between maternal blood PFOA and adiponectin in children {Ashley-Martin et al., 2017 3981371}. In contrast, a significant negative association with adiponectin was observed among adults in the Diabetes Prevention Program {Cardenas et al., 2017 4167229}. A *medium* confidence study reported a negative non-significant correlation between PFOA and plasma adiponectin {Sun et al., 2018 4241053}.

Two high confidence studies reported non-significant positive associations with adiponectin; no statistically significant effects were observed after stratifying by infant sex in either study {Buck et al., 2018 5080288; Minatoya et al., 2017 3981691}.

Five studies examined associations with leptin. One study reported a significant association.

Three *high* quality studies examined leptin {Buck et al., 2018 5080288; Minatoya et al., 2017 3981691; Ashley-Martin et al., 2017 3981371}, all of which sampled mother-child pairs and

observed positive, non-significant associations with children's leptin concentrations {Buck et al., 2018 5080288; Minatoya et al., 2017 3981691; Ashley-Martin et al., 2017 3981371}.

Two *medium* confidence studies examined leptin. One study, from the POUNDS LOST clinical trial, followed overweight and obese adults. A positive, significant correlation was observed between plasma PFOA and leptin concentrations {Liu et al., 2018 4238396}.

A non-significant, slightly positive association was observed between PFOA and soluble leptin receptors {Liu et al., 2018 4238396}

Eight studies examined hemoglobin and five reported an association. A *high* confidence study on participants in the Diabetes Prevention Program reported a significant positive association with HbA1c {Cardenas et al., 2017 4167229}. Two *medium* confidence studies reported positive, non-significant associations with HbA1c {Duan et al., 2020 5918597; Sun et al., 2018 4241053}. One *medium* confidence study of PFOA and HbA1c among 10,859 NHANES participants reported a negative, significant spearman correlation between serum PFOA and plasma hemoglobin {Huang et al., 2018 5024212}.

Another *medium* confidence cross-sectional study assessed the association between plasma PFOA and HbA1c among adults aged 20–60 {Su et al., 2016 3860116}. A negative, non-significant association between HbA1c and continuous PFOA was reported, but a significant decrease in average HbA1c was observed with increasing quartiles of PFOA {Su et al., 2016 3860116}. In the POUNDS LOST trial, a clinical trial of overweight and obese adults, negative, significant correlation was observed between PFOA and HbA1c {Liu et al., 2018 4238396}. Additionally, a *medium* confidence cross-sectional analysis of adults from NHANES reported a significant negative association with HbA1c {Liu et al., 2018 4238514}.

One *low* confidence study reported a statistically significant negative association with HbA1c among women with PCOS, and a non-significant positive association with HbA1c among women without PCOS {Heffernan et al., 2018 5079713}. Another *low* confidence study reported no significant association between PFOA and glycated hemoglobin {Chen et al., 2019 5387400}. *Low* confidence ratings were given to these studies due to failure to exclude participants using medications that could impact HbA1c {Chen et al., 2019 5387400} and concerns with participant selection and residual confounding {Heffernan et al., 2018 5079713}.

Eight studies evaluated body weight measures, and six reported an association.

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, non-significant association with weight loss in months 6–24 of the trial {Liu et al., 2018 4238396}. A significant increase in average weight gain during months 6–24 of the trial was observed with increasing tertiles of PFOA {Liu et al., 2018 4238396}.

Seven studies evaluated being overweight and one reported a significant association. A cohort study of mothers and children from the Faroe Islands followed mother-child pairs reported an increased, significant risk of being overweight at age 5 with increase in maternal PFOA and a non-significant increased risk of being overweight at 18 {Karlsen et al., 2017 3858520}. In a tertiles analysis, a non-significant negative association was observed with being overweight at 18 months, and a non-significant positive association was observed with being overweight at age 5

{Karlsen et al., 2017 3858520}. A significant increased risk of being obese at age 5 was observed in the highest tertile of maternal PFOA exposure {Karlsen et al., 2017 3858520}.

A *medium* confidence study reported significantly greater serum PFOA among obese adults compared to non-obese adults {Jain et al., 2019 5080621}. Five *medium* confidence studies evaluated maternal PFOA and risk of being overweight or obese in their children; these studies reported increased, non-significant risk or odds of being overweight {Braun et al., 2016 3859836; Lauritzen et al., 2018; Martinsson et al., 2020 6311645; Manzano-Salgado et al., 2017 4238509; Mora et al., 2017 3859823}. In a sex-stratified analysis, Mora et al. (2017) observed an increased, non-significant relative risk of being overweight or obese among boys, but a decreased, non-significant risk among girls.

In the *low* confidence studies, significant associations were seen between PFOA and being overweight {Tian et al., 2019 5080586} and being obese {Yang et al., 2018 4238462}. One study was given a *low* confidence rating due to concerns with BMI being related to PFOA; although this was acknowledged by the authors, this was not accounted for in the analysis {Tian et al., 2019 5080586}. Low confidence ratings were also given due to concerns with outcome misclassification and residual confounding by SES {Yang et al., 2018 4238462}.

One study observed a significant negative association with weight-for-age z-score among children {Braun et al., 2016 3859836}. A significant interaction between maternal PFOA and age was observed in the second tertile of maternal PFOA exposure, but not in the third tertile of maternal PFOA exposure {Braun et al., 2016 3859836}.

Five studies evaluated body fat measures, and one reported an association. Four studies of *medium* confidence evaluated body fat {Hartman et al., 2017 3859812; Mora et al., 2017 3859823; Braun et al., 2016 3859836; Liu et al., 2018 5881135}. A negative, non-significant association was observed between maternal plasma PFOA and body fat percentage in young girls in the ALSPAC, and this association persisted after stratification by age at menarche {Hartman et al., 2017 3859812}. However, the negative association between maternal plasma PFOA and trunk fat percentage in young girls was significant {Hartman et al., 2017 3859812}. Three *medium* confidence studies reported positive, non-significant associations with body fat measures {Mora et al., 2017 3859823; Braun et al., 2016 3859836; Liu et al., 2018 5881135}.

Two *medium* confidence studies evaluated fat mass, and no associations were reported. Non-significant, positive associations with fat mass were reported among children {Jeddy et al., 2018 5079850} and overweight and obese adults {Liu et al., 2018 5881135}.

Fifteen studies assessed BMI, and one reported a significant association.

In the HOME study, a cohort study of mother-child pairs, PFOA exposure was measured during pregnancy and BMI was recorded at age 8 {Braun et al., 2016 3859836}. Significant positive associations with BMI z-score were observed in the second tertile of maternal PFOA exposure, and a negative, non-significant association was observed in the third tertile of maternal PFOA exposure {Braun et al., 2016 3859836}. Additionally, significant increases in BMI z-score between ages 2 and 8 were observed in both the second and third tertile of maternal PFOA exposure {Braun et al., 2016 3859836}. Two *medium* confidence studies of mother-child pairs

observed positive, but non-significant association between maternal serum PFOA child's BMI z-score {Lauritzen et al., 2018 4217244; Jensen et al., 2020 6833719}.

Two *high* confidence studies and three *medium* confidence studies observed positive, non-significant associations with BMI {Cardenas et al., 2017 4167229; Chen et al., 2019 5387400; Mora et al., 2017 3859823; Domazet et al., 2016 3853465; Liu et al., 2018 4238396}. After sex-stratification, a negative, non-significant association with BMI was observed among male children in mid-childhood {Mora et al., 2017 3859823}. Domazet et al. (2016 3983465) reported a non-significant positive association between PFOA measured at age 15 and BMI at age 21.

In a *medium* confidence cohort study from the ALSPAC, a significant negative association with BMI was observed among mother-child pairs {Hartman et al., 2017 3859812}. In a *medium* confidence study from the Fernald Community Cohort, a repeated-measures analysis reported a non-significant percent decrease in BMI was observed per IQR increase in PFOA, while a latent-analysis reported a non-significant percent increase in BMI per IQR increase in PFOA {Blake et al., 2018 5080657}. In a sex-stratified analysis, non-significant percent decreases were observed for both males and females {Blake et al., 2018 5080657}.

In the single *low* confidence study, Tian et al. (2019 5080586) observed a statistically significant increase in BMI with increase in PFOA. In a sex-stratified analysis, a statistically significant positive association was reported between PFOA and BMI among men; the association between PFOA and BMI among women was positive, but not significant {Tian et al., 2019 5080586}. This study was given a *low* confidence rating due to concerns with BMI being related to PFOA; although this was acknowledged by the authors, this was not accounted for in the analysis.

Four studies examined anthropometric measurements, and one reported significant association with waist circumference. One *medium* confidence study reported a negative, non-significant association with hip-circumference {Chen et al., 2019}. Three *medium* confidence studies evaluated waist measurements and observed positive, non-significant associations with waist circumference {Chen et al., 2019 5387400; Braun et al. 2016 3859823; Liu et al., 2018 4238396}

A low confidence study from the Isomers of C8 Health project evaluated waist circumference among adults. A significant, positive association with waist circumference was observed. After stratification by sex, the association with waist circumference among men remained significant, but was not significant among women {Tian et al., 2019 5080586}. Significant increased odds of increased waist circumference were observed in the overall study population and among men; odds of increased waist circumference were increased but non-significant among women {Tian et al., 2019 5080586}. This study was given a *low* confidence rating due to concerns with waist circumference being related to PFOA; although this was acknowledged by the authors, this was not accounted for in the analysis.

3.3.7.1.6 Findings from Occupational Studies

There was one occupational study, which came from the C8 Health Project {Steenland et al., 2013}. A decreased, non-significant risk of type 1 diabetes was observed in the second and fourth quartiles of PFOA exposure in both lagged and unlagged analyses were observed. A non-significant increased risk of type 1 diabetes was observed in the third quartile in both lagged and unlagged analyses {Steenland et al., 2013 1937218}.

3.3.7.2 Animal Evidence

3.3.7.2.1 Metabolic Homeostasis

There are 3 studies from the most recent literature search conducted in 2020 and 2 key studies from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and metabolic homeostasis. Study quality evaluations for these 5 studies are shown in Figure 84.

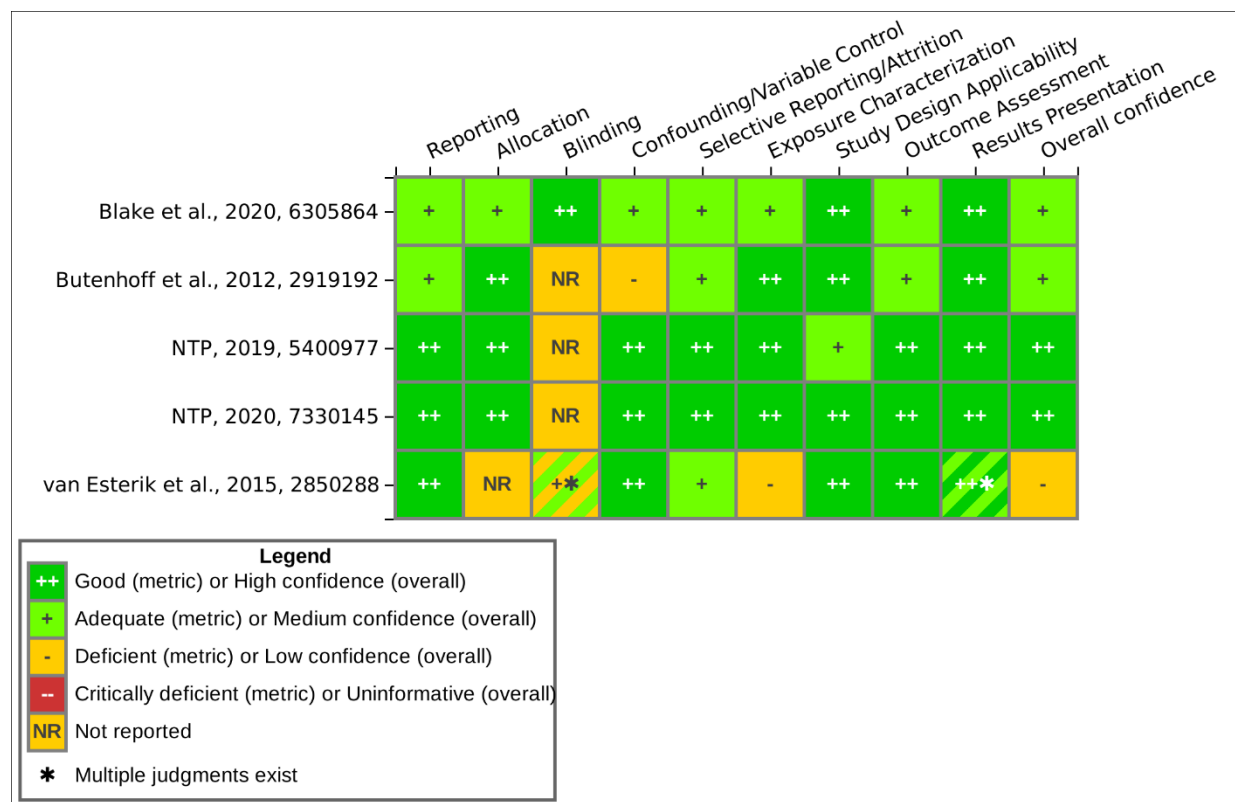


Figure 84. Summary of Study Evaluation for Toxicology Studies of PFOA and Metabolic Effects

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

PFOA has been observed to cause perturbations in metabolic homeostasis in rodents. However, there appears to be differences in responses depending on species, length of exposure, and sex. Overall, the effects on metabolic parameters following PFOA exposure are inconclusive.

In a 28-day study conducted by NTP (2019, 5400977), glucose was significantly decreased in male Sprague-Dawley rats following exposure to ≥ 2.5 mg/kg/day PFOA. No significant response was observed in the female rats treated with up to 100 mg/kg/day PFOA. In a single-dose study in male Sprague-Dawley rats, Elcombe et al. (2010, 2850034) similarly observed a significant decrease in serum glucose after administration of 300 ppm PFOA in feed (equivalent to approximately 19 mg/kg/day) for 28 days. However, a chronic study by Butenhoff et al. (2012, 2919192) observed increases in glucose when measured beginning at 3 months. The authors exposed Sprague-Dawley rats to 30 or 300 ppm PFOA in feed for a 24-month period. Serum

samples for clinical chemistry measurements were taken at 3, 6, 12, 18, and 24 months. For males in the 30 ppm group (~1.3 mg/kg/day PFOA), glucose levels were significantly higher than controls at 3, 6, and 12 months, then returned to baseline control levels at 18 and 24 months. Male rats in the 300 ppm group (~14.2 mg/kg/day PFOA) had significantly higher serum glucose levels than the control groups at the 3- and 24-month time points. In female rats, effects on serum glucose were only observed at the 6-month timepoint; in both the 30 and 300 ppm groups (~1.6 and ~16.1 mg/kg/day PFOA, respectively), serum glucose levels were significantly lower than controls.

In CD-1 mice, three independent studies investigated the effects of gestational PFOA exposure on adult offspring {Hines, 2009, 194816; Quist, 2015, 6570066} or pregnant dams {Blake et al., 2020, 6305864} and found no effect on glucose levels or glucose tolerance. Interestingly, Hines et al. (2009, 194816) observed weight gain in female offspring exposed to lower doses of PFOA (0.01, 0.1, and 0.3 mg/kg/day but not 1 mg/kg/day or controls) from GD1–17. This weight gain was correlated with mid-life (21-33 weeks of age) increased serum insulin and leptin levels in the 0.01 and 0.1 mg/kg/day groups, but not glucose tolerance in early (15-16 weeks of age) or late (70-74 weeks of age) adulthood. These results indicate potential susceptibility to metabolic dysfunction later in life after low-dose gestational PFOA exposure. However, in a similar study, Quist et al. (2015, 6570066) exposed pregnant mice to 0, 0.01, 0.1, 0.3, or 1 mg/kg/day from GD1–17 and observed no statistical differences in serum glucose or insulin levels in female offspring at postnatal week 13 (PNW13). Blake et al. (2020, 6305864) also saw no effect on dam serum glucose with gestational exposure to 1 or 5 mg/kg/day PFOA from GD1.5–11.5 or GD1.5–17.5.

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

There are 9 studies from the most recent literature search conducted in 2020 and 6 key study from the 2016 PFOA HESD {EPA, 2016, 3603279} that investigated the association between PFOA and systemic effects. Study quality evaluations for these 15 studies are shown in Figure 85.

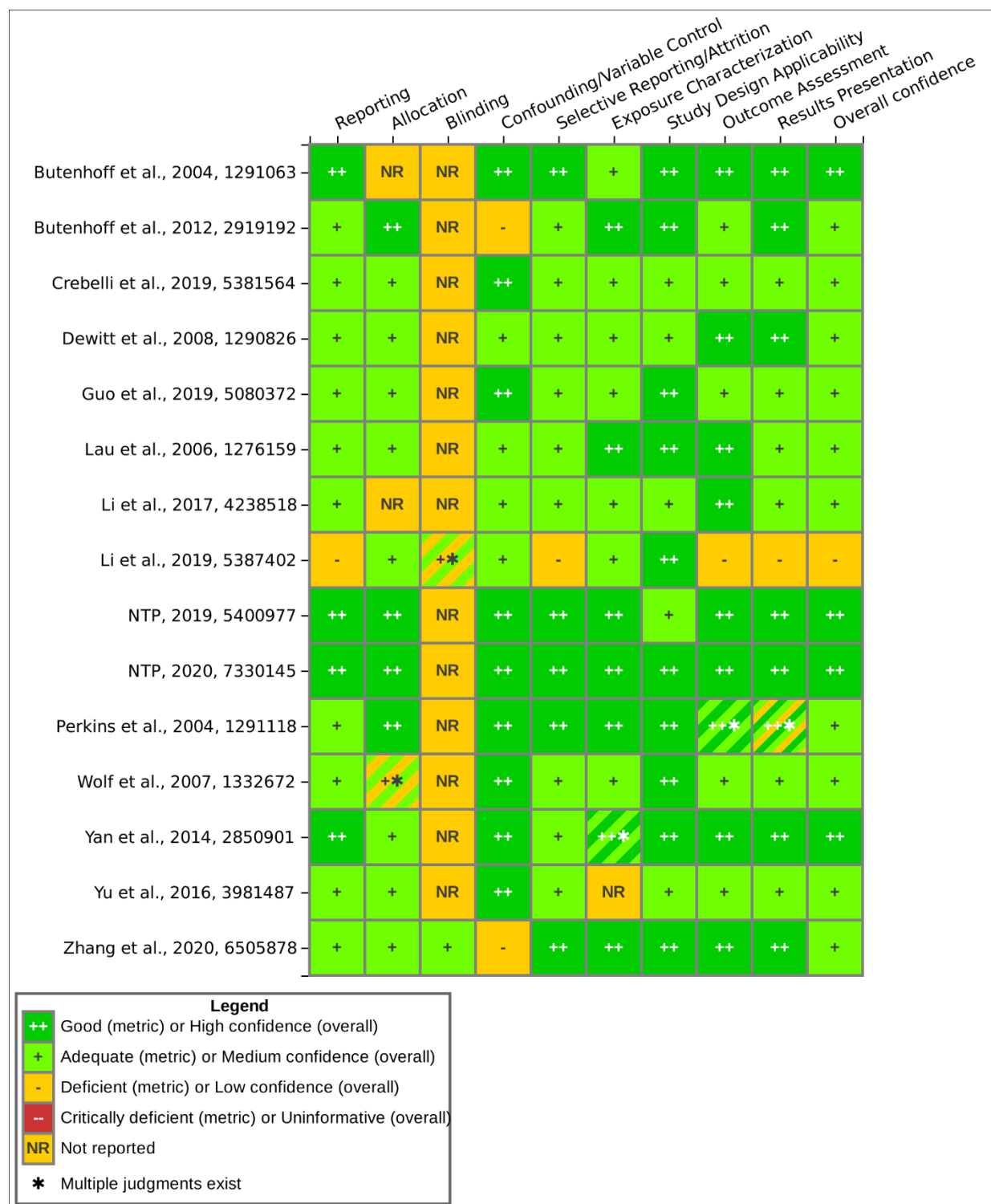


Figure 85. Summary of Study Evaluation for Toxicology Studies of PFOA and Systemic Effects

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

Available animal toxicity data suggest that PFOA exposure can elicit whole-body toxicity, which is reflected by changes in survival, body weights, food consumption, and other clinical observations. Reductions in survival precipitated only at higher doses of PFOA in a single non-human primate study. Reductions in terminal body weight and reductions in weight gain are consistently observed across studies of varying durations of oral exposure to PFOA. Prior to this updated assessment, the available literature measuring clinical outcomes, food and water consumption, body weight, and survival primarily consisted of acute studies {U.S. EPA 2016, 3603279}. Many of the findings were consistent with those in more recent literature and are included herein.

3.3.7.2.2.1 Survival

Although one subchronic toxicity study in non-human primates exposed to ≥ 30 mg/kg/day for 90 days PFOA showed reductions in survival (Goldenthal et al., 1987), survival rates were not affected in rodent studies across study durations and doses (NTP, 2019, 5400977; NTP, 2020, 7330145; Perkins et al., 2004, 1291118; Crebelli et al., 2019, 5381564; Thomford et al., 2001, 5432382). Interestingly, survival was increased in two studies: Butenhoff et al. (2012, 2919192) and Biegel et al. (2001, 673581). Butenhoff et al. fed male Sprague Dawley rats 0, 30, or 300 ppm PFOA via the diet (equivalent to 0, 1.3, or 14.2 mg/kg/day) for two years and observed that survival was increased in males at the highest dose (Figure 86). No significant effect was observed in female rats (exposure equivalents of 0, 1.6, or 16.1 mg/kg/day) in this study (Figure 86). Similarly, Biegel et al. (2001, 673581) observed increased survival in male rats fed 300 ppm PFOA each day at the end of another two-year study. In other studies of rats, mice, and non-human primates included in this updated assessment, all animals survived to the end of study (Figure 86).

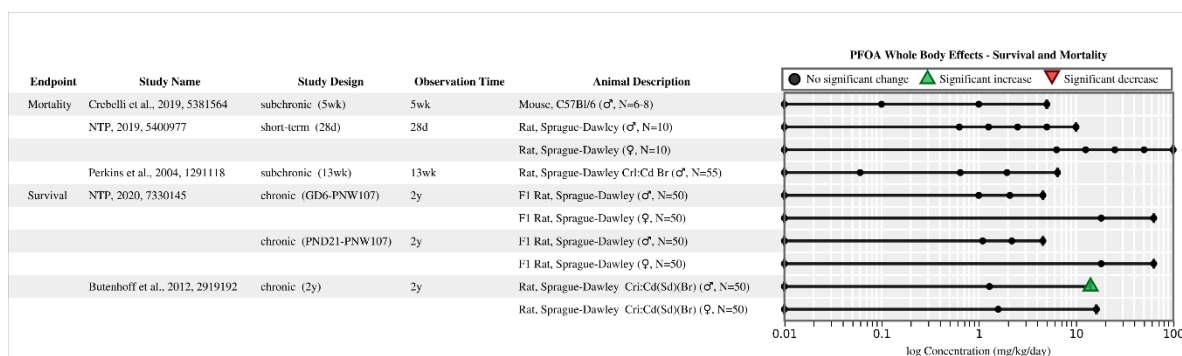


Figure 86. Effects on Survival and Mortality in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA 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; F1 = first generation; d = day; wk = week; y = year.

3.3.7.2.2.2 Clinical Observations

Clinical observations have been reported in animal studies of oral exposure to PFOA. Two 28-day studies described clinical assessments following 28 days of oral PFOA exposure via gavage in Sprague Dawley rats. Whereas NTP (2019, 5400977) did not observe any treatment-related clinical observations in 7- to 9-week-old Sprague Dawley rats exposed to PFOA (0–10

mg/kg/day, males; 0–50 mg/kg/day, females) for 28 days, Cui et al. (2009, 757868) described adverse clinical signs in male Sprague Dawley rats exposed to 5 mg/kg/day, including cachexia and lethargy in the third week of study.

The aforementioned study by Butenhoff et al. (2004, 1291063) reported that there were low incidences of dehydration, urine-stained abdominal fur, and ungroomed fur in at least three of the 30 P₀ male, but not female rats exposed for 70 days in the 30 mg/kg/day exposure group. No effects were noted in lower exposure groups (1–10 mg/kg/day), nor in the F₁ offspring at the end of study.

The chronic exposure study by Butenhoff et al. (2012, 2919192) checked for palpable masses daily during the two-year exposure, but the incidence was indistinguishable from controls in all exposure groups.

3.3.7.2.2.3 Body Weight in Adults

Reductions in body weight and/or reductions in weight gain have been observed in non-human primates as well as across rodent studies of varying exposure lengths (short-term, subchronic, chronic), species (rats or mice), and strains of mice.

In a short-term exposure study, Dewitt et al. (2008, 1290826) found that mean body weight was reduced in female C57BL/6N mice exposed to 15 or 30 mg/kg/day PFOA in drinking water for 15 days; no effects were observed at or below 7.5 mg/kg/day (Figure 87). Four independent studies reported body weights from BALB/c mice exposed to various doses (ranging from 0.5–20 mg/kg/day) of PFOA via gavage for 28 days; all exposures began around 6–8 weeks of age (Li et al., 2017, 4238518; Guo et al., 2019, 5080372; Yan et al., 2014, 2850901; Yu et al., 2016, 3981487). Of these, Yu et al., was the only study that did not observe any changes in body weight; mice were exposed to 0.5 or 2.5 mg/kg/day PFOA (Figure 87). Significant reductions in body weight that differed by more than 10% of control were observed only at the highest doses tested in the other studies: 2.5 mg/kg/day in Li et al. (2017, 4238518), 10 mg/kg/day in Guo et al. (2019, 5080372), and 5 or 20 mg/kg/day in Yan et al. (2014, 2850901) (Figure 87). Two studies reported weight reductions in ICR mice exposed for approximately one month. Zhang et al. (2020, 6505878) observed that 5 mg/kg/day, but not 0.5 or 2 mg/kg/day, PFOA was sufficient to reduce body weight in female ICR mice after 28 days of exposure (Figure 87). Males were not evaluated. Son et al. (2008, 1276157) observed similar results in male ICR mice exposed to 17.63 or 47.21 mg/kg/day for 21 days. Females were not evaluated.

Another short-term exposure study by Loveless et al. (2008, 988599) in CD-1 mice administered 0, 0.3, 1, 10, 30 mg/kg/day for 28 days via gavage noted that mean terminal body weights at the end of study were 86 and 78% of control at 10 or 30 mg/kg/day, respectively. In another study, 6- to 8-week-old C57BL/6 mice were exposed to 0, 0.1, 1, or 5 mg/kg/day PFOA in drinking water for 5 weeks. Whereas untreated control mice gained an average of 5.1 ± 0.2 g over the course of the 5-week study, mice treated with 5 mg/kg/day PFOA gained significantly less weight (3.0 ± 0.1 g) (Crebelli et al., 2019, 5381564).

Five short-term studies have determined the effect of PFOA on body weight in rats. Loveless et al. (2008, 988599) applied the aforementioned exposure paradigm for CD-1 mice in male Crl:CD(SD)IGS BR rats. Mean terminal body weights at the end of the 28-day study were 10 or 25% lower than control at 10 or 30 mg/kg/day. Another study exposed male and female Sprague

Dawley rats to PFOA for 28 days (0, 0.625, 1.25, 2.5, 5, or 10 mg/kg/day for males, 0, 6.25, 12.5, 25, 50, or 100 mg/kg/day for females). The mean body weights of 0.625, 1.25, and 2.5 mg/kg/day males and all treated females were within 10% of the respective vehicle control groups throughout the study. At the end of study, mean body weights of the 5 and 10 mg/kg/day males were 12% to 19% lower, respectively, than those of the vehicle control group. No effects on terminal body weight were observed in females (NTP 2019, 5400977).

The remaining three short-term PFOA exposure studies in rats (Pastoor et al., 1987, 3748971; Cui et al., 2009, 757868; Rigden et al., 2015, 7907801) also suggest a decrease in body weight following PFOA exposure, and are discussed in greater detail in the 2016 PFOA HESD {U.S. EPA 2016, 3603279}. Briefly, Pastoor et al. (1987, 3748971) reported a 17% decrease in body weight from controls in male Crl:CD (SD) BR rats that had been exposed to 50 mg/kg PFOA for 7 days. Females were not evaluated. Cui et al. (2009, 757868) found that terminal body weight was significantly reduced in male Sprague Dawley rats exposed to 20 mg/kg/day PFOA for 28 days, but the magnitude of this change (in comparison to controls) was less than 10%. No effects were observed at the 5 mg/kg/day group and females were not evaluated.

Rigden et al. (2015, 7907801) exposed male Sprague Dawley rats to 0, 10, 33, or 100 mg/kg/day PFOA via gavage for three days and recorded body weights each day throughout exposure as well as for four days after the end of exposure. Although body weight decreased on the last day of exposure in the 33 and 100 mg/kg/day exposure groups, growth resumed and the trajectory mirrored that of all other groups including controls during the 4 days after exposure (Rigden et al., 2015, 7907801).

In a subchronic exposure study, Perkins et al. (2004, 1291118) weighed male Sprague Dawley rats weekly over the course of a 13-week exposure to 0, 0.06, 0.64, 1.94, or 6.5 mg/kg/day. Body weight change and absolute body weight at study termination were both reduced in the highest exposure group (Figure 87). Another subchronic study in rhesus monkeys (two per sex per group) reported reductions in body weight following exposure to 30 or 100 mg/kg/day PFOA for 13 weeks (Goldenthal et al., 1978). The reduction in weight loss preceded death in one monkey of each sex. Changes in body weight were similar to controls in the other dose groups (3 or 10 mg/kg/day) (Goldenthal et al., 1978).

Absolute body weights of Parental (P)-generation male and female Sprague Dawley rats were measured in a reproductive toxicity study by Butenhoff et al. (2004, 1291063); six-week-old rats were exposed to 0, 1, 3, 10, or 30 mg/kg/day PFOA via gavage for at least 70 days prior to mating and until sacrificed. During the peripubertal period (through test day 15), body weight relative to the control group was reduced in males exposed to 10 or 30 mg/kg/day. Terminal body weight was reduced in P₀ males following 106 days of exposure at dosages of 3 mg/kg/day and above, and the changes were greater than 10% in groups exposed to 10 or 30 mg/kg/day (Figure 87). Body weights for the P₀ females were not significantly different (and generally within 10% from control) during the prehabitation period, body weights in the P₀ females at other time points are discussed in Section 3.3.1.2.1.

Two chronic exposure studies reported reduced body weights in male rats that were fed chow laden with 300 ppm PFOA for two years (Butenhoff et al., 2012, 2919192; Biegel et al., 2001, 673581). Whereas the Butenhoff et al. study was performed in Sprague Dawley rats and

evaluated the effects of PFOA on body weight in each sex, Biegel et al. used Crl:CD BR rats and only looked at males.

Of note, a few studies observed that the reductions in body weight and/or body weight change began around day 14–15 of exposure in BALB/c mice (Li et al., 2017) and in Sprague Dawley rats (NTP, 2019, 5400977). Although this observation was specific to males in one 28-day rat study (NTP, 2019, 5400977), it was common to both sexes in BALB/c mice (Li et al., 2017). Zhang et al. (2020, 6505878) observed a trending reduction in body weight in female ICR mice at day 15 of exposure to 5 mg/kg/day PFOA, however the effect did not reach significance until day 25 and males were not tested. More data are required to understand whether the reductions in body weight are more common in a particular sex.

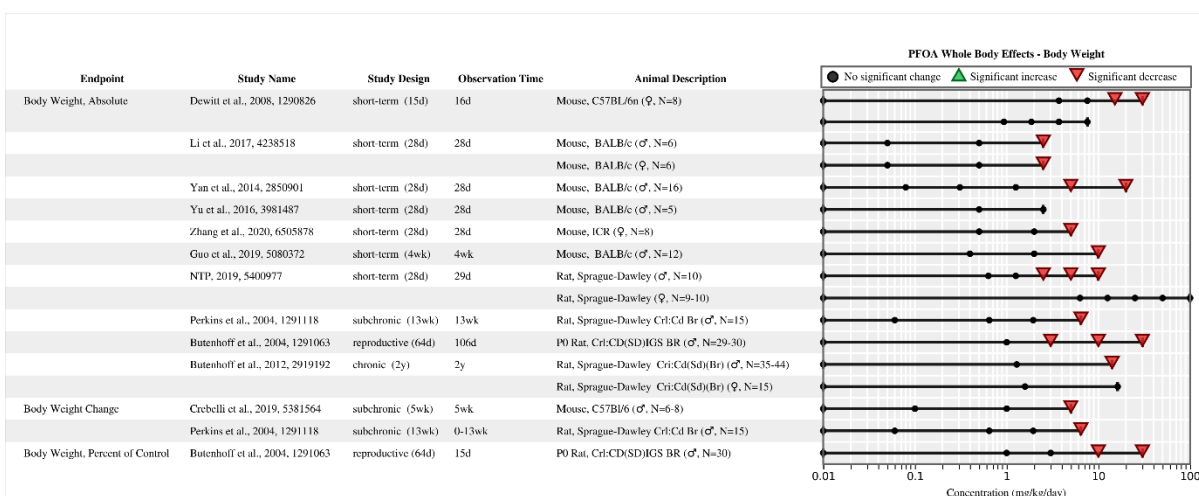


Figure 87. Effects on Body Weight in Rodents Following Exposure to PFOA (logarithmic scale)

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

GD = gestation day; PNW = postnatal week; PND = postnatal day; P₀ = parental generation; d = day; wk = week; y = year.

3.3.7.2.2.4 Body Weight in Adults Following Developmental Exposure

Studies with animals exposed perinatally prior to weaning (i.e., up to PND28) are described in Section 3.3.1.2.

Several developmental exposure studies have evaluated body weight changes after weaning in CD-1 mice, Kunming mice, and Sprague Dawley rats perinatally exposed to PFOA, most of which saw reductions in body weight (relative to litter) prior to weaning (Section 3.3.1.2). Lau et al. (2006, 1276159) exposed pregnant CD-1 mice to 0, 1, 3, 5, 10, 20, or 40 mg/kg/day PFOA from GD1–18 and weighed male and female pups at postnatal week 6.5 and 60 (PNW6.5 and PNW60), as well as the dams at GD18. Weight gain in dams that carried pregnancy to term is described in Section 3.3.1.2. Decrements in body weight of offspring were noted in the 10 mg/kg/day exposure group for PNW6.5 male pups only. No changes in body weight were observed in PNW6.5 females, and offspring from the 20 mg/kg/day group were precluded from the analysis due to low viability. The male-specific weight-loss did not persist to PNW60 in either sex (Figure 88). Similarly, Song et al. (2018, 5079725) observed reduced body weights in PND70 pups following gestational exposure to 1 mg/kg/day PFOA, where pregnant Kunming

mice were exposed to 0, 1, 2.5 or 5 mg/kg/day from GD1–17 (Figure 88). Interestingly, this reduction was not observed in the 2.5 or 5 mg/kg/day groups, which were significantly heavier than controls at an earlier timepoint, PND21 (Section 3.3.1.2).

Absolute body weights in adult F₁-generation rats were also measured in the aforementioned study by Butenhoff et al. (2004, 1291063). P₀ male and female Sprague Dawley rats were exposed to 0, 1, 3, 10, or 30 mg/kg/day PFOA for at least 70 days prior to mating and until sacrificed and their offspring (F₁ generation) were dosed similarly beginning at weaning. Relative body weights were reduced in F₁ male and female juvenile (PND35) rats, as well as peripubertal F₁ (PND56) male rats from the 30 mg/kg/day group. Additionally, male rats from the 10 mg/kg/day group had significantly reduced body weight (post-weaning) beginning at PND77 and lasting through the end of the study. A dose-dependent reduction in body weight at the end of the study (PND120) was observed in F₁ males (Figure 88) (Butenhoff et al., 2004, 1291063). Effects on maternal body weight and on offspring prior to weaning are described in Section 3.3.1.2.

Two rodent studies evaluated the relative sensitivities of body weight to perinatal and/or postnatal exposure of PFOA. NTP (2020, 7330145) evaluated the effects on body weight following perinatal and/or postweaning exposure to PFOA in Sprague Dawley rats. In that study, pregnant rats were exposed to 0, 150, or 300 ppm PFOA to constitute a perinatal exposure in offspring, and postnatal exposures (0, 150, or 300 ppm for males, 0, 300, or 1,000 ppm for females) were continued during the postweaning period for two years (see further study design details in Section 3.3.1.2.1.2). Body weights at the 16-week interim period tended to be lower in all F₁ gestational (GD6–PNW21; GD6–PNW107) and post-weaning (PND21–PNW21; PND21–PNW107) exposure groups and reached significance in all male exposure groups. At the end of the two-year study, there were no consistent effects of PFOA exposure on F₁ males. However, absolute body weight was reduced in F₁ females exposed during gestation plus after weaning (GD6–PNW107) as well as after weaning alone (PND21–PNW107).

Similar findings come from Wolf et al. (2007, 1332672), who investigated the relative contributions of gestational and lactational exposures to PFOA in CD-1 mice. Pregnant mice were given 0 or 5 mg/kg/day PFOA at staggered intervals of gestational development (GD7–17, 10–17, 13–17, or 15–17) and/or 0, 3, or 5 mg/kg/day during the lactational period (PND1–22). Body weights were determined in male and female pups on PND22 and PND92. While no reductions in absolute body weight in any group at PND92 were observed, an elevation in body weight was noted in PND92 mice exposed to 3 mg/kg/day from GD1–17, which had been significantly decreased from control when measured on PND22 (Section 3.3.1.2) (Wolf et al. 2007, 1332672).

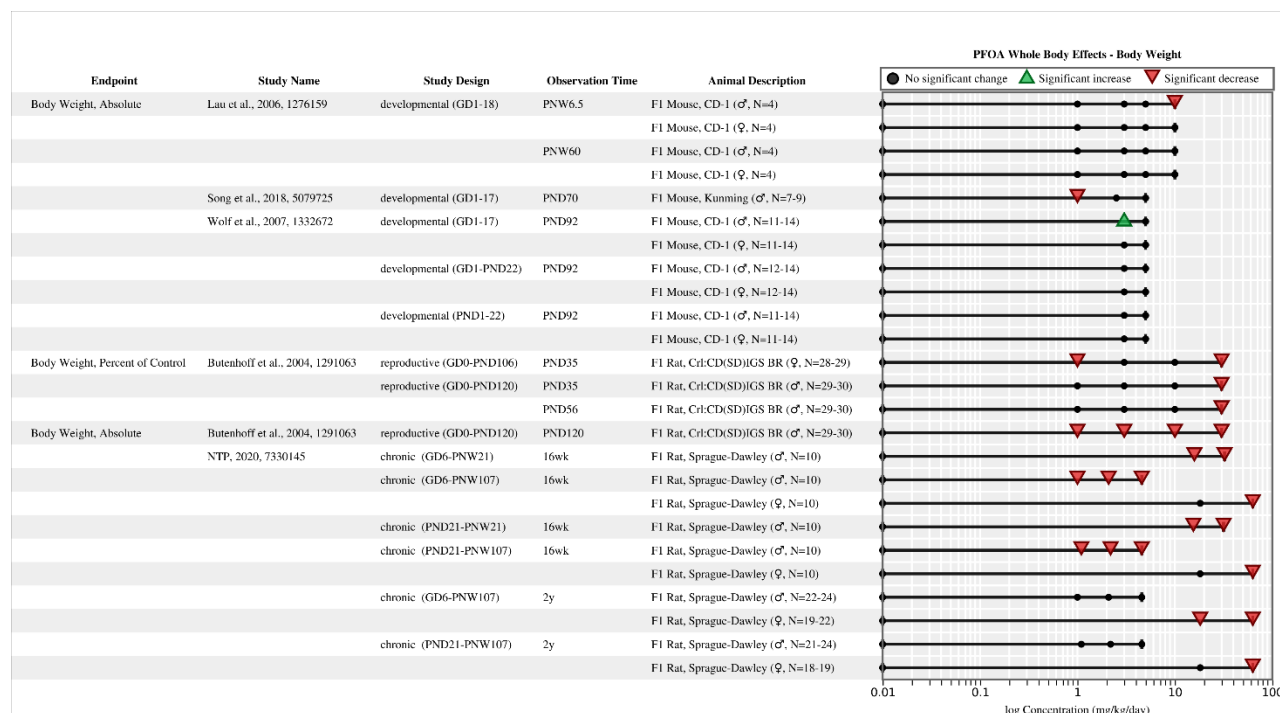


Figure 88. Effects on Body Weight in Rodents Following Developmental Exposure to PFOA (logarithmic scale)

PFOA 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; F1 = first generation; wk = week; y = year.

3.3.7.2.2.5 Food and Water Consumption

Reductions in body weight can be a consequence of reduced in food and/or water consumption, which have been reported in a few of the aforementioned rodent studies and two non-human primate studies following oral exposure to PFOA. Reductions in food or water consumption could not explain all the differences observed in body weight, however, and the limited number of studies that provided data on food consumption make it difficult to thoroughly evaluate the correlation between food consumption and effects on body weight.

Two drinking water studies of different durations reported water consumption in mice. Son et al. (2008, 1276157) reported that food and water consumption was reduced in male ICR mice exposed to 250 mg/L PFOA (equivalent to 47.21 mg/kg/day) for 21 days, but not at concentrations of 50 mg/L (equivalent to 17.63 mg/kg/day) or below. Therefore, the aforementioned reductions in weight loss at 17.63 mg/kg/day were unlikely related to reductions in food consumption or dehydration. A shorter duration (15 day) in C57BL/6N mice exposed to 0, 3.75, 7.5, 15, or 30 mg/kg/day PFOA reported that water consumption per cage did not vary statistically between exposure groups and controls (Dewitt et al., 2008, 1290826), despite reduced weight loss in the two highest exposure groups.

Studies of varying exposure durations in rats have also reported food and/or water consumption that in some cases support a relationship between reduced intake and weight loss. The study by Rigden et al (2015, 7907801) noted a slight decrease in food consumption (data were not

provided) and suggested dehydration related to decreased water consumption as an explanation for weight loss due to increased urine volume during the final two days of exposure. In another study of male Sprague Dawley rats exposed to 0, 5, or 20 mg/kg/day PFOA for 28 days via gavage exhibited decreased food consumption at the 5 mg/kg/day dose (Cui et al., 2009, 757868). However, this level of exposure did not coincide with an effect on weight loss. Elcombe et al. (2010, 2850034) also recorded food consumption (per gram basis) in male Sprague Dawley rats fed 300 ppm PFOA for 28 days. Rats exposed to PFOA consumed less food by day 28. No differences in food consumption were observed in another study of male Sprague Dawley rats fed 0, 1, 10, 30, or 100 ppm (equivalent to 0, 0.06, 0.64, 1.94, 6.5 mg/kg/day) for 13 weeks, despite reductions in body weight at the highest exposure level (Figure 89) (Perkins et al., 2004, 1291118). Females were not used in this study.

The reproductive toxicity study in Sprague Dawley rats by Butenhoff et al. (2004, 1291063) recorded food consumption of P₀ males as well as their male and female F₁ offspring at PND35 following exposure to 0, 1, 3, 10, or 30 mg/kg/day PFOA via gavage. Mean absolute feed consumption (as a percent of control) of male P₀ rats was reduced in the highest exposure group for a majority of the time across 106 days of study. However, given the aforementioned reductions in body weight for these animals, feed consumption relative to body weight was actually elevated at the 3, 10, and 30 mg/kg/day doses. For F₁ males and females, absolute feed consumption was reduced at the 30 mg/kg/day dose (Figure 89).

Two non-human primate studies covered in the 2016 PFOA HESD {U.S. EPA 2016, 3603279} reported reductions in food consumption. Male cynomolgus monkeys displayed overt toxicity, including reduced food consumption, after just 12 days of oral exposure to 30 mg/kg/day PFOA. As a result, the exposure was reduced to 20 mg/kg/day on day 22 for the remainder of the 26-week study (Butenhoff et al., 2002, 1276161). Male cynomolgus monkeys were used in another study that evaluated health effects including food consumption post exposure to 0, 2, or 20 mg/kg/day PFOA for 4 weeks. Low/no food consumption was observed in one male cynomolgus monkey from the 20 mg/kg/day exposure group (Thomford et al., 2001, 5432382).

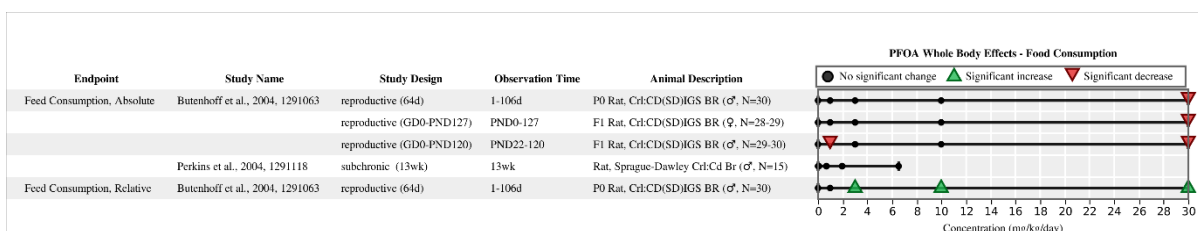


Figure 89. Effects on Food Consumption in Rodents Following Exposure to PFOA

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

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

3.3.7.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse metabolic outcomes is discussed in Sections 3.3.3 and 3.4.5 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 34 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to metabolic effects. A summary of these studies is

shown in Figure 90. Additional analysis on the mechanistic actions of PFOA on metabolic health outcomes is ongoing 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	2	4
Cell Growth, Differentiation, Proliferation, Or Viability	4	0	12	15
Cell Signaling Or Signal Transduction	1	1	4	6
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	8	2	8	18
Hormone Function	3	5	4	12
Inflammation And Immune Response	2	0	1	3
Oxidative Stress	2	1	3	6
Xenobiotic Metabolism	0	0	4	4
Not Specified (Review Article)	1	0	0	1
Grand Total	11	7	17	34

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

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

Mechanistic evidence linking PFOA exposure to adverse systemic outcomes are discussed in sections 3.3.2 and 3.4.5 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 26 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to systemic effects. A summary of these studies is shown in Figure 91. Additional analysis on the mechanistic actions of PFOA on systemic health outcomes is ongoing 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	0	0	3	3
Cell Growth, Differentiation, Proliferation, Or Viability	1	0	6	7
Cell Signaling Or Signal Transduction	2	1	5	8
Extracellular Matrix Or Molecules	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	2	1	6	8
Inflammation And Immune Response	0	0	3	3
Oxidative Stress	2	1	5	7
Xenobiotic Metabolism	1	1	2	4
Other	1	0	3	4
Not Specified (Review Article)	1	0	0	1
Grand Total	5	2	20	26

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

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

3.3.7.4 Evidence Integration

The 2016 EPA HA on PFOA did not report associations between PFOA and metabolic health outcomes in humans. In this review of 72 epidemiological studies, evidence for any association with PFOA and metabolic outcomes was inconsistent, but suggestive evidence was observed for diabetes, leptin, and some adiposity measures.

Contrary to the 2016 EPA Health Assessment for PFOA which concluded that there is no evidence of an association between PFOA and diabetes in humans, the available evidence in this review is suggestive of an association between PFOA and diabetes, particularly gestational diabetes, but more research is needed. The available human epidemiological evidence supports a suggestive association between PFOA and diabetes, including gestational diabetes. Eight studies examined gestational diabetes and five reported positive associations with gestational diabetes. A significant association with gestational diabetes was observed by Su et al. (2016 3860116), and the four other studies reported non-significant positive associations (Wang et al., 2018 5079666; Xu et al., 2020 6833677; Liu et al., 2019 588135; Rahman et al., 2019 5024206). This evidence suggests an association between PFOA and gestational diabetes.

Eight epidemiological studies examined diabetes, and five reported positive associations (four significant positive associations, one non-significant positive association). Mancini et al. (2018, 5079710) reported an increased risk of diabetes in the 4–8th deciles of PFOA exposure, with a significant increased risk in deciles 4 and 5, but a decreased risk in the lower 3 deciles. Two

studies reporting positive associations with diabetes performed sex-stratified analyses and observed conflicting results; one study of male anglers reported non-significantly increased risk odds of diabetes (Christensen et al., 2016, 3858533), and the other reported a non-significant decreased risk among males, but a non-significant increased risk among females (Cardenas et al., 2017, 4167229).

Eight human epidemiological studies examining leptin were identified since the 2016 Health Advisory: four measured leptin in the general population, three in children, and one in pregnant women. From these studies, there is evidence of a positive association with leptin in adults. All four studies examining leptin in the general population observed positive associations (Buck et al., 2018 5080288; Minatoya et al., 2017 3981691; Ashley-Martin et al., 2017 3981371; Liu et al., 2018 4238396). In the POUNDS LOST trial, a clinical trial of 562 overweight and obese adults, leptin was significantly correlated with PFOA. Among pregnant women, Mitro et al. (2020 6833625) observed a significant association between PFOA and leptin, with positive associations persisting after stratification by age during pregnancy. The two studies examining leptin levels among children both reported negative, non-significant associations, while another study of mother-child pairs reported a positive, non-significant association with leptin (Fleisch et al., 2017 3858513). This suggests that age may be a factor in the association between PFOA and leptin. Generally, this evidence suggests an association between PFOA and leptin among adults.

Since the 2016 Health Advisory, ten human epidemiological studies examining waist circumference were identified and nine reported positive associations. Four general population studies reported a positive association with waist circumference (Chen et al., 2019 5387400; Braun et al. 2016 3859823; Liu et al., 2018 4238396; Tian et al., 2019 5080586). A significant positive association with waist circumference was observed in a *low* confidence study of adults highly exposed to PFOA from the C8 Health Project (Tian et al., 2019 5080586). Among women, a positive, non-significant association was observed between PFOA measured during pregnancy and waist circumference at 3 years postpartum (Mitro et al., 2020 6833625). Five studies in children examined waist circumference (Hartman et al. 2017 3859812; Chen et al. 2019 5080578; Domazet et al., 2016 3981435; Mora et al., 2017 3859823). Four studies of children reported non-significant positive associations with waist circumference. A study from the ALSPAC observed a significant decrease in female children's waist circumference with increase maternal serum PFOA (Hartman et al. 2017 3859812). Based on these ten studies, there is suggestive evidence of an association between PFOA and waist circumference.

Three human epidemiological studies observed positive associations with gestational weight gain among pregnant women, with one association being 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 positive association between PFOA and gestational weight gain among under- or normal weight mothers, but a negative association among mothers who are overweight or obese (Marks et al., 2019, 5381534). While this evidence suggests a potential association with gestational weight gain, more evidence is needed.

Of the nine human epidemiological studies examining body fat, six reported positive associations (Hartman et al., 2017 3859812; Mora et al., 2017 3859823; Braun et al., 2016 3859836; Liu et

al., 2018 5881135; Chen et al., 2019 5080578; Jeddy). A significant positive association was observed between maternal PFOA exposure and trunk fat percentage in young (Hartman et al., 2017 3859812). All other positive associations with body fat among those in the general population were not significant (Mora et al., 2017, 3859823; Braun et al., 2016, 3859836; Liu et al., 2018, 5881135). In three studies of body fat in children, only one reported a positive association (Chen et al., 2019, 5080578); all associations between PFOA and body fat in children were non-significant. Generally, these results suggest a possible association between PFOA and body fat, particularly in adults, but more research is needed.

Evidence from the nine studies on being overweight identified since the 2016 Health Advisory suggest a positive association between PFOA and being overweight. All nine studies reported positive associations with being overweight, and of those, five studies reported increased odds of children being overweight with increasing maternal PFOA (Braun et al., 2016 3859836; Lauritzen et al., 2018; Martinsson et al., 2020 6311645; Manzano-Salgado et al., 2017 4238509; Mora et al., 2017 3859823). A study of mothers and children from the Faroe Islands observed a significantly increased risk of being overweight at age 5 with increase in maternal PFOA and a non-significant increased risk of being overweight at 18 (Karlsen et al., 2017 3858520). Among adults, a study from NHANES reported significantly greater serum PFOA among obese adults compared to non-obese adults (Jain et al., 2019 5080621). Additionally, two *low* confidence studies, significant associations were seen between PFOA and being overweight (Tian et al., 2019 5080586) and being obese (Yang et al., 2018 4238462). Overall, this evidence suggests a positive association between PFOA and being overweight.

There is suggestive evidence of a positive association between PFOA and BMI in humans. Since the 2016 Health Advisory, 17 epidemiological studies on BMI were identified, 14 of which reported a positive association between PFOA and BMI. Eight studies examined BMI in children, seven of which reported positive associations between PFOA and BMI. Six of the seventeen identified studies reported non-significant associations with BMI in the general population (Cardenas et al., 2017 4167229; Chen et al., 2019 5387400; Mora et al., 2017 3859823; Domazet et al., 2016 3853465; Liu et al., 2018 4238396; Blake et al., 2018 5080657). One study, which was of *low* confidence, reported a significant positive association with BMI (Tian et al., 2019 5080586). Additionally, the only study on BMI in pregnant women reported a significant positive association with BMI (Mitro et al., 2020 6833625). Overall, there is suggestive evidence of an association between PFOA and BMI, but more evidence is needed. No consistent evidence was observed for BMI z-score.

Similar to the evidence from the 2016 Health Advisory, the available human epidemiological evidence in this review does not support an association between PFOA and metabolic syndrome. Findings were mixed among the four studies identified since 2016; two reported negative associations with metabolic syndrome, and two reported positive associations, with the only significant negative association observed in a *low* confidence study. There is no evidence to suggest an association between PFOA and metabolic syndrome.

Findings on associations between PFOA and metabolic outcomes are inconsistent among high and medium confidence studies. As a result, no studies or endpoints were considered for the derivation of PODs.

Similar to the available human epidemiological evidence, though some alterations related to glucose homeostasis were reported in the available animal toxicity literature, the results were often inconsistent when comparing between species, sexes, length of exposure, and life stages. In male rats, changes in serum glucose levels appear to be influenced by exposure duration, with short-term exposure resulting in decreased serum glucose levels and chronic exposure resulting in increased serum glucose levels. In mice, there was no evidence of altered glucose levels due to PFOA exposure and conflicting reports of changes in serum insulin levels in studies with similar exposure paradigms. Therefore, due to inconsistencies in the available animal toxicity literature, no metabolic endpoints were considered for the derivation of PODs.

Evidence from animal studies suggests that exposure to PFOA of varying durations can elicit adverse whole-body effects, which primarily manifest as reductions in body weight that are not always explained by decreased food and/or water consumption or other clinical signs of toxicity. The effects are consistent across studies of varying exposures to PFOA, across species, and across sex. Reductions in body weight may serve as an early indicator of later PFOA toxicity because it can reflect poor health in the whole organism. The studies by Butenhoff et al. (2004, 1291063) and NTP (2019, 5400977) demonstrate linear dose-dependent reductions in body weight in adult male rats exposed to PFOA. Significant reductions in weight are noted at 1 mg/kg/day in F₁ males at PND120 (Butenhoff et al., 2004, 1291063) as well as 2.5 and 3 mg/kg/day in P₀ males from NTP, 2019 and Butenhoff et al., 2004, respectively. However, toxicological significance (i.e., greater than 10% change from controls) occurs at ≥ 5 mg/kg/day groups in each study. Nevertheless, EPA considered absolute body weight in F₁ and P₀ male rats from Butenhoff et al. (2004, 1291063) and male rats from NTP (2019, 5400977) to be relevant to potential toxicity in humans. Therefore, these endpoints 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} reported mixed results from the literature reviewed and emphasized 2012 C8 Science Panel (2012, 1430770) conclusions, which reported no probable link between PFOA exposure and neurodevelopmental disorders in children, including attention deficit hyperactivity disorder (ADHD) and learning disabilities. Among the studies reviewed for the 2016 Health Assessment, evidence of a significant positive association for child PFOA levels and parent reported ADHD was observed in children aged 12-15 in the general population {Hoffman, 2010, 1291112}, and a positive association with ADHD-like behaviors and decreased executive function in children in a highly exposed community {Stein, 2013, 2721873}. The relationship between PFOA exposure and ADHD-related behavior was also observed in a single country from the INUENDO cohort, showing a significant increase in hyperactivity among children ages 7 to 9 with elevated PFOA exposure (Hoyer, 2015, 2851038). A significant increase in risk of development of cerebral palsy in males associated with maternal PFOA was observed in a case-control study of maternal PFOA levels of participants within the DNBC {Liew, 2014, 2852208}. Studies on outcomes such as Apgar score, fine motor skills, gross motor skills, cognitive skills, behavioral problems, and coordination problems did not find significant evidence for an effect of PFOA exposure {Fei,

2008, 1290822; Fei, 2011, 758428}. Data interpretations within these studies were limited in some cases by use of a cross-sectional analysis {Fei, 2008, 1290822; Hoffman, 2010, 1291112; Stein, 2013, 2721873}, potential random misclassification error resulting from using current PFOA levels as proxy measures of etiologically relevant exposures {Hoffman, 2010, 1291112; Stein, 2013, 2721873}, outcomes defined by parental report {Fei, 2008, 1290822; Fei, 2011, 758428; Hoyer, 2015, 2851038; Hoffman, 2010, 1291112} or parent and teacher report {Stein, 2013, 2721873}, and limited sample sizes in some sub-analyses {Hoyer, 2015, 2851038}.

For this updated review, 38 studies (38 publications) investigated the association between PFOA and neurological outcomes. Two were conducted in high-exposure communities (Spratlen, 2020, 6364693; Stein, 2013, 2850964). One publication (Vuong, 2020, 6356876) was conducted in pregnant women. The remainder were conducted on 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 (Table C-17). The studies measured PFOA in different matrices, including blood, cord blood, breast milk {Forns, 2015, 3228833; Lenters, 2019, 5080366}, maternal serum, amniotic fluid {Long, 2019, 5080602}, and maternal plasma. Eight 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 examined in these studies included clinical conditions such as ADHD, autism spectrum disorder (ASD), MS, and hearing loss. Neurological function was also assessed by performance on numerous neuropsychological tests evaluating neurological domains, including development, general intelligence (i.e., intelligence quotient [IQ]), social-emotional, executive function, ADHD and attention, ASD and intellectual disability (ID), memory, and visuospatial performance.

3.3.8.1.2 Study Quality

Of the 38 studies identified since the 2016 assessment, three studies {Niu, 2019, 5381527; Oulhote, 2016, 3789517; Harris, 2018, 4442261} were classified as having *high* confidence, thirty studies as *medium* confidence, and five as *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 PFOA. Small sample size, temporality and reporting concerns 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.

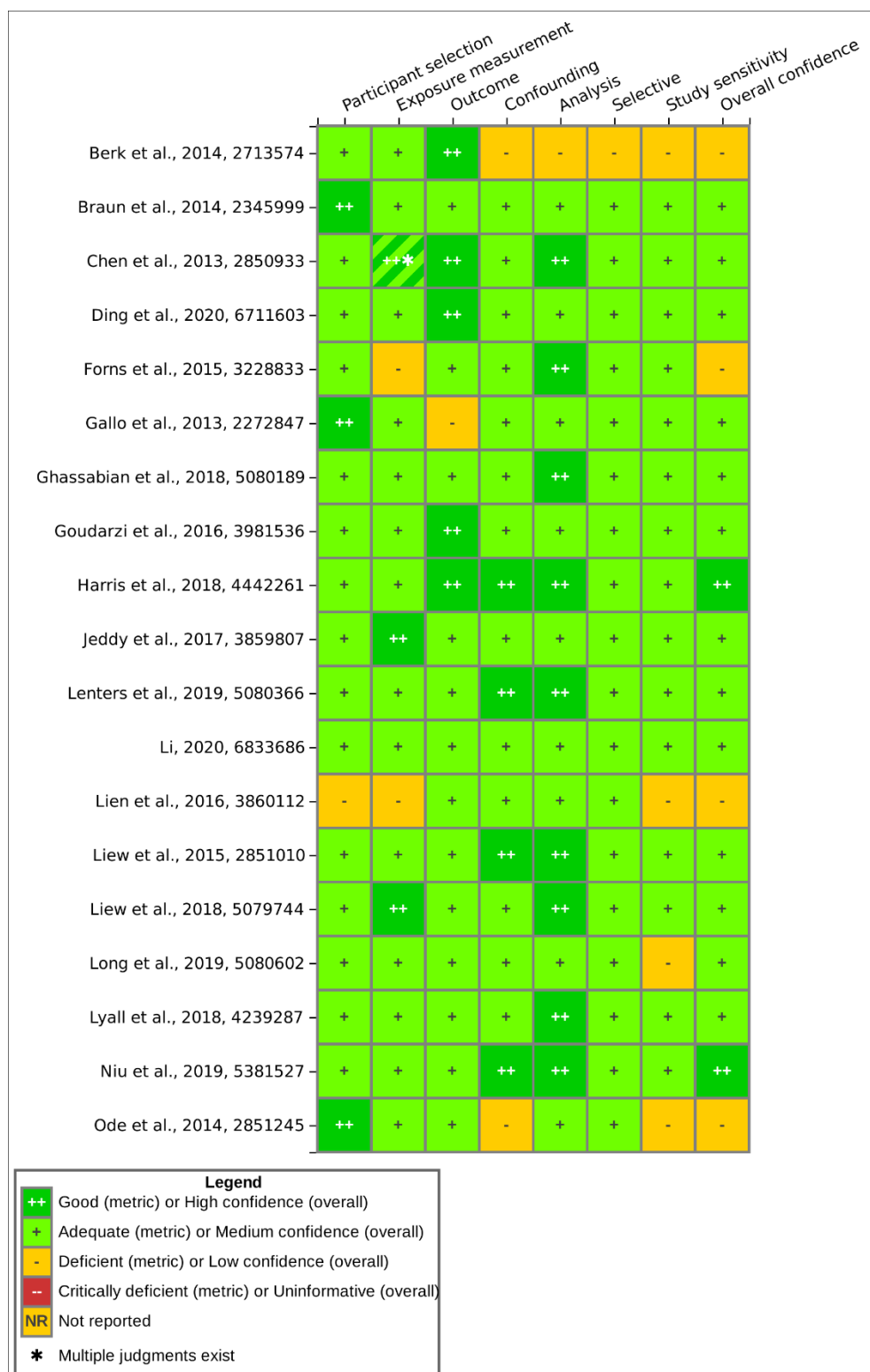


Figure 92. Summary of Study Evaluation for Epidemiology Studies of PFOA and Neurological Effects

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

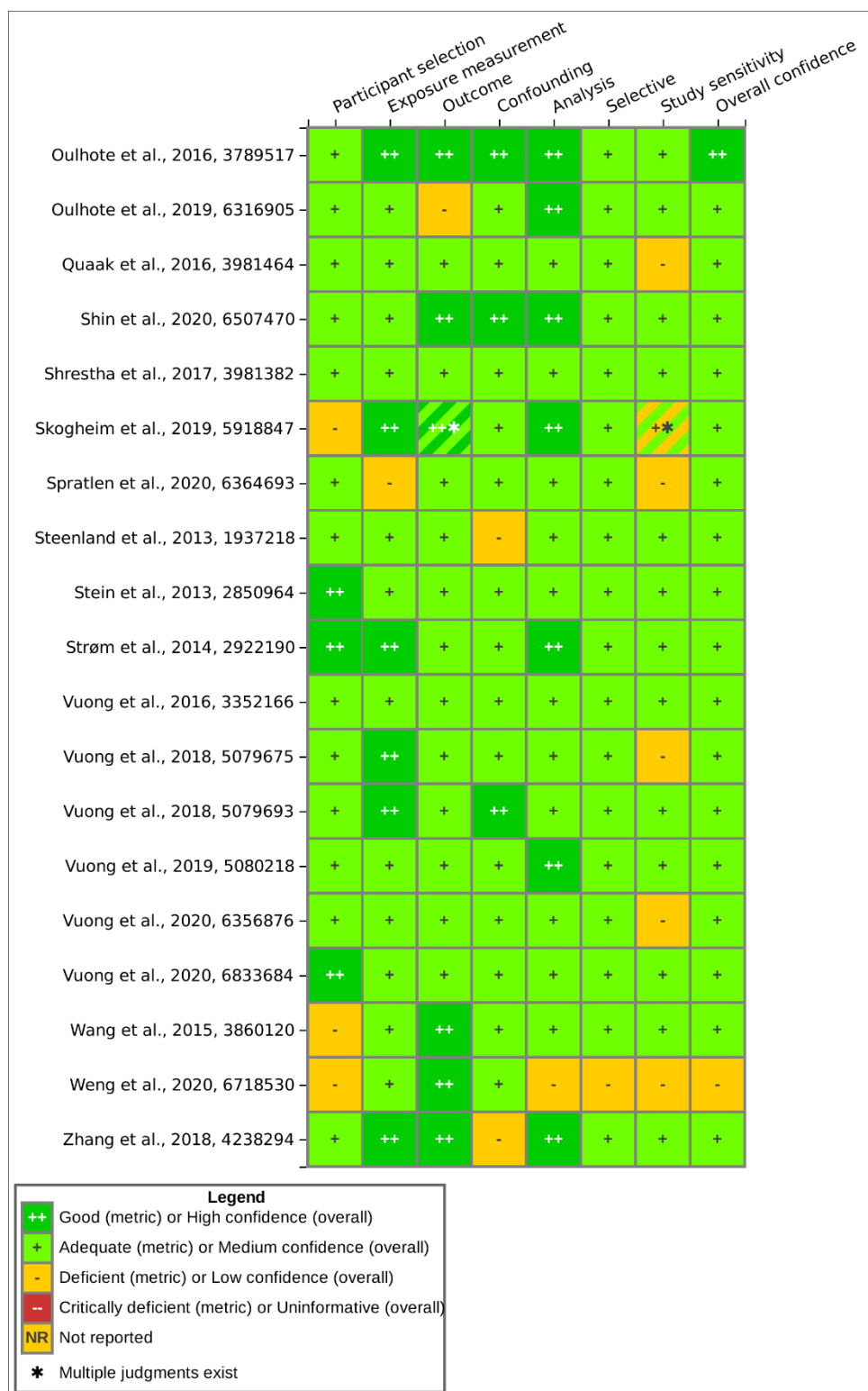


Figure 93. Summary of Study Evaluation for Epidemiology Studies of PFOA and Neurological Effects (Continued)

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

3.3.8.1.3 Findings in 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 two high-exposure community cohort studies {Stein, 2013, 2850964; 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 PFOA concentrations (median = 19.9 ng/mL) during pregnancy were consistently associated with increased risk of problems with personal-social skills in 4-year-old girls (but not in boys), as assessed by the ASQ. In boys, significant decreases in risk for problems with gross motor development were observed among boys, and the risk of problems with problem solving skills were non-significantly elevated. Results from a *medium* confidence study {Goudarzi, 2016, 3981536} reported prenatal PFOA (median = 1.2 ng/mL) concentrations were associated with statistically significantly lower Mental Development Index (MDI) scores for female (but not male) infants at 6 months of age. In contrast, no apparent trends with neurodevelopmental indices from the Bayley Scales of Infant Development (BSID-II) at one year of age were reported in a high-exposure community study of children prenatally exposed to the WTC Disaster {Spratlen, 2020, 6364693}. Adverse associations at 2 and 3 years were not observed, however, a significant positive association was reported for the MDI at 3 years {Spratlen, 2020, 6364693}. A *medium* confidence study {Jeddy, 2017, 3859807} using data from the ALSPAC observed inconsistent patterns of association between prenatal PFOA concentrations (median = 3.7 ng/mL) and neurodevelopmental indices in 15-month-olds as assessed by an adapted version of the MacArthur Communicative Development Inventories for Infants (MCDI). An inverse association was reported for intelligibility scores among 38-month-olds, but there were no associations with maternal PFOA for language or communicative scores in 38-month-olds. Results varied by maternal age at delivery, as a statistically significantly inverse association was observed for vocabulary comprehension and production scores in 15-month infants with mothers younger than 25 years of age, and a significant inverse association for intelligibility scores in children 38 months of age with mothers older than 30 years of age (Jeddy, 2017, 3859807). Results did not suggest an adverse association between estimated or measured PFOA exposures and performance on neuropsychological tests (NEPSY-II) in a high-exposure community study of children participating in the C8 Health Project {Stein, 2013, 2850964}. In one *low* confidence study, which assessed perinatal PFOA breast milk exposures (median = 40 ng/mL) and child neuropsychological development at 6, 12 and 24 months of mother-child pairs in the HUMIS {Forns, 2015, 3228833}, no association was reported between perinatal PFOA exposures and early neuropsychological development.

Eleven studies evaluated cognitive function and IQ measures among children, with most conducted within the general population {Vuong, 2020, 6833684; Zhang, 2018, 4238294; Strøm, 2014, 2922190; Harris, 2018, 4442261; Oulhote, 2019, 6316905; Skogheim, 2019, 5918847; Vuong, 2019, 5080218; Liew, 2018, 5079744; Wang, 2015, 3860120; Lyall, 2018, 4239287} and two within high-exposure communities {Stein, 2013, 2850964; Spratlen, 2020, 6364693}. In a *medium* confidence study (Stein, 2013, 2850964) of children from the C8 Health Project, girls aged 6 to 12 years with measured childhood PFOA (median = 35.0 ng/mL) exposure above the median had a 4.1 score decrease in the Wechsler Individual Achievement Test-II (WIAT-II) Numerical Operations scaled score as compared with girls below the median. A significant 4.9 score increase was observed among boys for the same measure. Overall, children in the highest

versus the lowest quartile of estimated in utero PFOA (110.8–649.2 ng/mL versus 4.5–<11.7 ng/mL) had significant increases in full scale IQ. Across all administered tests, no consistent adverse associations between measured childhood PFOA (median = 35.0 ng/mL) and cognitive function {Stein, 2013, 2850964} were observed. Positive associations between prenatal PFOA (median = 5.2 ng/mL) and reading skills were reported in a *medium* confidence study in children aged eight years utilizing data from the HOME study {Vuong, 2020, 6833684}. Childhood serum PFOA concentrations at ages three and eight were statistically significantly associated with higher children's reading scores at ages five and eight years, respectively in a *medium* confidence prospective study of data within the HOME study {Zhang, 2018, 4238294}. No significant associations between prenatal PFOA and offspring scholastic achievement were reported in a *medium* confidence prebirth cohort study of children (up to age 20) participants within the Danish Fetal Origins Cohort {Strøm, 2014, 2922190}. Maternal prenatal PFOA (median = 3.3 ng/mL) concentrations were statistically significantly associated with lower cognitive function as assessed by the Boston Naming Test with cues in a *medium* confidence study of children aged seven years {Oulhote, 2019, 6316905}.

Skogheim (2019, 5918847) examined cognitive dysfunction in preschool children from the Norwegian Mother, Father, and Child Cohort Study (MoBa) and evidence was inconsistent. Significant decreases in non-verbal working memory were observed only in the highest quintile and significant increases in verbal working memory only in the third quintile of PFOA prenatal exposure (median = 2.5 ng/mL) {Skogheim, 2019, 5918847}. No adverse associations between prenatal (geometric mean = 5.2 ng/mL) and childhood (geometric mean = 2.4 ng/mL) PFOA and cognitive function at eight years were reported, and a statistically significant increase of 4.1 points in working memory associated with an increase in prenatal PFOA was reported in a *medium* confidence study utilizing data from the HOME study {Vuong, 2019, 5080218}. Child sex modified the positive association {Vuong, 2019, 5080218}, with higher full-scale IQ in female children, and no association in male children. In another *medium* confidence study in a highly exposed community study, statistically significant sex-specific trends between exposures and some cognitive outcomes (verbal and full-scale IQ) at four and six years were observed, suggesting stronger positive associations for females compared to males {Spratlen, 2020, 6364693}. No consistent associations between prenatal PFOA and child IQ at five years of age were reported in a *medium* confidence study of children from the DNBC {Liew, 2018, 5079744}. Data from a *medium* confidence study (Wang, 2015, 3860120) on the Taiwan Maternal and Infant Cohort Study showed no consistent associations between maternal serum PFOA (median = 2.5 ng/mL) and IQ measurements in children five or eight years of age.

Six studies examined the potential relationship between PFOA and social-emotional and behavioral regulation problems {Quaak, 2016, 3981464; Oulhote, 2019, 6316905; Ghassabian, 2018, 5080189; Vuong, 2018, 5079693; Oulhote, 2016, 3789517; Weng, 2020, 6718530}. The relationship between prenatal PFOA (median = 870.0 ng/L) exposures and behavioral development at age 18 months using the Child Behavior Checklist 1.5–5 (CBCL 1.5–5) was explored in a *high* confidence study utilizing data from the Dutch cohort Linking Maternal Nutrition to Child Health (LINC) {Quaak, 2016, 3981464}. Results indicated prenatal exposure to PFOA was statistically significantly negatively associated with externalizing behavior problems in boys, indicating less problems. Statistically significant associations between serum PFOA (median = 4.1 µg/L) in children aged five years and total Strengths and Difficulties Questionnaire (SDQ) behavioral survey scores assessed at age seven were reported in a *high*

confidence study {Oulhote, 2016, 3789517}. Maternal prenatal PFOA concentrations (median = 3.3 ng/mL) were positively associated with total SDQ scores, indicating more behavioral problems, in a *medium* confidence study of children seven years of age {Oulhote, 2019, 6316905}. Higher newborn PFOA levels (median = 1.1 ng/mL) in dried blood spots were associated with difficulties in prosocial behavior, but not total behavioral difficulties, as assessed by the maternal completed SDQ at age 7 in another *medium* confidence study {Ghassabian, 2018, 5080189}. Evidence was mixed and insufficient to support an overall association between prenatal PFOA (median = 5.2 ng/mL) and inattention, impulsivity as assessed by the Connors' Continuous Performance Test-II (CCPT-II) in a *medium* confidence study {Vuong, 2018, 5079693}. A *low* confidence study on adolescents reported no significant correlations between prenatal PFOA levels (mean = 2.9 ng/mL) and brain activity in regions associated with impulsive behavior as assessed by MRI imaging in teenage offspring {Weng, 2020, 6718530}.

One *medium* confidence study {Strøm, 2014, 2922190} from the Danish Fetal Origins Cohort examined the association between prenatal PFOA exposure and depression among offspring with 20 years of follow-up. No significant association was observed between clinical depression and maternal PFOA (3.8 ng/mL) levels.

Two *medium* confidence studies {Vuong, 2016, 3352166; Vuong, 2018, 5079675} examined the relationship between PFOA 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 both 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. No associations were observed between prenatal PFOA levels and executive function (Vuong, 2016, 3352166). In analyses using childhood (8 years old) serum PFOA levels (Vuong, 2018, 5079675), results indicated higher PFOA levels were significantly associated with increased odds of being at risk of having clinical impairments—specifically for the metacognition index at age eight.

Six *medium* confidence studies among the general population {Strøm, 2014, 2922190; Liew, 2015, 2851010; Quaak, 2016, 3981464; Skogheim, 2019, 5918847; Lenters, 2019, 5080366}, and one in a high-exposure community {Stein, 2013, 2850964}, examined ADHD and measures of attention in children. A *medium* confidence study of participants in the C8 Health Study observed consistently lower Clinical Confidence Index scores, indicating less probability of ADHD, on the CCPT-II in children (mean age = 9.9 years) associated with increased estimated in utero PFOA levels (median = 43.7 ng/mL) and increased measured childhood PFOA (median = 35.0 ng/mL) {Stein, 2013, 2850964}. Strøm, 2014 (2014, 2922190) investigated the association between prenatal PFOA exposure and ADHD among offspring (follow-up to age 20) of participants within the Danish Fetal Origins Cohort. No association between prenatal PFOA 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 PFOA exposures (case median = 4.1 ng/mL; control median = 4.0 ng/mL) and ADHD in children. No consistent evidence was observed to suggest that prenatal PFOA exposures increase the risk of ADHD. Quaak, 2016 (2016, 3981464) explored the relationship between prenatal PFOA 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 observed between prenatal PFOA exposures and ADHD scores in the whole population as well as within

the sex-stratified analyses. One *medium* confidence study (Lenters, 2019, 5080366) examined early life high PFOA exposures in breast milk in relation to ADHD among children (range: 7.2–14.1 years old) from the HUMIS and observed positive non-significant associations with odds of ADHD (OR: 1.35, 95% CI: 0.87, 2.11), but not consistently in various models.

Two *low* confidence studies {Ode, 2014, 2851245; Lien, 2016, 3860112} examined ADHD and ADHD-related measures, but no significant associations were observed. Lien, 2016 (2016, 3860112) evaluated the association between cord blood PFOA (mean = 1.6 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. No significant associations or trends were observed; however, the direction of association was primarily negative. Ode, 2014 (2014, 2851245) investigated the association in a case-control study between cord blood PFOA (median = 1.8 ng/mL for cases; 1.83 ng/mL for controls) exposures and ADHD diagnosis in childhood (age range 5–17 years), but no consistent pattern was observed. Deficiencies identified in these studies included the reliability of exposure measures, limited study sensitivity, and potential for residual confounding.

Six *medium* confidence studies evaluated PFOA 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 PFOA (median = 4.06 µg/L) at age five was associated with significantly higher SDQ autism screening scores at age seven in a *high* confidence study {Oulhote, 2016, 3789517}. In a *medium* confidence study from the HOME study, increasing maternal serum PFOA concentrations (median = 5.5 ug/L) were non-significantly associated with fewer autistic behaviors in children 4 to 5 years of age as assessed by maternal completed Social Responsiveness Scale (SRS) scores {Braun, 2014, 2345999}. No consistent evidence of an association between maternal plasma PFOA (median = 3.9 ng/mL for cases; 4.0 ng/mL for controls) and diagnosed childhood autism was reported in a *medium* confidence study of mother-child pairs with an average of ten years of follow-up within the DNBC {Liew, 2015, 2851010}. No association was observed in a *medium* confidence case-control study of amniotic fluid PFOA (median = 0.3 ng/mL for cases; 0.3 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 PFOA (geometric mean = 3.6 ng/mL for ASD cases; 3.3 ng/mL for ID cases; 3.7 ng/mL for controls) was inversely associated with diagnosed ASD and ID in a *medium* confidence study of children aged 4.5–9 years {Lyall, 2018, 4239287}. No significant association was observed in a *medium* confidence study of modeled prenatal maternal PFOA (median = 1.1 ng/mL for ASD cases; 1.2 ng/mL for controls) and clinically confirmed ASD among children (age 2–5 years) in the Childhood Autism Risk from Genetics and Environment (CHARGE) study {Shin, 2020, 6507470}.

The effects on visuospatial performance were evaluated in one *high* confidence study (Harris, 2018, 4442261) which observed associations, and one *medium* confidence study (Vuong, 2018, 5079693) which observed no associations. In participants from Project Viva (Harris, 2018, 4442261) observed that children scored consistently lower on visual-motor tests (Wide Range Assessment of Visual Motor Abilities) with increasing prenatal PFOA exposure. No clear patterns were observed using early childhood (median age = 3.2 years) test performance, but significant inverse associations for mid-childhood (median age = 7.7 years) test performance

were observed for the second (4.1–5.6 ng/mL) and fourth (> 7.7 ng/mL) quartiles of prenatal PFOA exposure. 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 the General Adult Population

The effects of PFOA 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 a significant association between serum PFOA and performance on tests for memory and learning corresponding to a 6% higher (better memory and learning) mean score.

Findings of a *medium* confidence study {Shrestha, 2017, 3981382}, described above, indicated higher serum PFOA in adults was associated with significantly better performance executive function measured by the Wisconsin Card Sorting Test (WCST).

Two studies (Berk, 2014, 2713574; Shrestha, 2017, 3981382) examined the effects of PFOA exposure on depression among adults. No associations were reported in a *medium* confidence study of depression, assessed by the Beck Depression Inventory (BDI), and serum PFOA (median = 8.1 ng/mL) in a cross-sectional study of adults aged 55 to 74 years {Shrestha, 2017, 3981382}. One *low* confidence NHANES study {Berk, 2014, 2713574} observed a lower prevalence of depression with increasing PFOA exposure as assessed by the nine-item depression module of the Patient Health Questionnaire (PHQ-9).

Only one *medium* confidence study (Vuong, 2020, 6356876) examined social-emotional effects in pregnant women. No evidence was reported to support an adverse relationship between serum PFOA during pregnancy and maternal depressive symptoms assessed by the Beck Depression Inventory-II (BDI-II) from pregnancy to 8 years postpartum.

Two *medium* confidence studies explored the relationship between PFOA and memory impairment {Gallo, 2013, 2272847; Shrestha, 2017, 3981382} and observed mixed effects. Gallo (2013, 2272847) observed statistically significant inverse associations with memory impairment in adults from the C8 Health Project. However, no adverse effects of PFOA on memory impairment were observed in adults (ages 55–74 years) in New York State {Shrestha, 2017, 3981382}.

Two *medium* confidence cross-sectional studies investigated PFOA and hearing impairment in analyses of adult NHANES participants and observed mixed effects. Li, 2020 (2020, 6833686) reported significant positive associations between PFOA and hearing impairment, while Ding, 2020 (2020, 6711603) reported no significant associations.

3.3.8.2 Animal Evidence

There are 4 studies from the most recent literature search conducted in 2020 and 3 key studies from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and nervous effects. Study quality evaluations for these 7 studies are shown in Figure 94.

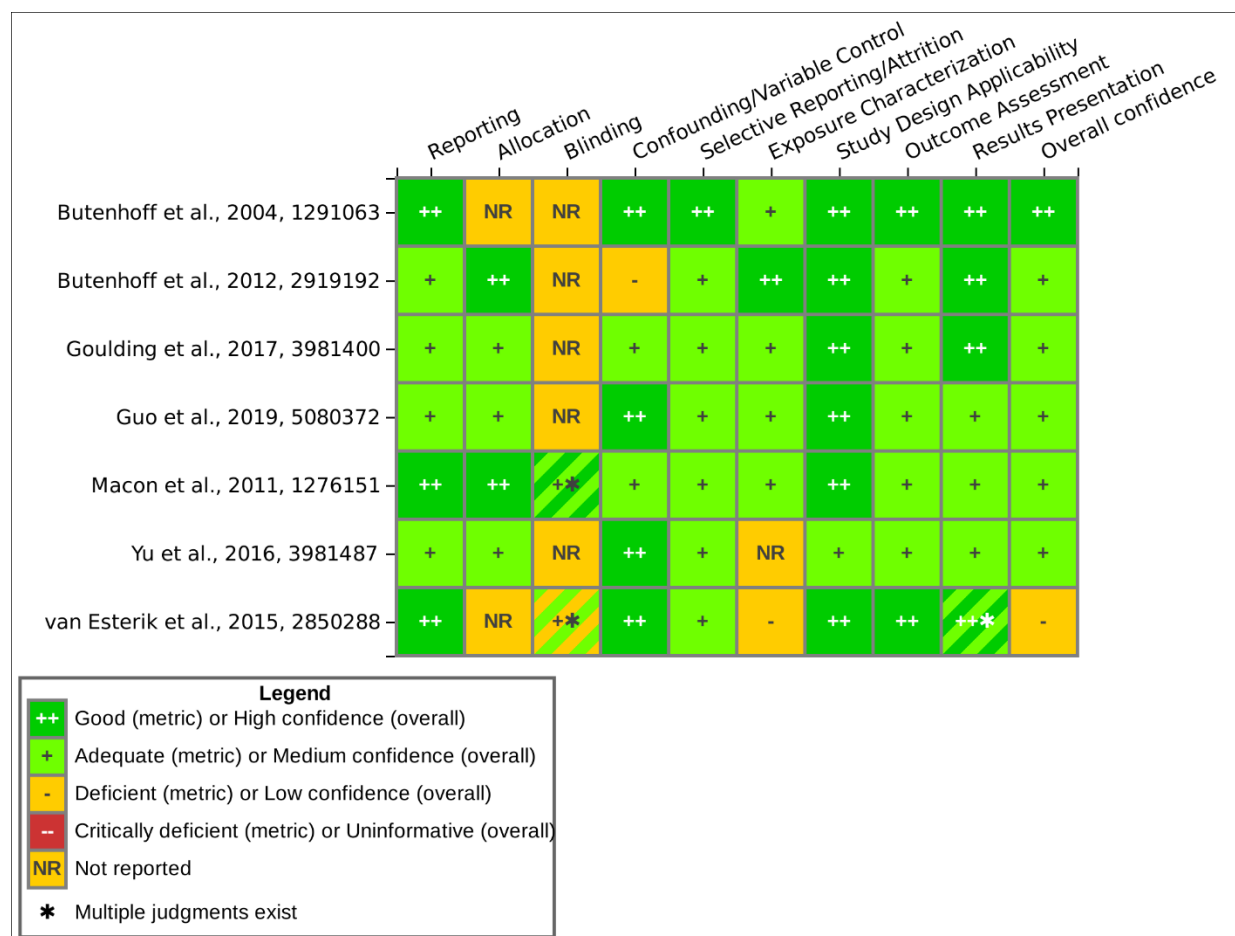


Figure 94. Summary of Study Evaluation for Toxicology Studies of PFOA and Nervous Effects

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

There are few studies available that evaluate neurotoxicity, including neurodevelopmental toxicity, with short-term, chronic, or gestational exposure to PFOA in experimental models. From the available literature, there is little evidence of morphological changes or damage that can be attributed to PFOA exposure. However, there is some evidence suggesting that PFOA exposure may be associated with behavioral and physiological effects, areas of research that may warrant further analysis. Additionally, several single-dose studies indicate that neurodevelopmental endpoints may be sensitive indicators of PFOA toxicity.

Absolute and relative brain weights, as well as brain histopathology, were reported in studies using mice, rats, and monkeys; these studies generally reported null or inconsistent results across dose-groups, generations, sexes, or studies {Perkins, 2004, 1291118; Yu, 2016, 3981487; Goldenthal, 1978, 1291068; Yahia, 2010, 1332451; Macon, 2011, 1276151; Butenhoff, 2004, 1291063; Butenhoff, 2012, 2919192}. Statistically significant changes in brain weight were often not consistent across sexes or generations, were transient, were not dose-dependent, or occurred at relatively high doses compared to other health outcomes. For example, in a 2-year rat feeding study, Butenhoff et al. (2012, 2919192) observed significantly increased absolute brain

weights in males from the low dose group (1.3 mg/kg/day) but not the high dose group (14.2 mg/kg/day) or either female treatment groups. In a rat reproductive study, Butenhoff et al. (2004, 1291063) observed no change in absolute or relative brain weight in males or females from the P₀ and no change in females from the F₁ generation, but reported a significant decrease in absolute brain weight in the high-dose F₁ males (30 mg/kg/day) at PND120. There was no change observed in relative brain weight in F₁ males. Similarly, Macon et al. (2011, 1276151) reported a transient significant decrease in absolute brain weight in F₁ male mice exposed to 1 and 3 mg/kg/day during gestation at PND63 (time points measured ranged from PND7–84). There were no differences in absolute brain weight in females or in relative brain weight in either sex. Dam mice in the highest dose group reported by Yahia et al. (2010, 1332451) in a gestational study (10 mg/kg/day) had significantly decreased absolute brain weight (approximately 7% decrease) and no statistical difference in relative brain weight. A 28-day study in male mice with doses up to 2.5 mg/kg/day {Yu, 2016, 3981487} and a 13-week study with interim sacrifices at 4 and 7 weeks in male mice with doses up to 6.5 mg/kg/day {Perkins, 2004, 1291118} also found no evidence of altered absolute or relative brain weights after PFOA exposure. One monkey study with a limited sample size (n=2/sex/group) reported decreased absolute brain weight in females dosed with 10 mg/kg/day PFOA for 90 days (highest dose tested that did not induce mortality) {Goldenthal, 1978, 1291068}. There were no significant effects on brain weight in males from the same study. Despite several noted changes in brain weight, there were no reports of altered brain histopathology due to PFOA exposure in the available literature {Butenhoff et al., 2004, 1291063; Yahia, 2010, 1332451; Butenhoff, 2012, 2919192; Li, 2017, 4238518; NTP, 2019, 5400977; NTP, 2020, 7330145}.

There is a single study available to assess neurodevelopmental outcomes. Goulding et al. (2017, 3981400) assessed behavioral effects in F₁ male offspring gestationally exposed to 0, 0.1, 0.3, or 1 mg/kg/day PFOA from GD1–17. The authors conducted different behavioral assays across multiple periods of development through adulthood (~3 weeks–6 months of age). Significant effects were only observed in the highest dose group (1 mg/kg/day). Ambulatory activity in an open-field chamber, reported as the number of photocell breaks, was measured on PND18–20. There was a significant increase in the number of photocell breaks in the 1 mg/kg/day dose group on PND18, however, this response was not observed on PND19 or PND20. On PND60, Goulding et al. (2017, 3981400) reported no significant effects due to PFOA exposures in the auditory startle response, habituation, prepulse startle inhibition, and running wheel tests. The running wheel assay was repeated at PND72 with similar results. On PND168, mice were monitored for ambulatory activity following an acute injection of methamphetamine; the authors reported a significantly decreased number of photocell breaks in the 1 mg/kg/day group compared to controls. A few studies report clinical signs of toxicity that exhibit neurotoxicity including ataxia in potentially moribund animals {Goldenthal, 1978, 1291068; Butenhoff, 2012, 2919192}.

Yu et al. (2016, 3981487) analyzed tissue concentrations of four neurotransmitters in the brains of male mice exposed to 0, 0.5, or 2.5 mg/kg/day PFOA for 28 days. Concentrations of dopamine, serotonin, and norepinephrine were significantly altered in the 0.5 mg/kg/day dose group compared to controls but not the high dose group; dopamine and serotonin were both increased while norepinephrine was decreased. Glutamate concentrations in the 2.5 mg/kg/day dose group were significantly decreased compared to controls.

Other behavioral and neurochemical effects were observed in multiple single-dose studies. Onishchenko et al. (2011, 758427) and Sobolewski et al. (2014, 2851072) observed behavioral effects including altered locomotor activity, exploratory behavior, circadian activity, and motor coordination in mouse offspring following gestational or perinatal exposure to single dose levels of PFOA (0.3 and 0.1 mg/kg/day in the respective studies). Cheng et al. (2013, 2304777) administered 10 ppm PFOA to pregnant rats from GD1–PND21 and similarly observed altered motor coordination and locomotor activity in male and female offspring. This study did not report drinking water consumption or body weights of the dams. Johansson et al. (2008, 1276156; 2009, 757874) also observed behavioral (spontaneous behavior and locomotion) and neurochemical effects (altered cholinergic system responses and brain enzyme and protein levels) in adult mouse offspring after a single PFOA dose of either 0.58 or 8.7 mg/kg on PND10.

3.3.8.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse nervous outcomes is discussed in Sections 3.2.4 and 3.4.1 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 20 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to nervous effects. A summary of these studies is shown in Figure 95. Additional analysis on the mechanistic actions of PFOA on nervous health outcomes is ongoing and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	2	0	0	2
Cell Growth, Differentiation, Proliferation, Or Viability	2	0	5	6
Cell Signaling Or Signal Transduction	4	0	5	9
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	2	0	1	3
Hormone Function	1	0	2	3
Inflammation And Immune Response	0	1	0	1
Oxidative Stress	0	0	4	4
Xenobiotic Metabolism	0	0	1	1
Other	1	0	0	1
Not Specified (Review Article)	4	0	1	4
Grand Total	11	1	10	20

Figure 95. Summary of Mechanistic Studies of PFOA 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 Health Assessment provide mostly mixed results on the associations between PFOA and neurological outcomes. There were no new neurological studies identified that evaluated cerebral palsy. Outcomes investigated include those of depression, memory impairment, hearing impairment, ASD, and ID.

The recent human epidemiological studies provide limited indication of adverse effects of PFOA on neurodevelopment, neuropsychological development {Goudarzi, 2016, 3981536; Niu, 2019, 5381527}, cognitive development {Harris, 2018, 4442261; Oulhote, 2019, 6316905}, and executive function {Vuong, 2018, 5079675}. Results for IQ were largely non-significant and inconsistent. There was no evidence of an association with depression; only two studies observed effects of PFOA on hearing {Li, 2020, 6833686} and memory impairment {Gallo 2013, 2272847}. Overall, results for neurodevelopmental, neuropsychological, cognitive, and executive function outcomes were somewhat mixed and limited in number, and establishment of a relationship with PFOA warrants further study.

The recent human epidemiological studies also provide limited indication of adverse effects of PFOA on behavioral problems, ADHD, ASD and ID. There was suggestive evidence of an association between PFOA exposure and behavioral problems associated {Oulhote, 2016, 3789517; Outhote, 2019, 6316905; Ghassabian, 2018, 5080189}, however overall results were mixed. Of the multiple studies examining associations between PFOA and ADHD, only one {Lenters, 2019, 5080366} observed associations with PFOA in a high-exposed population. No adverse associations of ID with PFOA were observed. Oulhote, 2016, 3789517 observed a two-fold increase in serum PFOA at age five was associated with significantly higher SDQ autism screening scores at age seven, but no associations between PFOA and autism screening scores were observed in other studies. However, many studies have methodological concerns, as PFOA 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 many 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 indicates mixed results for links between PFOA exposure and neurodevelopmental disorders in children, including ADHD and learning disabilities. Therefore, the suggestive nervous system effects identified in the epidemiological literature were not considered for the derivation of PODs.

In animal models, some changes in absolute brain weight were noted after PFOA exposure however, these changes in brain weight were not associated with histopathological effects. However, there is limited, but compelling evidence from several single-dose studies indicating neurodevelopmental consequences of PFOA exposure during perinatal periods, though these studies cannot be modeled for this assessment due to the exposure paradigm. In a multi-dose study, Goulding et al. (2017, 3981400) assessed neurodevelopmental consequences of PFOA exposure, but the observed effects in neonates were transient and therefore, are difficult to interpret. This study also reported a suppression of ambulatory activity in mice from the high dose group following an acute injection of methamphetamine on PND168. The biological

significance of the alterations in neurotransmitter levels observed in a separate study is unclear (Yu et al., 2016, 3981487); however, these effects indicate a potential alteration of neural signaling and could be an additional outcome related to PFOA neurotoxicity or a potential toxicological mechanism underlying the observed behavioral changes. Further studies correlating changes in absolute brain weight with potential neurological or behavioral consequences, as well as studies on neurodevelopment, are highlighted here as a research need. Due to the uncertainties related to the biological significance of the observed effects, the neurotoxicological endpoints described in animals were not considered for the derivation of PODs.

3.3.9 Renal

3.3.9.1 Human Evidence

3.3.9.1.1 Introduction

PFOA has the potential to affect the kidney's function of tubular resorption because of it uses tubular transporters for excretion and resorption (U.S. EPA, 2016, 3603279). Biomarkers of renal function include blood urea nitrogen (BUN), serum creatinine, estimated glomerular filtration rate (eGFR), and uric acid levels. eGFR is a marker of non-malignant renal disease.

The 2016 HESD for PFOA (U.S. EPA, 2016, 3603279) concluded there was evidence of a suggestive association between PFOA and two renal outcomes (i.e., uric acid levels and eGFR) based on one occupational (Costa, 2009, 1429922), two studies in high-exposed communities (Steenland, 2010, 1290810; Watkins, 2013, 2850974), and one general population study (Shankar, 2011, 2919232). Kidney function was measured by eGFR, hyperuricemia, and uric acid levels. However, given the cross-sectional study designs, reverse causality as an explanation could not be ruled out. The report also concluded there was no probable link between PFOA exposure and kidney disease based on three occupational studies (Steenland, 2015, 2851015; Steenland and Woskie 2012, 2919168; Raleigh, 2014, 2850270)

For this updated review, 23 studies examined the association between PFOA and renal health outcomes. Five studies were in children and adolescents (Geiger, 2013, 2919148; Kataria, 2015, 3859835; Qin, 2016, 3981721; Khalil, 2018, 4238547), two in pregnant women (Nielsen, 2020, 6833687; Gyllenhammer, 2018, 4238300), one study was in occupational workers (Rotander, 2015, 3859842) and the remainder of the studies were in general population. Seventeen of the studies utilized a cross-sectional study design; the remaining studies included five cohort study designs (Blake, 2018, 5080657; Conway, 2018, 5080465; Dhingra, 2016, 3981521; Gyllenhammer, 2018, 4238300; Nielsen, 2020, 6833687), and one controlled trial (Convertino, 2018, 5080342) (Table C-18). All studies measured PFOA 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 those studying populations in the United States, five studies utilized data from the NHANES (Geiger, 2013, 2919148; Jain, 2019, 5080378; Jain, 2019, 5381566; Kataria, 2015, 3859835; Lee, 2020, 6833761; Scinicariello, 2020, 6833670). Outcomes evaluated in these studies included clinical conditions, such as chronic kidney disease (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 PFOA is removed from the blood by the kidney, cross-sectional analyses using serum PFOA as the exposure measure are problematic if individuals with compromised kidney function are included: PFOA 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 23 studies identified since the 2016 assessment, one was classified as *high* confidence (Dhingra, 2016, 3981521), two as *medium* confidence (Dhingra, 2017, 3981432; Gyllenhammar, 2018, 4238300), nineteen as *low* confidence, and one as *uninformative* (Seo, 2018, 4238334) (Figure 96). The main concerns with the *low* confidence studies included potential for residual confounding, selection bias, and reverse causality. Other concerns included small sample sizes (Khalil, 2018, 4238547; Nielsen, 2020, 6833687), selective reporting of significant results (Lee, 2020, 6833761), and potential for selection bias (Lin, 2013, 2850967; Rotander, 2015, 3859842). Additionally, *low* confidence studies utilizing cross-sectional analyses of kidney function with serum PFOA were impacted by the potential for reverse causation.

Seo, 2018 (4238334) was considered *uninformative* due to use of bivariate statistical analyses, limiting the ability to interpret the results. Additionally, other potential sources of bias were identified, including a lack of information on participant recruitment and selection, unexplained discrepancies in sample sizes, and missing details on outcome assessment methods.

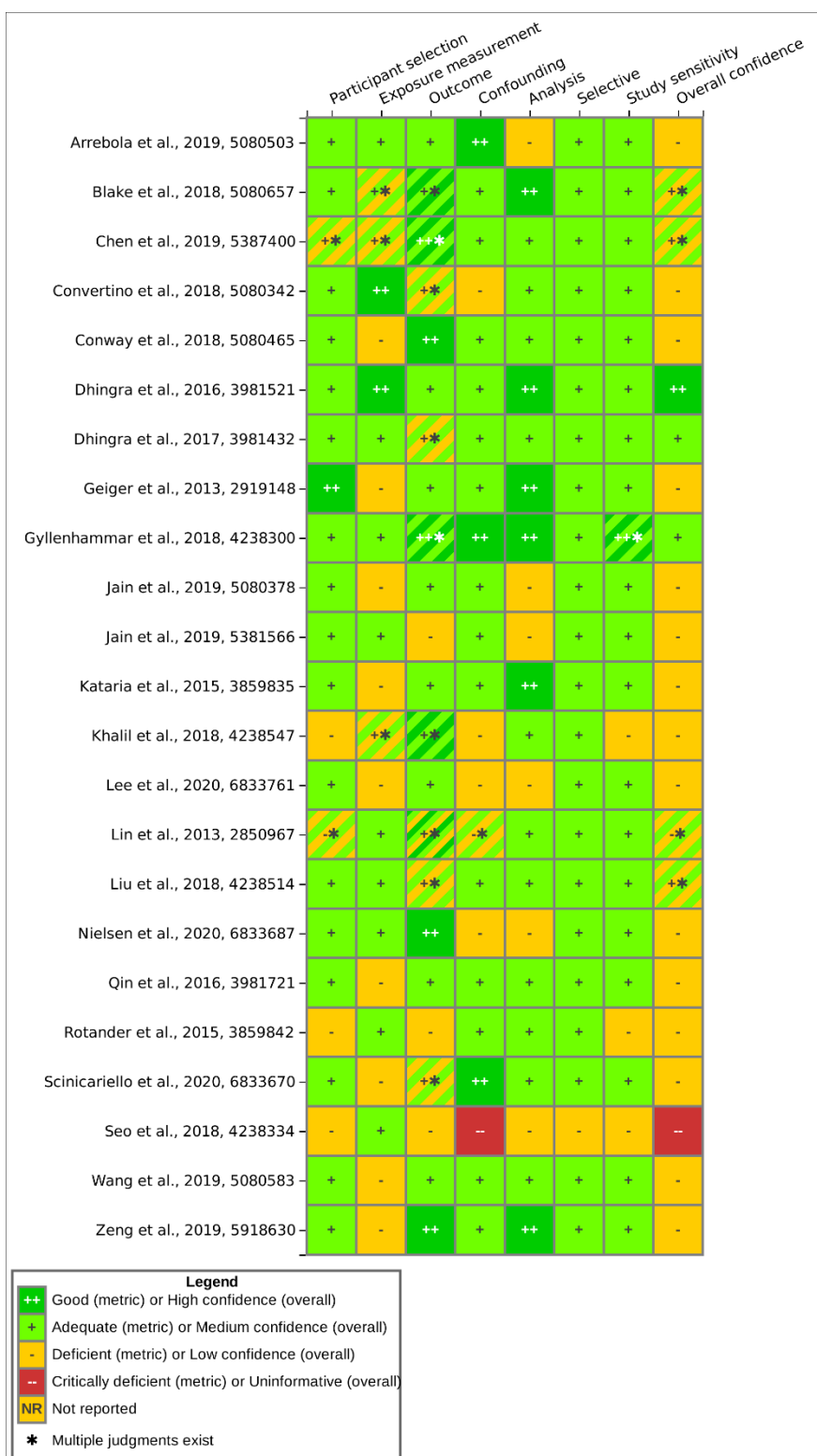


Figure 96. Summary of Study Evaluation for Epidemiology Studies of PFOA and Renal Effects

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

3.3.9.1.3 Findings in Children and Adolescents

Three *low* confidence studies examined 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. Geiger, 2013, (2919148) used NHANES data from 1999–2000 and 2003–2008 to assess the association between serum PFOA levels and hyperuricemia in children aged 12 to 18 years. A statistically significant positive association was observed between increasing quartiles of PFOA and hyperuricemia (p-trend = 0.0071), and serum uric acid (p-trend = 0.0001). An overlapping NHANES (2003–2010) study {Kataria, 2015, 3859835} also observed a significant positive association for uric acid for the highest quartile of PFOA exposure (≥ 4.7 ng/mL) compared to the lowest (< 2.5 ng/mL). Qin, 2016 (3981721) reported significant positive associations with uric acid and hyperuricemia in children aged 12 to 15 years from the GBCA in Taiwan. Positive associations were observed when the highest compared to the lowest PFOA quartiles. When stratified by sex, the associations were only evident among boys, including an increasing trend (p-trend = 0.033) {Qin, 2016, 3981721}.

One *low* confidence study {Kataria, 2015, 3859835} reported on GFRs among children (12–19 years old) from NHANES (2003–2010). A negative association was reported between PFOA and eGFR, where the fourth quartile was associated with a statistically significant decrease in eGFR compared to the lowest exposure quartile, and the second and third quartiles showed a non-significant decrease.

Two *low* confidence studies investigated associations between PFOA and serum creatinine among children and adolescents {Khalil, 2018, 4238547; Kataria, 2015, 3859835}. Kataria, 2015 (3859835) reported a significant positive association with serum creatinine in the highest PFOA quartile when compared with the lowest quartile. Khalil, 2018 (4238547) observed weak, non-significant negative association with serum creatinine in obese children (8–12 years).

3.3.9.1.4 Findings from the General Adult Population

Three studies examined CKD and no significant associations were observed {Conway, 2018, 5080465; Dhingra, 2016, 3981521; Wang, 2019, 5080583}. CKD was defined as an eGFR of < 60 mL/min/1.73 m². A *high* confidence C8 Health Project community study {Dhingra, 2016, 3981521} observed positive non-significant increases in the risk of CKD in both retrospective and prospective analyses, and among non-diabetic participants. In retrospective analyses, the magnitude of effect was diminished and inconsistent when modeling exposure using increasing lag periods (5-, 10-, and 20-year lag). In contrast, negative associations were observed in two *low* confidence studies {Wang, 2019, 5080583; Conway, 2018, 5080465}. Analyses of participants in the Isomers of C8 Health Project in China {Wang, 2019, 5080583} observed a significant negative association with odds of CKD. Analyses of diabetic individuals in the U.S.-based C8 Health Project {Conway, 2018, 5080465} also showed significantly reduced odds, but this effect was not observed in non-diabetic participants. However, a concern for reverse causality makes interpretation of the results difficult in both *low* confidence studies.

Gout was examined in one *low* confidence study {Scinicariello, 2020, 6833670} on adults from NHANES (2009–2014) and a significant increased trend in risk of self-reported gout across PFOA quartiles was observed (p-value = 0.01). The observed effects were similar when stratifying by CKD status.

Seven *low* confidence general population studies {Arrebola, 2019, 5080503; Chen, 2019, 5387400; Lin, 2013, 2850967; Scinicariello, 2020, 6833670; Seo, 2018, 4238334; Zeng, 2019, 5918630; Jain, 2019, 5080378} and one *low* confidence occupational study {Rotander, 2015, 3859842} examined uric acid levels, and three of these studies reported specifically on hyperuricemia {Arrebola, 2019, 5080503; Scinicariello, 2020, 6833670; Zeng, 2019, 5918630}. Significant findings were found in three studies, indicating a positive association with uric acid or increased odds of hyperuricemia, while non-significant positive associations were observed for uric acid in three general population confidence studies and one occupational study.

A *low* confidence NHANES (2009–2014) study {Scinicariello, 2020, 6833670} on adults reported a significant positive association between serum PFOA and serum uric acid in quartile analyses, and the trend was significant (p -trend = 0.0001). The association remained when restricted to participants without CKD, but the association was not consistent among those with CKD. Analyses of hyperuricemia were similar. A significant increasing trend in the odds of hyperuricemia was observed among the whole sample and those without CKD. Similarly, a positive association with serum uric acid was observed in a *low* confidence study on participants from the Isomers of C8 Health Project {Zeng, 2019, 5918630}. In addition, a significant positive association was observed for hyperuricemia and total-PFOA exposure {Zeng, 2019, 5918630}. Results were similar among men and women in sex-stratified analyses. Utilizing NHANES data from 2007–2014, a *low* confidence study {Jain, 2019, 5080378} assessed the associations between serum PFOA and uric acid across gender and stages of GF. For males, serum PFOA and uric acid were positively associated ($p < 0.01$) at stage GF-1 and GF-2 and negatively associated ($p < 0.01$) at stage GF-3B/4. For females, all associations were positive but only reached significance for GF-1 and GF-3A. Two *low* confidence study {Chen, 2019, 5387400; Lin, 2013, 2850967} did not observe associations with plasma uric acid in Croatian adults aged 44–56 years, or in adolescents and young adults aged 12 to 30 years in the Young Taiwanese Cohort Study. A *low* confidence study {Arrebola, 2019, 5080503} from the BIOAMBIENT.ES study observed a non-significant increase in risk of hyperuricemia.

One *low* confidence occupational study examined serum uric acid levels among firefighters with past exposure to AFFF {Rotander, 2015, 3859842}. Uric acid levels were elevated with increasing PFOA exposure in firefighters, but the result did not reach significance.

One *medium* and two *low* confidence studies in high exposed populations examined eGFR, and two studies reported negative associations {Blake, 2018, 5080657; Dhingra, 2017, 3981432}, while one reported a positive association {Wang, 2019, 5080583}. Dhingra (2017, 3981432) reported a significant negative association with measured but not modelled PFOA and a negative trend in eGFR across measured serum PFOA quintiles in women from the Women from C8 Science Panel Project. The study used modelled PFOA as an approach to demonstrate that cross-sectional analyses using measured PFOA are affected by reverse causation {Dhingra, 2017, 3981432}. Blake (2018, 5080657) observed negative non-significant associations in participants of the Fernald Community Cohort (FCC) with high exposure to PFAS from their household water supplies. Wang (2019, 5080583) observed positive associations in a high-exposed population from the Isomers of C8 Health Project.

The evidence on PFOA and renal effects among pregnant women was limited. Only two studies on pregnant women examined effects on eGFR {Nielsen, 2020, 6833687; Gyllenhammer, 2018, 4238300}. One *medium* confidence study {Gyllenhammer, 2018, 4238300} assessed the

relationship between maternal PFOA during pregnancy and maternal eGFR three weeks after delivery, calculated using both creatinine- and cystatin C-based estimates of GFR. A significant positive relationship between cystatin C-GFR and maternal PFOA was reported ($\beta = 0.004 \pm 0.002$, $p = 0.022$). Changes in kidney function during pregnancy were evaluated in a small group of pregnant women ($n = 73$) using creatinine-GFR and cystatin C-GFR in a *low* confidence study (Nielsen, 2020, 6833687), but no significant effects were observed using partial Spearman rank correlations. While the *medium* confidence study in pregnant women reported a positive association between PFOA and eGFR {Gyllenhammer, 2018, 4238300}, given the limited number of studies, there is not enough evidence to determine conclusive associations between PFOA renal function among pregnant women and an occupational group of firefighters.

Four *low* confidence studies examined albumin and creatinine as biomarkers for renal function {Convertino, 2018, 5080342; Chen, 2019, 5387400; Jain, 2019, 5381566; Lee, 2020, 6833761}. The four studies provided differing conclusions. Jain (2019, 5381566) reported statistically significant positive with serum and urine creatinine, and serum albumin in NHANES (2005–2014) participants. Statistically significant negative associations were observed 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 PFOA, stratification by stages of GF had inconsistent effects. However, Lee (2020, 6833761) observed a decreased risk of albuminuria (defined as urine albumin-to-creatinine ratio ≥ 30 mg/g) Chen (2019, 5387400) did not observe significant associations with plasma creatinine. Convertino (2018, 5080342) did not observe any associations with serum creatinine during a phase 1 controlled trial assessing the chemotherapeutic potential of APFO.

One *low* confidence study {Liu, 2018 4238514} examined serum proteins among NHANES (2013–2014) participants and reported a significant positive association using linear PFOA exposure levels. The result was similar for total PFOA but did not reach significance.

3.3.9.2 Animal Evidence

There are 4 studies from the most recent literature search conducted in 2020 and 2 key studies from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and renal effects. Study quality evaluations for these 6 studies are shown in Figure 97.

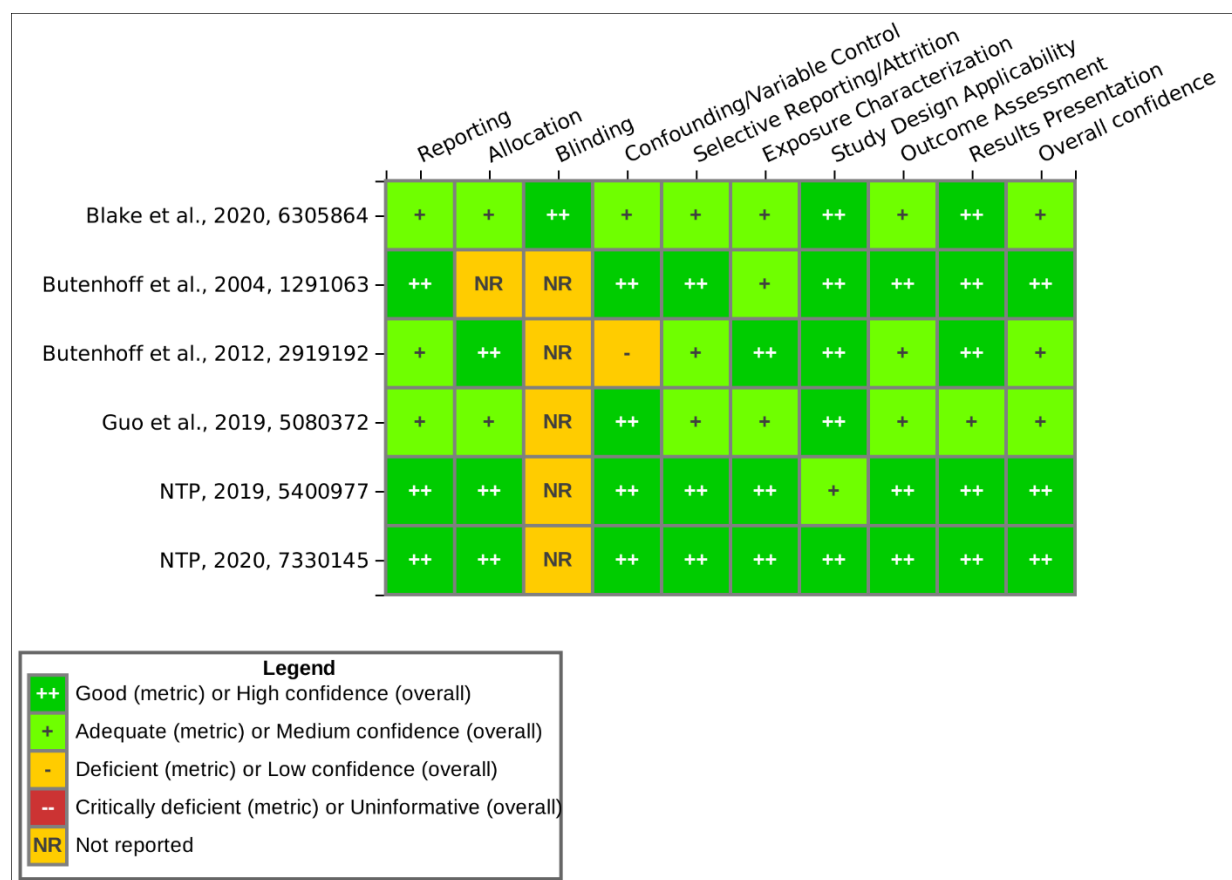


Figure 97. Summary of Study Evaluation for Toxicology Studies of PFOA and Renal Effects

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

The available data suggest the renal system may be adversely affected by PFOA exposure, but the evidence all comes from studies conducted in rats. One study in mice {Blake, 2020, 6305864} and one study in monkeys {Butenhoff, 2002, 1276161} both reported no effects on the renal system. In contrast, several short-term and chronic studies in rats reported significant increases in absolute and/or relative kidney weights {Cui, 2009, 757868; NTP, 2019, 5400977; Butenhoff, 2004, 1291063; Butenhoff, 2012, 2919192; NTP, 2020, 7330145} and/or alterations in renal clinical chemistry/urinalysis parameters {Cui, 2009, 757868; NTP, 2019, 5400977; NTP, 2020, 7330145; Guo, 2019, 5080372}. However, only two studies reported concurring histological changes in the kidney {Cui, 2009, 757868; NTP, 2020, 7330145}.

Effects on kidney weight were predominately observed in male rats rather than female rats, regardless of study design and exposure duration (Figure 98 (absolute kidney weight), Figure 99 (relative kidney weight in males), Figure 100 (relative kidney weight in females)). This is true of both absolute and relative kidney weight metrics. However, across both sexes, several studies observed statistically significant decreases in absolute kidney weight at the highest doses tested (Figure 98), which often corresponded to doses resulting in reduced body weight (Sections 3.3.1.2 and 3.3.7.2). These changes in body weight may influence the interpretation of absolute and relative kidney weight changes.

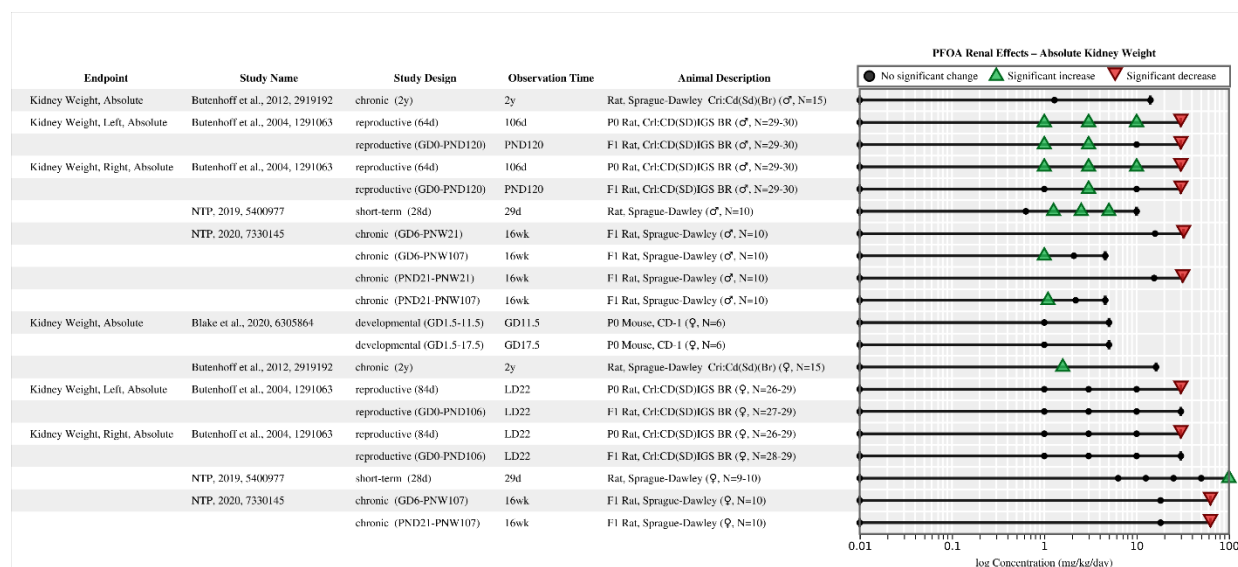


Figure 98. Absolute Kidney Weights in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA 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; F1 = first generation; PND = postnatal day; PNW = postnatal week; d = day; wk = week; y = year.

NTP (2019, 5400977) observed dose-dependent increases in the absolute and relative kidney weights of male Sprague Dawley rats treated with PFOA for 28 days. Absolute and relative kidney weights were increased in all treated groups (doses of 0.625–10 mg/kg/day), though the increase in absolute weight was only significant for the three middle dose groups (1.25, 2.5, and 5 mg/kg/day). The highest dose group (10 mg/kg/day) resulted in the largest increase in relative kidney weight of approximately 36% control weight. The lack of a clear dose-response trend in absolute kidney weights was likely related to decreased body weights observed at doses ≥ 2.5 mg/kg/day. Despite the increases observed in kidney weights, there were no significant histological changes observed in the kidneys of PFOA-treated rats {NTP, 2019, 5400977}. Cui et al. (2009, 757868) similarly observed increased relative kidney weights in male rats administered 5 or 20 mg/kg/day for 28 days, though the increases were not dose-dependent (absolute weights were not reported); however, histological changes were observed in the kidneys of the high-dose group, including turbidness and tumefaction in the epithelia of the proximal convoluted tubule (reported qualitatively without incidence data).

A similar trend in kidney weight was observed for male rats in a two-generation reproduction study {Butenhoff, 2004, 1291063}. Adult P0 and F1 males had significantly increased absolute kidney weights at 1, 3 and 10 mg/kg/day, but decreased kidney weights at the highest dose level of 30 mg/kg/day. Relative kidney weights were significantly increased in all treated males (increases of 16–27% and 11–19% change in P0 and F1 males, respectively). Kidney weights relative to brain weights were increased at 1, 3, and 10 mg/kg/day, but not 30 mg/kg/day. In the high-dose male group, absolute and relative kidney weight changes occurred in a pattern typically associated with decrements in body weight. However, in the lower-dose groups of males, significant increases in absolute kidney weight and relative-to-body and brain weights

appear to be treatment-related and are consistent with the results reported for male rats in the 28-day study by NTP (2019, 5400977). Increased kidney weights observed following exposure to PFOA may be a response to the challenge of providing transporters for renal removal of the foreign molecule {U.S. EPA, 2016, 3603279}. Increased kidney weight can be regarded as an adaptive response to the transport challenge. It is beneficial for the individual but adverse in the sense that it signifies the need to upregulate tubular transporters in the kidney to excrete the foreign material and a reflection of PFOA bioaccumulation in serum and tissues. Butenhoff et al. (2004, 1291063) did not report conducting kidney histopathology in this reproductive study.

Two chronic dietary studies in Sprague Dawley rats evaluated effects on the renal system, but the results were not consistent across studies. Butenhoff et al. (2012, 2919192) observed no dose-related changes in kidney weight or histopathology in male rats administered 30 or 300 ppm PFOA in the diet for two years (equivalent to 1.3 and 14.2 mg/kg/day, respectively). In contrast, a two-year study by NTP (2020, 7330145) observed altered kidney weights and increased incidences of nonneoplastic lesions in the kidneys of male rats exposed to postweaning dietary concentrations of 20, 40, 80, 150, or 300 ppm with or without perinatal exposure to 150 or 300 ppm (see study design details in Section 3.3.1.2.1.2). At the 16-week interim evaluation, absolute kidney weights were increased in males of the 0/20 and 300/20 ppm groups (perinatal/postweaning concentrations, equivalent to postweaning doses of 1.1, and 1.0 mg/kg/day, respectively) and decreased in males of the 0/300 and 300/300 ppm groups (31.7 and 32.1 mg/kg/day, respectively), but not significantly altered compared to controls in any of the intermediate dose groups. However, relative kidney weights were significantly increased in all treated groups (range of 21–35% increases across all groups); body weights were also significantly reduced in all treatment groups (dose-dependent range of 9–45% decreases across all groups). Substantially reduced body weights in treated males makes interpretation of kidney weight effects difficult.

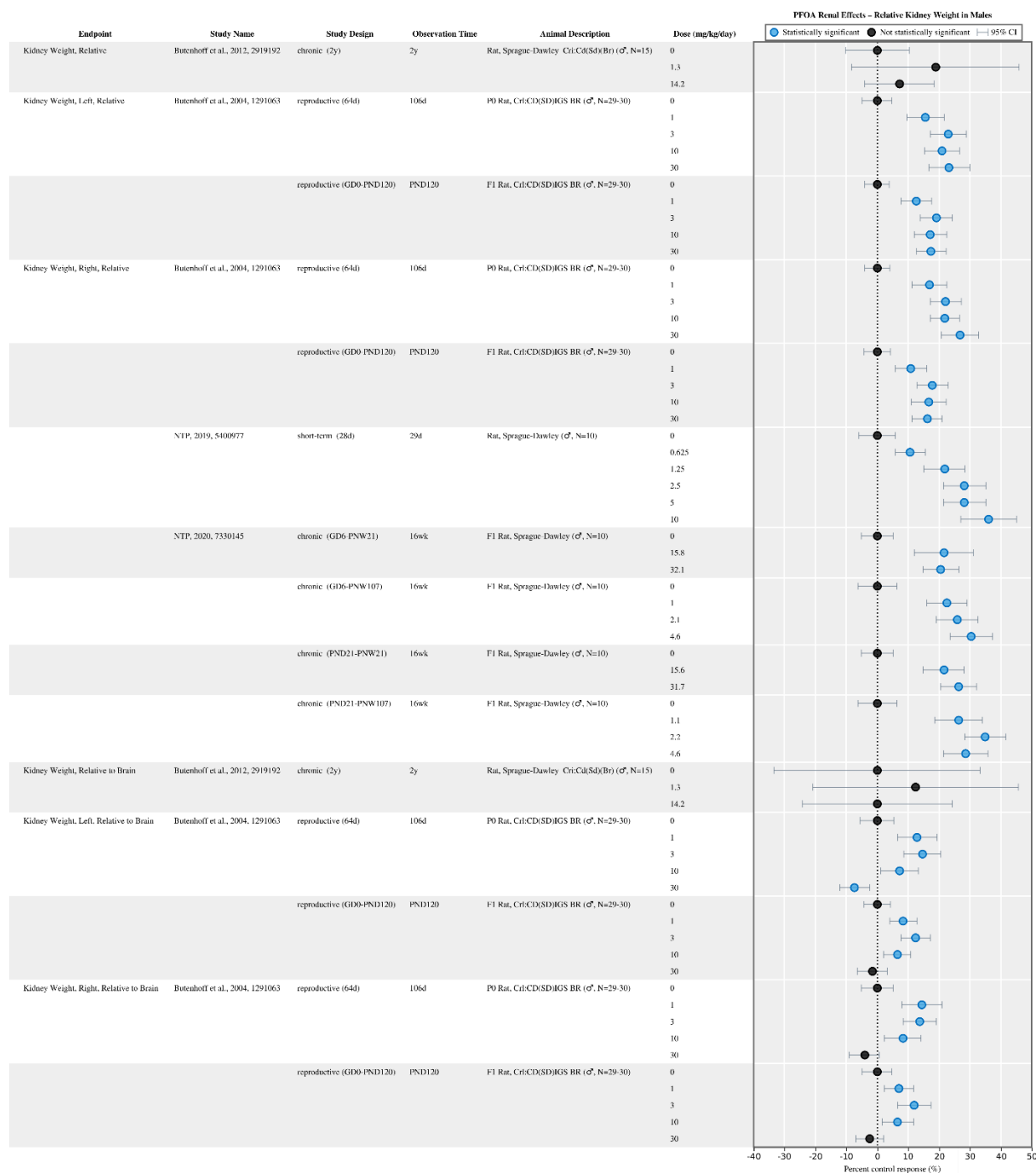


Figure 99. Percent Change in Relative Kidney Weights of Male Rats Following Exposure to PFOA

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

GD = gestation day; P₀ = parental generation; F₁ = first generation; PND = postnatal day; PNW = postnatal week; d = day; wk = week; y = year; CI = confidence interval.

Female rats were generally less sensitive to changes in kidney weights compared to male rats, with most differences occurring in the highest dose groups only (Figure 99, Figure 100). NTP (2019, 5400977) observed dose-dependent increases in absolute and relative kidney weights of female rats treated with PFOA for 28 days. Absolute kidney weight was only increased at the highest dose of 100 mg/kg/day (11% increase) while relative kidney weight was increased at 50 and 100 mg/kg/day (7% and 17% increases, respectively). Similar to males from this study, there were no significant histological changes observed in the kidneys of PFOA-treated rats (NTP, 2019, 5400977). In contrast, in a two-generation reproduction study {Butenhoff, 2004, 1291063}, absolute and relative kidney weights of P₀ females were significantly decreased at 30 mg/kg/day (decreases of approximately 5–8% change), and no effects were observed on kidney weight in F₁ females. There were no significant effects on the body weight of these animals at terminal sacrifice.

Butenhoff et al. (2012, 2919192) observed an increase in absolute kidney weight (11% change) in female rats administered 30 but not 300 ppm PFOA in the diet for two years (equivalent to 1.6 and 16.1 mg/kg/day, respectively). In contrast, the authors reported significant increases in relative kidney weights of rats administered 300 but not 30 ppm (increase of 15% change). Rats administered 300 ppm experienced decreases in body weight $\leq 11\%$ of control weights by the time of terminal sacrifice. The authors reported no change in renal histopathology in female rats {Butenhoff et al., 2012, 2919192}. A second two-year feeding study by NTP (2020, 7330145) found alterations in absolute kidney weight and increased incidences of nonneoplastic lesions in the kidneys of female rats exposed to postweaning dietary concentrations of 300 or 1,000 ppm with or without perinatal exposure to 300 ppm (see study design details in Section 3.3.1.2.1.2). At the 16-week interim evaluation, absolute kidney weights were decreased in females of the 0/1,000 ppm and 300/1,000 ppm groups (equivalent to 63.4 and 63.5 mg/kg/day postweaning doses); however, relative kidney weights were unaltered in females. Body weights were significantly reduced in females exposed to 1,000 ppm postweaning (by 12%). Decreased absolute kidney weights observed in females exposed to 1,000 ppm were likely related to reduced body weights as there was no change in relative kidney weight.

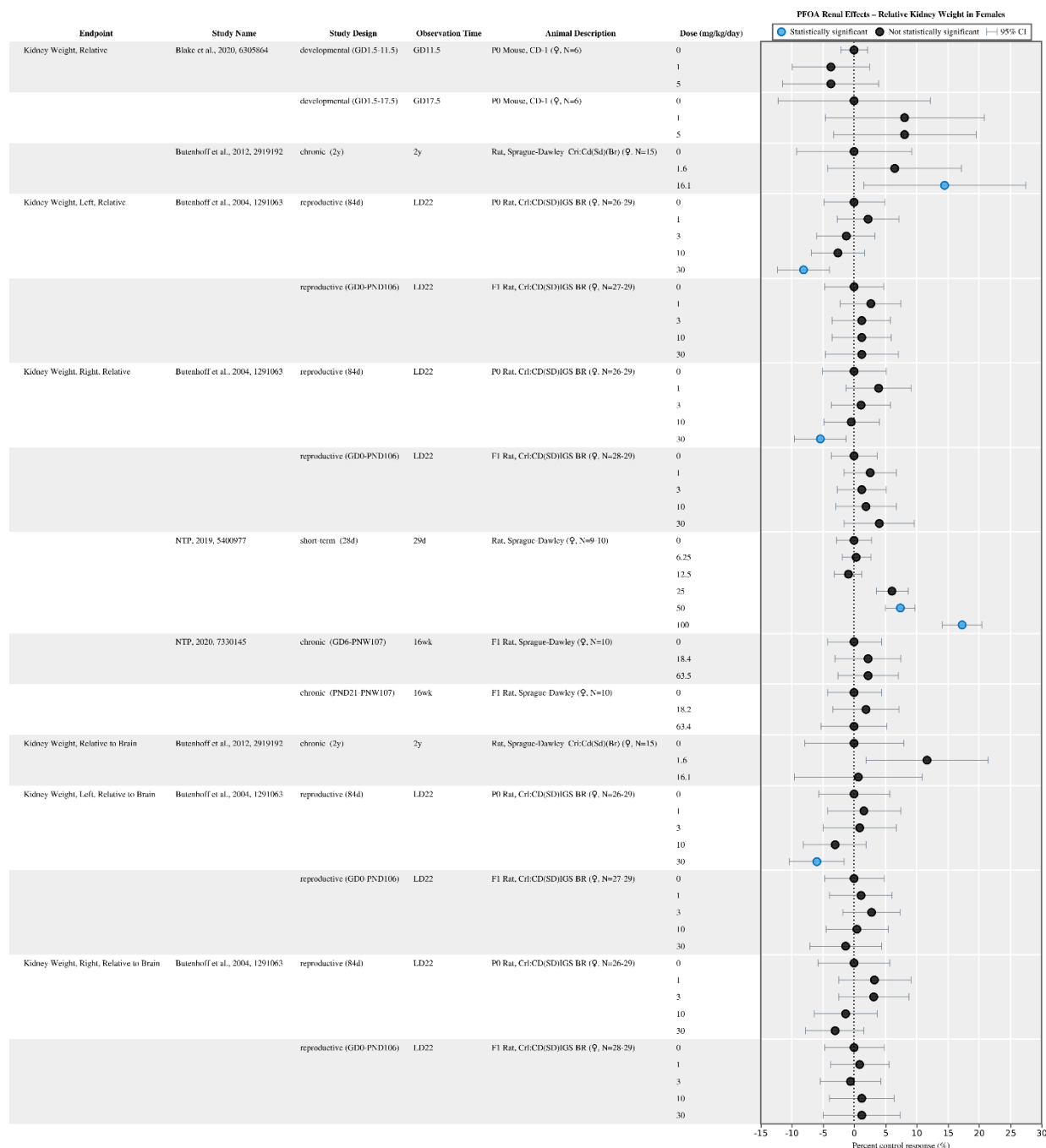


Figure 100. Percent Change in Relative Kidney Weights of Female Rodents Following Exposure to PFOA

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

GD = gestation day; P0 = parental generation; F1 = first generation; PND = postnatal day; PNW = postnatal week; d = day; wk = week; y = year; CI = confidence interval.

Histopathological examination of male rats at the 16-week interim of a 2-year dietary study showed increased incidences of renal tubule mineralization in the 0/150, 0/300, and 300/300 ppm

groups compared to the 0/0 ppm control group (incidences of 40%, 50%, and 60%, respectively, compared to 0% incidence in the control group) {NTP, 2020, 7330145}. No other significant histological changes were observed in males, and the male groups were removed from that study shortly after the interim. However, examination of female rats revealed treatment-related increased incidences of renal tubule mineralization, hyperplasia of the urothelium that lines the renal papilla, and necrosis of the renal papilla (that was observed only after 2 years). As shown in Table 10 **Error! Reference source not found.**, these lesions were mainly found in the female groups with the highest postweaning exposure (1,000 ppm, equivalent to approximately 63 mg/kg/day).

Table 10. Incidences of Nonneoplastic Lesions in the Kidneys of Female Sprague-Dawley Rats as Reported by NTP (2020, 7330145)

A	Postweaning Dose		
Perinatal Dose	0 ppm	300 ppm	1,000 ppm
16 Weeks			
Renal Tubule, Mineralization			
0 ppm	2/10 (20%) (1.0) ^a	1/10 (10%) (1.0)	7/10* (70%) (1.0)
150 ppm	—	2/10 (20%) (1.0)	—
300 ppm	—	—	5/10 (50%) (1.2)
Renal Papilla Urothelium, Hyperplasia			
0 ppm	0/10 (0%)	0/10 (0%)	4/10* (40%) (1.3)
150 ppm	—	0/10 (0%)	—
300 ppm	—	—	4/10* (40%) (1.0)
Renal Papilla, Necrosis			
0 ppm	0/10 (0%)	0/10 (0%)	0/10 (0%)
150 ppm	—	0/10 (0%)	—
300 ppm	—	—	0/10 (0%)
107 Weeks			
Renal Tubule, Mineralization			
0 ppm	5/50 (10%) (1.2)	6/50 (12%) (1.3)	16/50** (32%) (1.0)
150 ppm	—	8/50 (16%) (1.0)	—
300 ppm	—	—	8/50 (16%) (1.5)
Renal Papilla Urothelium, Hyperplasia			
0 ppm	4/50 (8%) (1.0)	21/50** (42%) (1.0)	40/50** (80%) (1.9)
150 ppm	—	8/50 (16%) (1.0)	—
300 ppm	—	—	45/50** (90%) (1.8)
Renal Papilla, Necrosis			
0 ppm	0/50 (0%)	0/50 (0%)	12/50** (24%) (2.3)
150 ppm	—	0/50 (0%)	—
300 ppm	—	—	22/50** (44%) (2.1)

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

^aAverage severity grade of lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

In a second similar study conducted by NTP in male rats only due to high mortality in the initial study, relative kidney weights of all groups exposed to postweaning dietary concentrations of 20, 40, or 80 ppm (equivalent to approximately 1, 2, or 4.6 mg/kg/day) for 16 weeks were significantly greater than the 0/0 ppm control group, but absolute kidney weights were significantly increased only in the groups exposed to 20 ppm postweaning {NTP, 2020, 7330145}. Body weights were significantly decreased in all treated groups (by 9–21%), and that could explain why absolute kidney weights did not achieve statistical significance in the higher dose groups in these growing rats. These patterns in kidney weights are similar to those observed for male rats in the studies by NTP (2019, 5400977) and Butenhoff et al. (2004, 1291063). There were no significant histological changes in the kidneys found at the interim or two-year terminal evaluations.

In contrast to results found in studies with rats, no treatment-related effects were reported for kidney weight or histopathology in female mice administered PFOA during gestation {Blake, 2020, 6305864} or in male monkeys administered PFOA for 6 months by oral capsule {Butenhoff, 2002, 1276161}. One short-term study in rats {NTP, 2019, 5400977} and three chronic studies in rats or monkeys also examined the urinary bladder for histopathology after exposure to PFOA, and no treatment-related effects were reported {Butenhoff, 2012, 2919192; NTP, 2020, 7330145; Butenhoff, 2002, 1276161}.

Several studies analyzed clinical chemistry and urinalysis endpoints related to renal toxicity, though there is uncertainty regarding adversity of the observed effects. In two separate studies, NTP observed increased concentrations of urea nitrogen in the blood of male and female rats following 28 days or 16 weeks of exposure {NTP, 2019, 5400977; NTP, 2020, 7330145}. However, without concomitant increases in blood creatinine concentrations, NTP concluded that the slight increases in urea nitrogen were likely due to a decrease in water intake {NTP, 2019, 5400977; NTP, 2020, 7330145}. In fact, creatinine concentrations were significantly decreased in male rats administered ≥ 0.625 mg/kg/day in the 28-day study, though NTP considered this change to be related to decreased food intake and body weight rather than a direct treatment effect {NTP, 2019, 5400977}.

Butenhoff et al. (2012, 2919192) also observed slight increases in BUN in male and female rats, but only at the 3- and 6-month evaluations of the 2-year study; creatinine was not measured. No significant differences were observed in BUN or creatinine in female mice administered PFOA during gestation {Blake, 2020, 6305864} or in male monkeys administered PFOA for 6 months {Butenhoff, 2002, 1276161}. However, a 28-day study in male mice found significant, dose-dependent decreases in BUN and increases in serum ammonia levels in all treated groups (0.4–10 mg/kg/day) compared to controls; the authors of this study suggest these changes are signs of urea cycle dysfunction caused by PFOA {Guo, 2019, 5080372}.

Two studies found that the activity of creatine kinase was decreased in male rats administered PFOA for 28 days or up to 2 years {NTP, 2019, 5400977; Butenhoff, 2012, 2919192}. NTP considered this effect to be treatment-related but not toxicologically relevant {NTP, 2019, 5400977}.

No apparent treatment-related effects were observed on urinalysis endpoints (e.g., volume, pH, specific gravity, protein, blood) measured in male or female rats over the course of two years of

treatment {Butenhoff, 2012, 2919192} or in male monkeys over the course of 6 months of treatment {Butenhoff, 2002, 1276161}.

3.3.9.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse renal outcomes is discussed in Sections 3.1.1.4, 3.2.5, 3.3.4, and 3.4.3 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 4 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to renal effects. A summary of these studies is shown in Figure 101. Additional analysis on the mechanistic actions of PFOA on renal health outcomes is ongoing 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	0	1	1
Cell Signaling Or Signal Transduction	1	1	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	2	0	2
Other	1	0	1
Grand Total	3	1	4

Figure 101. Summary of Mechanistic Studies of PFOA and Renal Effects

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

3.3.9.4 Evidence Integration

In summary, the present review of the epidemiological literature found limited evidence of an association between PFOA and decreased renal function. In comparison to the 2016 Health Assessment, the available 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 glomerular filtration and shared renal transporters for perfluoroalkyls and uric acid) cannot be ruled out. There were mixed results across the measures of renal function. A positive association was observed for CKD in a C8 Health Project population including among non-diabetics {Dhingra, 2016, 3981521}; while two studies reported negative associations {Wang, 2019, 5080583; Conway, 2018, 5080465}. The results were also inconsistent when assessing eGFR, in three high exposed population studies, with two reporting negative associations {Blake, 2018, 5080657; Dhingra, 2017, 3981432} and one positive association {Wang, 2019, 5080583}. Regarding hyperuricemia and uric acid levels, results varied across gender and GF. In children, there were mixed results for associations between PFOA and creatinine and uric acid. One *low* confidence study reported a statistically significance decrease in eGFR in adolescents across PFOA quartiles {Kataria, 2015, 3859835}.

No epidemiological studies assessing alterations in renal function due to PFOA exposure were considered for the derivation of PODs since there is limited evidence of an association between PFOA and decreased renal function.

The available evidence in animal models suggests that the kidney can be a target of PFOA toxicity, although effects have only been observed in rats. Clinical chemistry and urinalysis endpoints do not provide strong evidence of damage to kidney structure or function; however, kidney weights, particularly in male rats, were significantly increased following short-term and chronic exposure. The observed increases in kidney weights may indicate an adaptive response that is adverse in the sense that it signifies the need to upregulate tubular transporters in the kidney to excrete the foreign material and is a reflection of PFOA bioaccumulation in serum and tissues. However, kidney weights appear to be heavily influenced by changes in body weight which impacts the ability to interpret and model these responses.

Studies in animals generally found no histological changes correlating with increased kidney weight. The NTP chronic study {NTP, 2020, 7330145} in rats provides the most convincing evidence that the kidney can be damaged by exposure to PFOA, although the doses with effects observed were relatively high (approximately 18 and 63 mg/kg/day in females and 16 and 32 mg/kg/day in males). Renal lesions were mainly observed in treated females, except for increased tubule mineralization which was observed in both sexes. Cui et al. (2009, 757868) also observed kidney damage in male rats treated with 20 mg/kg/day PFOA for 28 days, but the incidences of specific lesions were not reported. The mechanisms of this kidney damage are unknown, but it may be related to direct cytotoxicity from the high concentration of PFOA in the urine {NTP, 2020, 7330145}. EPA concluded that these effects indicate a toxicity relevant to humans, but the renal lesions observed by NTP (2020, 7330145) were not considered for the derivation of PODs because the effects were observed at relatively high administered dose levels.

3.3.10 Hematological

3.3.10.1 Human Evidence

3.3.10.1.1 Introduction

The mechanisms for PFOA 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}. PFOA 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 PFOA 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 PFOA {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 PFOA 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 PFOA and hematological health outcomes (Figure 102). 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 PFOA using biomarkers in blood. Samples were taken from pregnant women, children, adolescents, or adults. Most included studies were cross-sectional, meaning exposures and outcomes were evaluated during the same period. 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, the NHANES {Etzel, 2019, 5043582; Jain, 2020, 6333438; Jain, 2020, 6833623}. Etzel et al., 2019 (N = 7,040) used 2003–2010 NHANES data for adolescents and adults 12 and over {Etzel, 2019, 5043582}, and Jain, 2020a (N = 11,251) and 2020b (N = 10,644), 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} used data on 48 obese children at 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. Convertino et al. (2018, 5080342) conducted a six-week trial with experimental exposure to APFO among late-stage cancer patients at two medical centers in Glasgow and Dundee, Scotland.

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 socioeconomic. In particular, the duration of breastfeeding is expected to be associated with both PFOA exposure and nutrition intake {Abraham, 2020, 6506041}. The blood matrix (whole blood versus plasma or serum) could also affect the interpretation of results. Measuring PFOA and serum lipids concurrently was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOA (median half-life = 2.7 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 102). The *low* confidence had deficiencies in confounding and limited sample sizes. Convertino et al., 2018 (2018, 5080342) did not control for confounding, although this concern is somewhat attenuated by the prospective trial study design wherein investigators manipulated the exposure levels. Khalil et al., 2018 (2018, 4238547) was affected by a small sample size, and potential residual confounding attributable to differences in participants' SES. Three studies were rated as *uninformative* for hematological outcomes. For Jain (2020, 6833623), the use of PFOA 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 (2020, 6506041) and Jiang et al., 2014 (2014, 2850910) only performed unadjusted correlation analyses and therefore did not consider the influence of potential confounding factors.

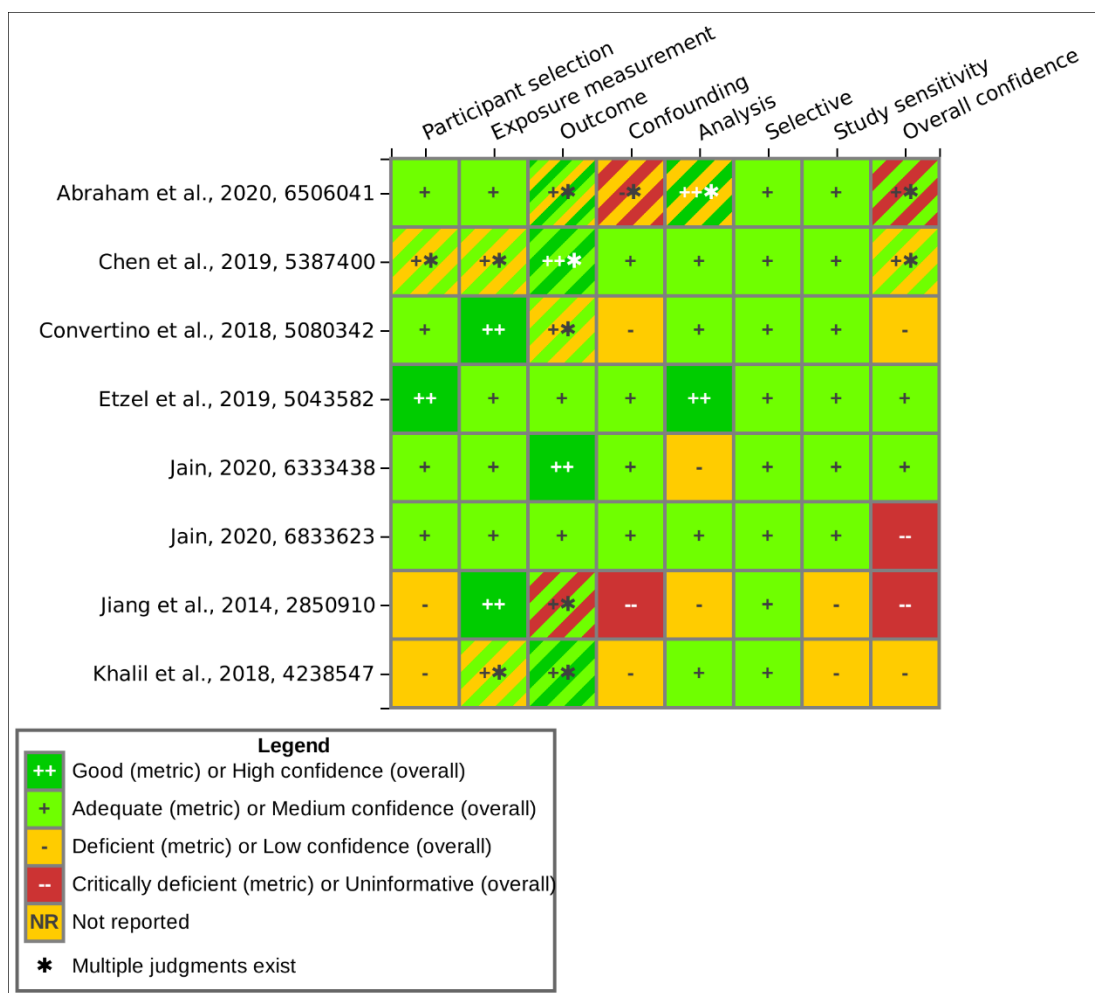


Figure 102. Summary of Study Evaluation for Epidemiology Studies of PFOA and Hematological Effects

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

3.3.10.1.3 Findings

Two studies examined levels of 25-hydroxy vitamin D or vitamin D deficiency and observed no associations. In adolescents and adults from NHANES (2003–2010), Etzel et al., 2019 (2019, 5043582) observed non-significant positive prevalence ORs for vitamin D deficiency and decreases in levels 25-hydroxy vitamin D pre doubling of PFOA. A *low* confidence study, Khalil et al., 2018 (2018, 4238547) observed a non-significant positive association between PFOA exposure and 25-hydroxy vitamin D levels in 8–12-year-old United States children.

In adults from NHANES (2003–2016), Jain, (2020, 6333438) observed small statistically significant increases in whole blood hemoglobin (WBHGB) levels (Table C-19). This was true for participants with or without anemia, and the magnitude of the association was larger among those anemics. For example, associations (slopes) between PFOA and WBHGB for anemic females were more than five times higher as compared to nonanemic females (beta = 0.03413 vs. 0.00605). 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 females, association between WBHGB and PFOA concentrations were uniformly positive across worsening stages of renal failure. For anemic males, association between WBHGB and PFOA concentrations were positive except at GF-3A ($45 \leq \text{eGFR} < 60 \text{ mL/min/1.73 m}^2$). Overall, the association between WBHGB and PFOA followed U-shaped distributions. Hemoglobin levels were also examined in pregnant women in Jiang et al., 2014 (2014, 2850910). Significant positive correlations were observed between total PFOA and hemoglobin levels ($r = 0.192$, $p < 0.05$) and albumin ($r = 0.251$, $p < 0.01$), although these results did not consider the influence of confounding factors and should be interpreted with caution.

Chen et al., 2019 (2019, 5387400) observed non-significant decreases in serum calcium levels among Croatian adults.

Several markers of liver function blood clotting tests were examined in a phase 1 dose-calculation trial using APFO. Convertino et al., 2018 (2018, 5080342), observed no clear differences in plotted probabilistic fibrinogen, prothrombin time (PPT), or activated partial thromboplastin time (aPPT) at various PFOA concentrations.

3.3.10.2 Animal Evidence

There is 1 study from the most recent literature search conducted in 2020 and 1 key study from the 2016 PFOA HESD {EPA, 2016, 3603279} that investigated the association between PFOA and hematological effects. Study quality evaluations for these 2 studies are shown in Figure 103.

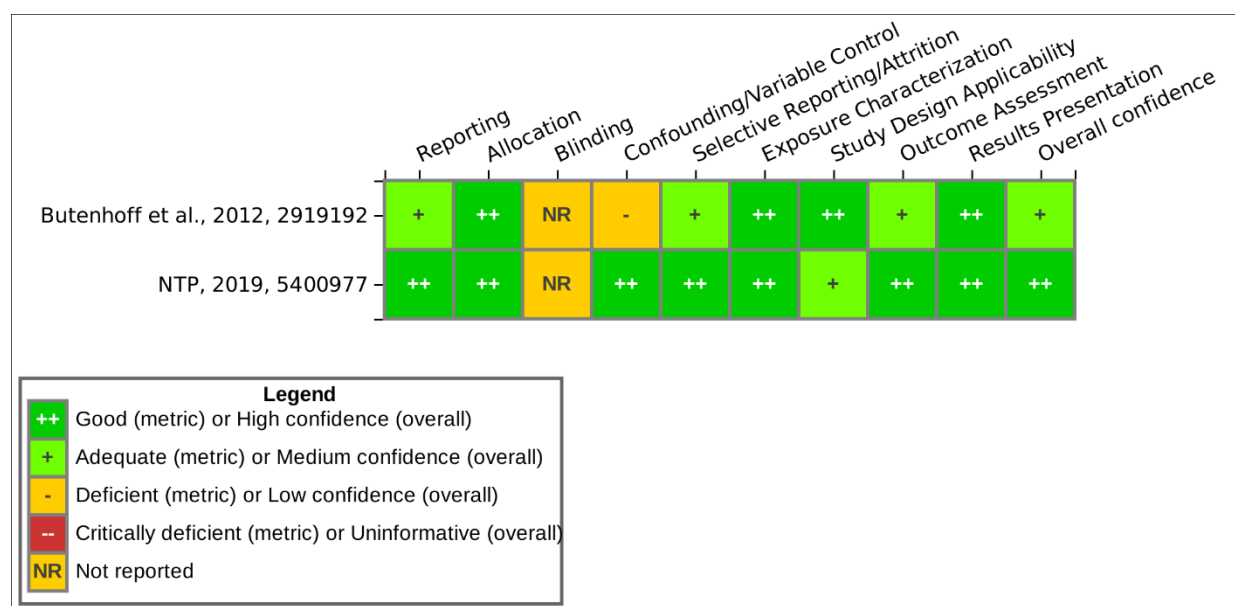


Figure 103. Summary of Study Evaluation for Toxicology Studies of PFOA and Hematological Effects

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. Five oral studies in rodents or monkeys with short-term to chronic exposure durations evaluated the effects of PFOA on the hematological system. Significant changes in some erythron parameters following PFOA exposure to rats at dose levels as low as 0.625 mg/kg/day {NTP, 2019, 5400977} and increases in aPPT and PPT in monkeys exposed to 30 mg/kg/day {Butenhoff et al., 2002, 1276161} suggest the potential for the hematological system to be a target of PFOA toxicity.

In a 28-day study, significant decreases in erythrocyte count, hematocrit, and hemoglobin (≥ 1.25 mg/kg/day), reticulocytes (≥ 0.625 mg/kg/day), and mean cell volume (10 mg/kg/day) were observed in male Sprague Dawley rats (Figure 104) {NTP, 2019, 5400977}; however, the majority of these effects, except reticulocyte counts, were within 10% of control levels. Significant decreases in erythrocyte count (100 mg/kg/day), hematocrit (≥ 6.25 mg/kg/day), and hemoglobin (≥ 12.5 mg/kg/day) were observed in female rats from the same 28-day study, but the effects were also within 10% of control levels (Figure 104) {NTP, 2019, 5400977}. Loveless et al. {2008, 988599} administered PFOA to male Sprague-Dawley rats or male CD-1 mice at dose levels 0, 0.3, 1, 10, or 30 mg/kg/day for 29 days. In rats, hemoglobin and hematocrit were significantly decreased (91–92% of control) at 10 and 30 mg/kg/day and a significant increase in reticulocytes (197% of control) was observed with 30 mg/kg/day. No other altered hematological effects were reported in rats or mice, though there was a slight increase in granulocytic bone marrow hyperplasia in mice dosed with 10 or 30 mg/kg/day.

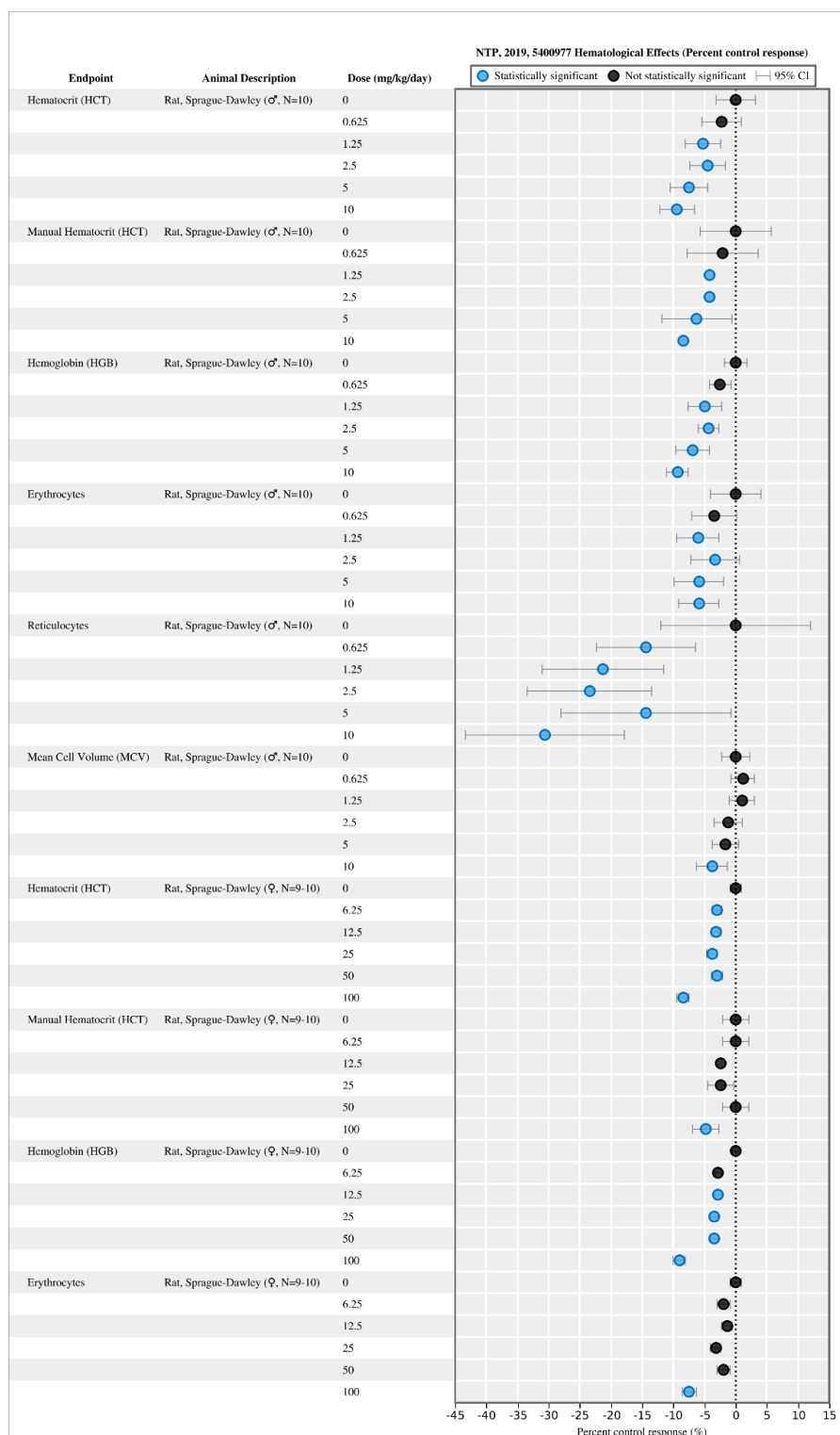


Figure 104. Hematological Effects in Male and Female Sprague Dawley Rats Dosed with PFOA for 28 Days as Reported by NTP, 2019, 5400977

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

HCT = hematocrit; HGB = hemoglobin; MCV = mean cell volume; CI = confidence interval.

Dietary administration of 30 or 300 ppm PFOA (equivalent to 1.3 or 14.2 mg/kg/day in males and 1.6 or 16.1 mg/kg/day in females) to male and female Sprague-Dawley rats for 2 years produced mild or inconsistent effects on hematology {Butenhoff et al., 2012, 2919192}. The authors provided data on red blood cell counts, hemoglobin, and hematocrit at 3, 6, 12, 18, and 24 months, though only time points prior to 52 weeks are considered as clinical pathology testing in aging rodents may be affected by naturally occurring disease {Weingand et al., 1992, 670731}. In males, Butenhoff et al. (2012, 2919192) reported significant decreases in red blood cell counts in both dose groups at 6 months and in the 14.2 mg/kg/day group at 12 months. These decreases did not exceed 10% change from controls. Similarly, the authors reported significant decreases in hematocrit in both dose groups at 3 months and with 14.2 mg/kg/day at 12 months, but these changes also did not exceed 10% difference from controls. There was no observed effect on hemoglobin levels at any time point. In females, significant changes were often noted in the 1.6 mg/kg/day dose group but not the 16.1 mg/kg/day group. For example, minimal decreases in hemoglobin, hematocrit, and red blood cell counts were observed at 6 months in the 1.6 mg/kg/day group but not the high dose group. Dose-dependent decreases in these three parameters were observed at the 12-month time point, though the magnitude of change did not exceed 10% difference from controls. Discussions on other parameters related to immune system function from this study are provided in Section 3.3.4.2.

In a 90-day study with rhesus monkeys, significant increases in aPPT and PPT were observed at 30 mg/kg/day at 1-month analyses {Goldenthal, 1978, 1291068}; at 3 months, the same effects were seen in the lone surviving monkey at 30 mg/kg/day (early mortality at the high dose level of 100 mg/kg/day precluded hematological analyses). A 182-day oral (capsule) study in male cynomolgus monkeys reported no hematological findings at dose levels up to 20 mg/kg/day {Butenhoff et al., 2002, 1276161}.

3.3.10.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse hematological outcomes in the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 5 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to hematological effects. A summary of these studies is shown in Figure 105. Additional analysis on the mechanistic actions of PFOA on hematological health outcomes is ongoing and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Atherogenesis And Clot Formation	0	1	1	2
Big Data, Non-Targeted Analysis	0	0	1	1
Cell Growth, Differentiation, Proliferation, Or Viability	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	1	2
Oxidative Stress	0	0	1	1
Grand Total	1	1	3	5

Figure 105. Summary of Mechanistic Studies of PFOA and Hematological Effects

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

3.3.10.4 Evidence Integration

Evidence from recent human epidemiological studies does not support a relationship between PFOA exposure and hematological alterations. Many of the outcomes were not studied in more than one study. Two studies that examined 25-hydroxy vitamin D levels reported mixed non-significant effects. There is evidence of an association between increased PFOA and slightly increased WBHGB, particularly among anemic adults from a large NHANES study {Jain, 2020, 6333438}. Increases in hemoglobin and albumin may also affect pregnant women {Jiang 2014, 2850910}. However, it is unclear whether the observed changes are clinically adverse.

Similarly, the data regarding potential for the hematological system to be a target for PFOA exposure in animal models is also limited. Results were inconsistent between sexes and species and effects were generally minimal (within 10% of the control). In the three studies that reported effects on red blood cells in rats {NTP, 2019, 5400977; Loveless et al., 2008, 988599; Butenhoff et al., 2012, 2919192}, results were all within 10% of the controls except for the decrease in reticulocytes observed in male rats in NTP, 2019 {5400977}. Based on the minimal changes observed and the possible transient nature of the effect, the hematological endpoints were not considered for the derivation of PODs.

Overall, the number of human and animal studies were limited, suggesting further research is needed to draw conclusions about the hematological health effects of PFOA. 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 or sensitivity.

In the 2016 Health Assessment for PFOA {U.S. EPA, 2016, 3603279}, no epidemiological evidence on pulmonary function was available; the C8 Science Panel concluded there was no probable link between PFOA exposure and respiratory health effects (e.g., chronic obstructive pulmonary disease (COPD)) {C8 Science Panel, 2012, 1430770}.

For this updated review, six new epidemiologic studies investigated the association between PFOA and respiratory effects: five studies targeting the general population reported on several lung function outcomes, and one occupational study examined COPD {Steenland, 2015, 2851015} (Table C-20). All studies measured PFOA using biomarkers in blood. Three studies were mother-child cohort studies conducted in Europe {Agier, 2019, 5043613; Impinen, 2018, 4238440; and Manzano-Salgado, 2019, 5412076}, one was a cross-sectional case-control study

conducted in Taiwan {Qin, 2017, 3869265}; one was a cross-sectional study of adolescents and young adults residing near the WTC {Gaylord, 2019, 5080201}, and one was an occupational cohort study of workers and former workers at a chemical plant in West Virginia {Steenland, 2015, 2851015}. Five studies examined lung function measures in children and young adults, including forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1/FVC ratio, forced expiratory flow at 25-75% (FEF 25-75%), peak expiratory flow rate (PEF) measured, lung volume and resistance at oscillation frequencies of 5 Hz or 20Hz, lung function at birth and severity of obstructive airways disease.

Studies that examined respiratory illnesses or symptoms reflecting immune system responses (e.g., asthma and allergies) and respiratory tract infections (e.g., cough) are discussed in the immune system section.

3.3.11.1.2 Study Quality

Of the six studies identified since the last assessment, five were classified *medium* confidence, and the occupational cohort study (Steenland, 2015, 2851015) was classified as *low* confidence mainly because of concerns for survival bias and potential for outcome misclassification (Figure 106). The *medium* confidence 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}.

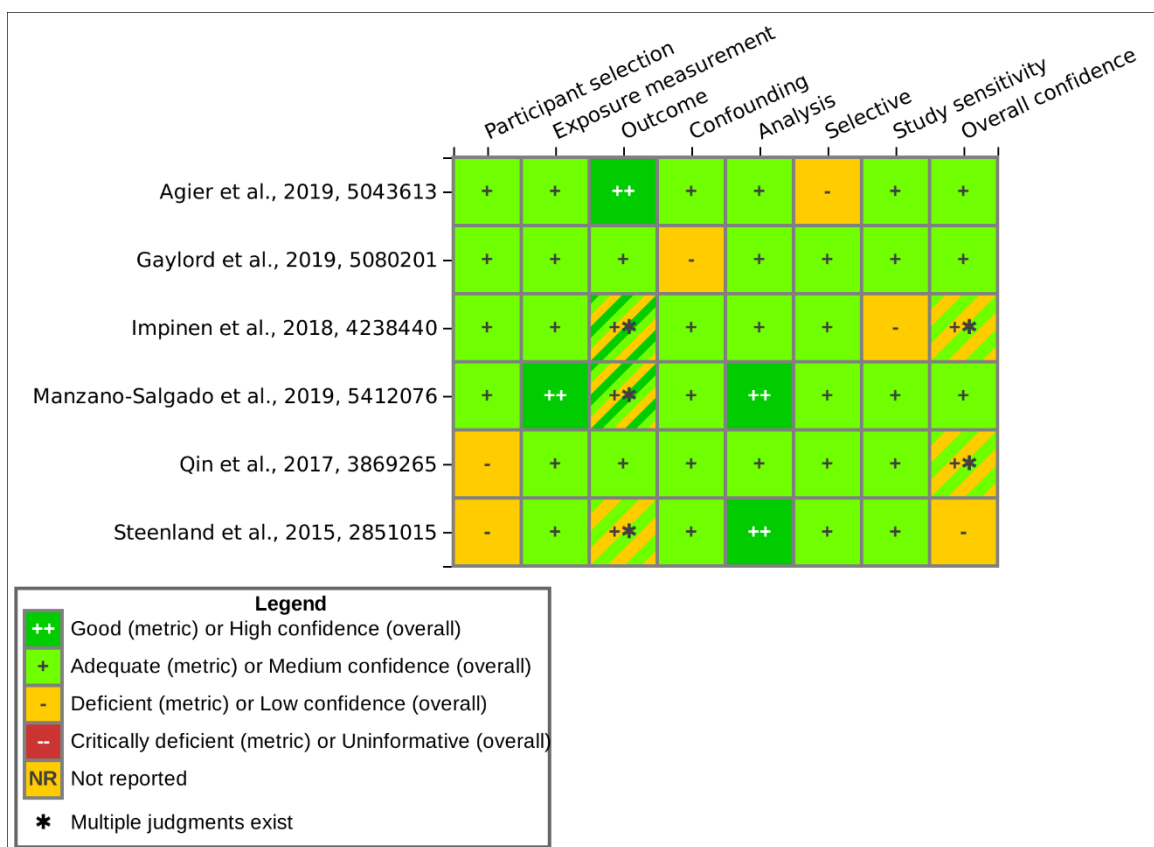


Figure 106. Summary of Study Evaluation for Epidemiology Studies of PFOA and Respiratory Effects

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

3.3.11.1.3 Findings from 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, 54120,76; 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, all reported negative associations (i.e., decrease in FEV1 with higher PFOA levels). In children ages 6–12 years, Agier (2019, 5043613) reported statistically significant associations with prenatal exposure (beta per log2 increase PFOA = -1.4 , 95% CI: $-2.7, -0.1$), but not for postnatal exposure. Qin (2017, 3869265) observed statistically significant associations for children ages 10–15 years with asthma (beta per ln increase PFOA = -0.10 , 95% CI: $-0.19, -0.02$), and in boys with asthma, but not in girls with asthma. There was also a significantly decreasing trend by quartiles of PFOA in children with asthma (p-trend = 0.002), but not observed in children without asthma. Negative non-significant associations were observed in two of the four studies {Manzano-Salgado, 2019, 5412076; Gaylord, 2019, 5080201}.

For other lung function measures examined, there was limited evidence of associations. Manzano-Salgado (2019, 5412076) reported a statistically significant association between

maternal PFOA concentrations and FVC at age 4 (beta = -0.17, 95% CI: -0.34, -0.01), but not for FVC at age 7 or for other measures of lung function, at either age 4 or age 7. Qin, 2017, 3869265 observed statistically significant associations for FEF25-75% (beta = -0.223, 95% CI: -0.4, -0.045) and a significant decreasing trend with quartiles of PFOA (p-value = 0.014) in children with asthma, but not for FVC or PEF or for any lung function measures in children without asthma. Impinen, 2018, 4238440 reported a statistically significant association between prenatal PFOA exposure and 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 PFOA = 1.43, 95% CI: 1.03, 1.98). The study also reported a non-significant increase in odds of reduced lung function at birth, as measured by tidal flow volume. Other lung function measures (i.e., FVC, FVC/FEV1, lung resistance, total lung capacity, functional residual capacity, and residual volume) in adolescents and young adults residing near the WTC were all inversely associated with PFOA exposure, but none were significant {Gaylord, 2019, 5080201}.

3.3.11.1.4 Findings from the General Adult Population

One occupational cohort study (Steenland, 2015, 2851015) assessed incidence of COPD and cumulative PFOA exposure in adult workers and former workers at a chemical plant in West Virginia. The study observed a non-significant increased risk of COPD in no-lag models, but no clear pattern of association in 10-year lag models.

3.3.11.2 Animal Evidence

There is 1 study from the most recent literature search conducted in 2020 and 1 key study from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and respiratory effects. Study quality evaluations for these 2 studies are shown in Figure 107.

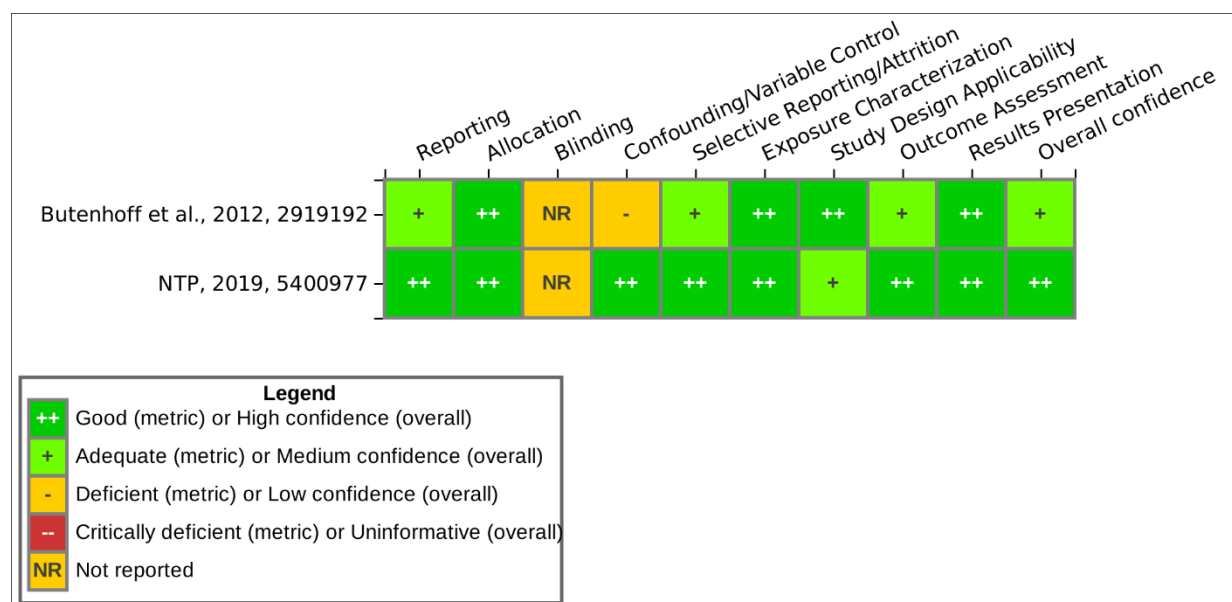


Figure 107. Summary of Study Evaluation for Toxicology Studies of PFOA and Respiratory Effects

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

There is evidence suggesting oral PFOA exposure may adversely affect the nasal, olfactory, and pulmonary systems, though the database examining respiratory toxicity is generally limited. Adverse histopathological effects in the lung and nose were observed in short-term and chronic studies in adult rats {Cui, 2009, 757868; Butenhoff, 2012, 2919192; NTP, 2019, 540097}. However, several other studies, including two chronic toxicity studies in rats and one developmental toxicity study in mice, did not report treatment-related alterations in the respiratory system of adults or neonates after treatment with PFOA {Perkins, 2004, 1291118; Yahia, 2010, 1332451; NTP, 2020, 7330145}.

In a 2-year rat feeding study, Butenhoff et al. (2012, 2919192) observed significantly increased incidences of alveolar macrophages and pulmonary hemorrhage in males in the high-dose group (300 ppm, equivalent to 14.2 mg/kg/day) (Table 11 **Error! Reference source not found.**). However, the incidences of perivascular mononuclear infiltrate and interstitial pneumonia were decreased in both exposure groups. Incidence of perivascular mononuclear infiltrate was also reduced in females, though only in the low-dose group (1.6 mg/kg/day, 4% incidence compared to 26% in controls). There was also a significant increase in the incidence of lung vascular mineralization in females, though this was again observed only in the low-dose group (44%, 76%, and 52% incidence in the 0, 1.6, and 16.1 mg/kg/day groups, respectively). Altered lung histopathology in males was considered a co-critical effect for this study in derivation of candidate RfDs for PFOA {U.S. EPA, 2016, 3603279}, though Butenhoff et al. (2012, 2919192) questioned whether these effects were directly related to PFOA treatment. Two additional chronic dietary studies in rats found no treatment-related effects on lung weight or histopathology {Perkins, 2004, 1291118; NTP, 2020, 7330145}. NTP (2020, 7330145) reported significant effects on lung weight in males and females that were considered secondary to decreased body weight and not direct toxicological effects of PFOA.

Table 11. Incidences of Non-Neoplastic Pulmonary Lesions in Male Rats as Reported by Butenhoff et al. (2012, 2919192)

Pulmonary Lesion	Dose		
	0 ppm (0 mg/kg/day)	30 ppm (1.3 mg/kg/day)	300 ppm (14.2 mg/kg/day)
Alveolar Macrophages	10/49 (20%)	16/50 (32%)	31/49 (63%)*
Hemorrhage	10/49 (20%)	14/49 (29%)	22/50 (44%)*
Vascular Mineralization	43/49 (88%)	43/49 (88%)	47/50 (94%)
Perivascular Mononuclear Infiltrate	21/49 (43%)	3/49 (6%)*	7/50 (14%)*
Interstitial Pneumonia	16/49 (33%)	5/49 (10%)*	7/50 (14%)

*Statistically significant at $p \leq 0.05$.

Cui et al. (2009, 757868) observed pulmonary congestion and focal or diffuse thickening of epithelial walls in the lungs of male rats gavaged with 5 or 20 mg/kg/day PFOA for 28 days (incidence data not provided). While NTP (2019, 5400977) did not report alterations in lung weight or histopathology after dosing for 28 days, there were several effects on the nasal cavity and olfactory system that were not suggestive of gavage-related reflux (Figure 108). Chronic active inflammation of the nasal respiratory epithelium was observed in both males and females,

though these effects did not exhibit a linear dose-response relationship. Similarly, olfactory epithelial inflammation and degeneration were observed in females. Increases in nasal and olfactory hyperplasia were thought to be a result of the observed epithelial degradation and/or inflammation {NTP, 2019, 5400977}. Interestingly, these nasal and olfactory effects were observed across multiple PFAS (PFOA, perfluorohexanoic acid (PFHxA), PFNA, PFBS, PFHxS) in toxicity studies conducted by NTP (2019, 5400977; 2019, 5400978), though not in the chronic PFOA feeding study {NTP, 2020, 7330145}. No other studies identified during this assessment reported examinations of nasal or olfactory systems in animal models.

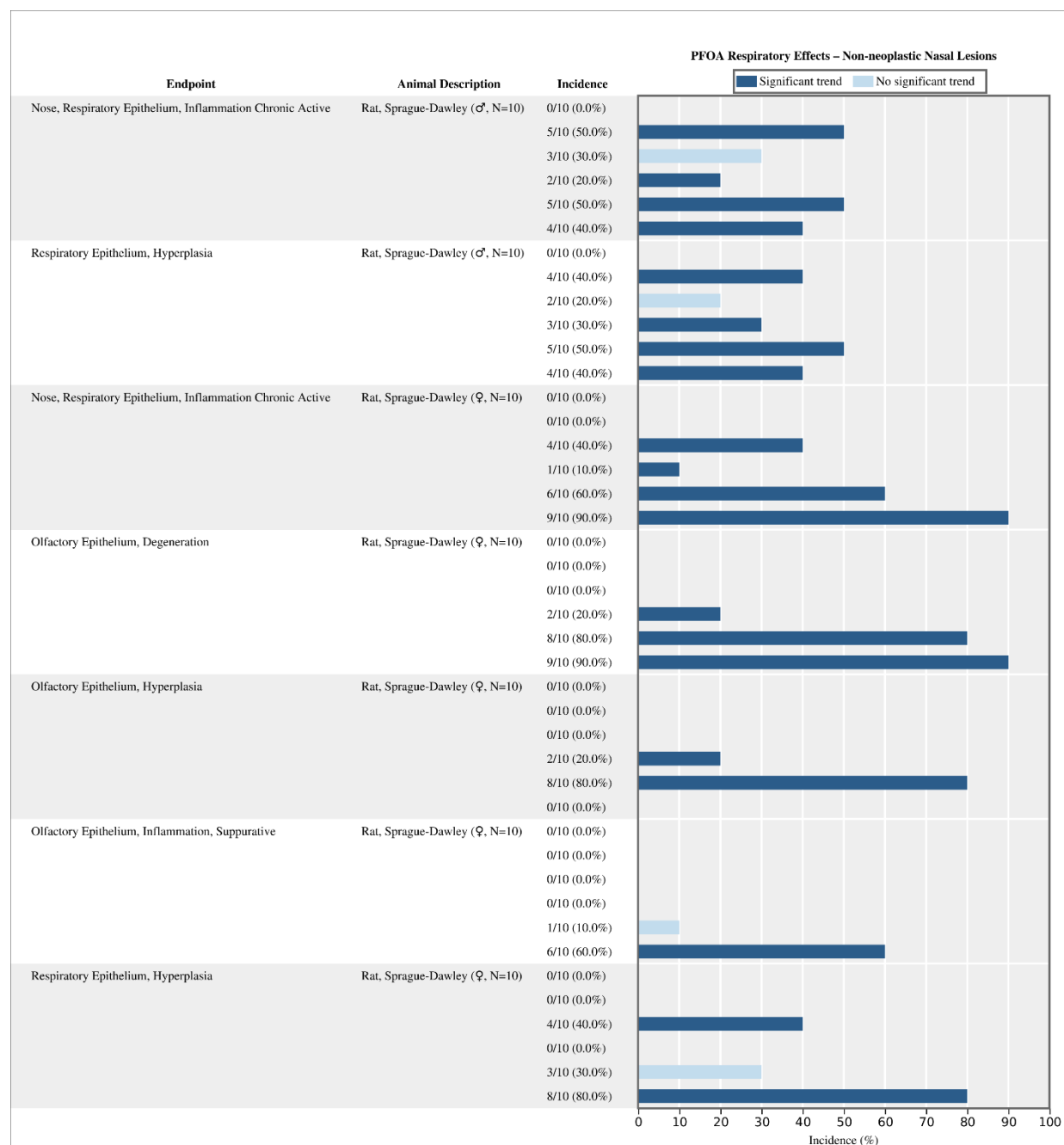


Figure 108. Incidence of Nonneoplastic Nasal Lesions in Male and Female Sprague-Dawley Rats Following 28-day Oral Exposure to PFOA, as Reported by NTP (2019, 540097)

Interactive figure and additional study details available on [HAWC](#).
Statistical significance reached at $p \leq 0.05$.

There is one available study in mice that assessed potential pulmonary effects of PFOA exposure. In this developmental toxicity study, Yahia et al. (2010, 1332451) saw no effect on the lungs of maternal or neonatal mice after up to 10 mg/kg/day PFOA treatment from GD0–18.

3.3.11.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse respiratory outcomes is discussed in Section 3.3.4 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 2 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to respiratory effects. A summary of these studies is shown in Figure 109. Additional analysis on the mechanistic actions of PFOA on respiratory health outcomes is ongoing and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	2	2
Cell Signaling Or Signal Transduction	1	1
Inflammation And Immune Response	1	1
Oxidative Stress	1	1
Grand Total	2	2

Figure 109. Summary of Mechanistic Studies of PFOA 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 epidemiological evidence to support an association between PFOA and respiratory health effects in adults or children. Although most studies observed some evidence of an association between prenatal exposure to PFOA and reduced lung function in children, these results were limited and inconsistent. While there is a suggestion of detrimental respiratory health effects particularly in children with asthma, the available epidemiological evidence does not support a probable link between PFOA exposure and respiratory health effects.

In addition, the available evidence for respiratory effects in animal models is also inconsistent. While the increases in alveolar macrophages and hemorrhaging reported by Butenhoff et al. (2012, 2919192) are suggestive of pulmonary damage, these results were not observed in two other chronic feeding studies in rats {Perkins, 2004, 1291118; NTP, 2020, 7330145}. The authors of the study also call into question whether those effects were related to PFOA treatment {Butenhoff, 2012, 2919192}. NTP (2019, 5400977) provides data suggestive of nasal toxicity due to PFOA exposure, though the positive results in males do not follow a linear dose-response and are difficult to interpret. The significant effects in females (i.e., olfactory epithelium degeneration and inflammation) occur at relatively high doses (50 mg/kg/day) compared to effects seen for other health outcomes. Therefore, it does not appear that respiratory effects are sensitive or replicable outcomes of PFOA toxicity.

Overall, evidence was inconsistent for respiratory effects in children and animal models, and no studies or endpoints were identified 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 PFOA {U.S. EPA, 2016, 3603279} did not previously evaluate musculoskeletal health outcomes in humans. The C8 Science Panel {C8 Science Panel, 2012, 1430770} concluded there is no probable link between PFOA and osteoarthritis.

For this updated review, nine studies (nine publications) examined the association between PFOA exposure and musculoskeletal health outcomes. Different study designs were used; one was a cohort study {Jeddy, 2018, 5079850}, one used cross-sectional and prospective analyses {Hu, 2019, 6315798}, and the remainder were cross-sectional. All studies measured PFOA in blood components (i.e., blood, plasma, or serum), and one study {Di Nisio, 2019, 5080655} measured PFOA in semen. Three studies {Khalil, 2016, 3229485; Lin, 2014, 5079772; Uhl, 2013, 1937226} used data from participants in 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 mineral density; bone area, thickness (e.g., endosteal and periosteal thickness), or circumference; bone mineral content (BMC); bone stiffness; ultrasound attenuation and speed of sound (indicators of bone quality); lean body mass; height; arm span; bone fracture; and plasma concentrations of β -C-telopeptides of type I collagen, a marker for bone turnover.

3.3.12.1.2 Study quality

Considerations specific to evaluating the quality of studies on musculoskeletal outcomes relate to the causal pathways for PFOA 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 PFOA 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 PFOA 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. Measuring PFOA concentrations and musculoskeletal outcomes concurrently was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOA (median half-life = 2.7 years) {Li, 2018, 4238434}, current blood concentrations are expected to correlate well with past exposures.

Based on these considerations, six studies were classified as *medium* confidence and three as *low* confidence (Figure 110).

Three cross-sectional or retrospective studies {Di Nisio, 2019, 5080655; Khalil, 2018, 4238547; Steenland, 2015, 2851015} classified as *low* confidence had deficiencies in participant selection, confounding, outcome measurement, and study sensitivity. Participant selection was considered a deficiency mainly due to underreporting about participation rates and participant characteristics relative to non-participants (e.g., those who died before the retrospective study was conducted). Other deficiencies included potential for outcome misclassification when the musculoskeletal outcome (taking medication for osteoarthritis) was not validated using medical records {Steenland, 2015, 2851015}; potential for residual confounding by SES; small sample sizes and limited ranges of participant exposure to PFOA {Di Nisio, 2019, 5080655; Khalil et al., 2018, 4238547}.

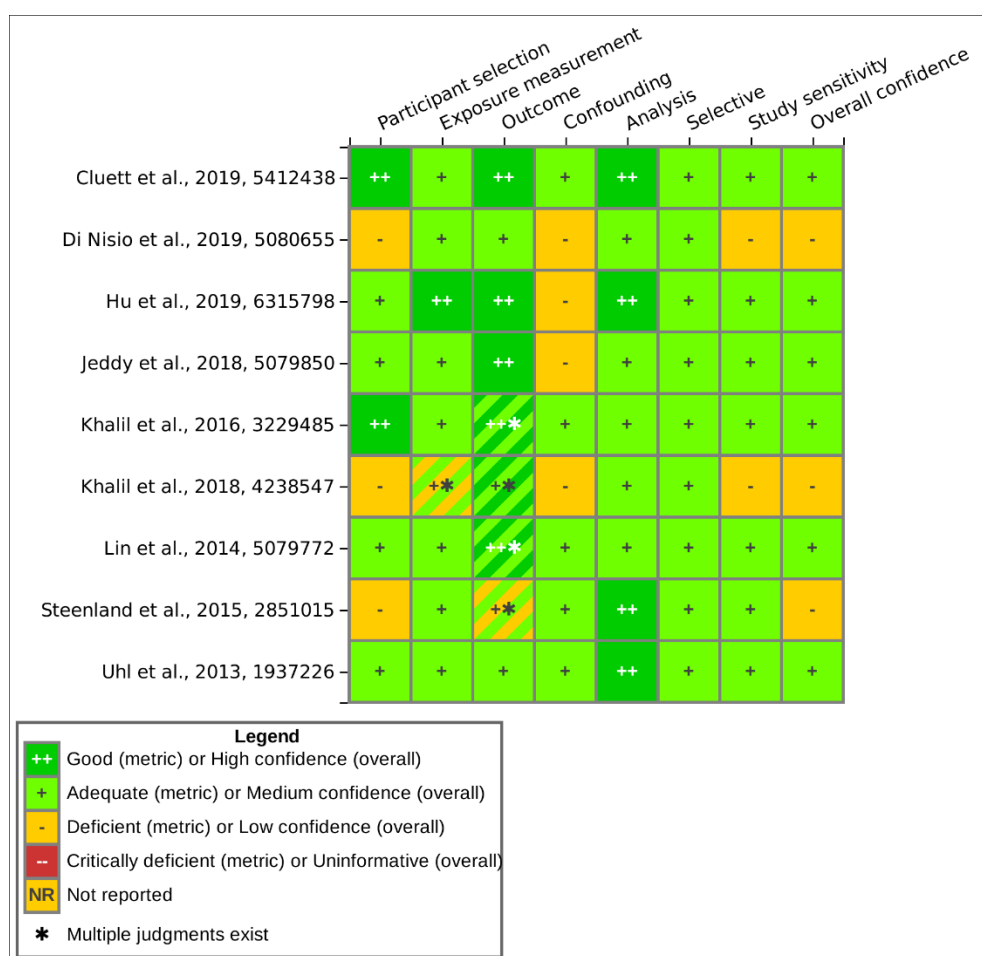


Figure 110. Summary of Study Evaluation for Epidemiology Studies of PFOA and Musculoskeletal Effects

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 (Table C-21). While the *medium* confidence studies observed few statistically significant associations between PFOA and the musculoskeletal health outcomes examined, the associations supported a harmful, rather than beneficial, direction of effect. Cluett et al. (2019, 5412438) observed a statistically significant negative association with the areal bone mineral density (aBMD) z-score (a standardized measure of bone mineral amount relative to bone area) in children aged 6–10 years, with a greater magnitude of effect for females and was not significant for males. Negative significant associations were also observed for BMC z-score. Jeddy et al. (2018, 5079850) observed a statistically significant negative association between prenatal PFOA exposure and height in 17-year-old girls. A statistically significant negative association was also observed with whole-body bone area, but this was no longer 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. Khalil, 2016, 322948 observed higher odds of osteoporosis in women aged 12–80 years from NHANES (2009–2010). Uhl et al. (2013, 1937226) observed statistically significantly increased odds of osteoarthritis in women aged 20–84 years in NHANES cycles (2003–2008). This was most apparent among younger premenopausal women aged 20–49, who may have differing susceptibility to endocrine disruption. An overlapping NHANES study {Lin, 2014, 5079772} observed no statistically significant associations with history of bone fractures in women aged 20 and older. In adults aged 30–70 years from the POUNDS LOST study, Hu et al. (2019, 6315798) observed small but statistically significant negative associations with bone mineral density (or two-year change in bone mineral density) in five of six sites examined: the spine, total hip, femoral neck, hip trochanter, 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 PFOA exposure and arm span.

3.3.12.1.5 Findings from Occupational Studies

One *low* confidence study of occupational exposure {Steenland, 2015, 2851015} reported limited, conflicting evidence related to osteoarthritis in predominantly male workers: participants with elevated PFOA exposure had lower odds of self-reported osteoarthritis after a 10-year time lag, but this finding was not supported across exposure quartiles.

3.3.12.2 Animal Evidence

There is one study from the most recent literature search conducted in 2020 that investigated the association between PFOA and musculoskeletal effects. The study quality evaluation for this study is shown in Figure 111.

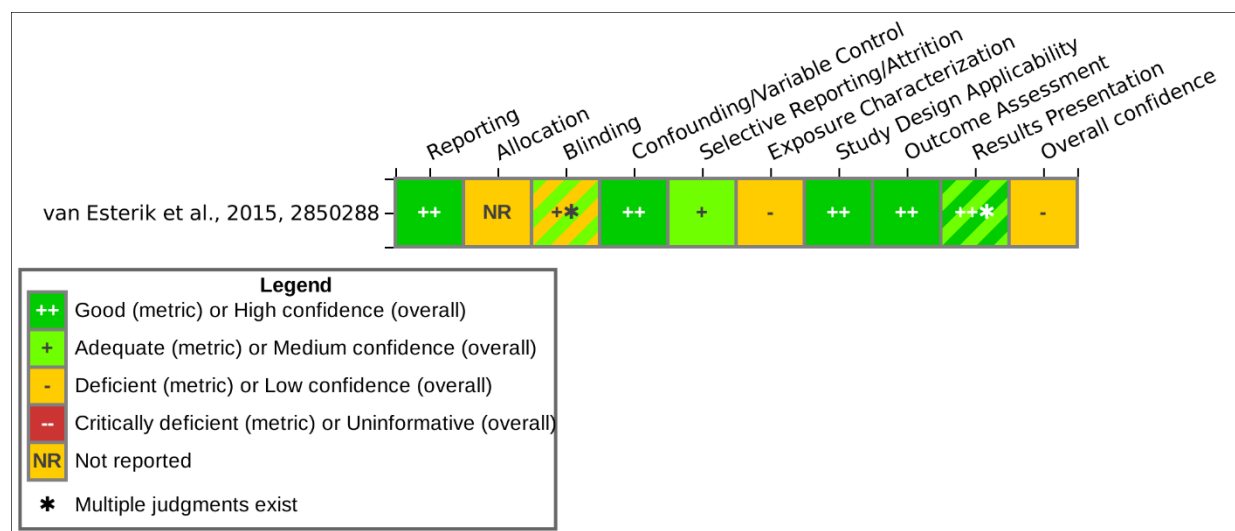


Figure 111. Summary of Study Evaluation for Toxicology Studies of PFOA and Musculoskeletal Effects

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

Limited data is available on the effect of PFOA 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 PFOA between GD1–PND0 and subsequently carried out a muscle strength test on pups. There was no effect of PFOA on muscle strength in exposed pups. Since publication of the HESD for PFOA {U.S. EPA, 2016, 3603279}, 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 PFOA exposure to adverse musculoskeletal outcomes in the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 7 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to musculoskeletal effects. A summary of these studies is shown in Figure 112.

Additional analysis on the mechanistic actions of PFOA on musculoskeletal health outcomes is ongoing and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	0	1	1
Cell Growth, Differentiation, Proliferation, Or Viability	0	6	6
Cell Signaling Or Signal Transduction	0	3	3
Extracellular Matrix Or Molecules	1	1	2
Oxidative Stress	0	2	2
Grand Total	1	7	7

Figure 112. Summary of Mechanistic Studies of PFOA and Musculoskeletal Effects

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

3.3.12.4 Evidence Integration

In summary, the present review of epidemiological data identified suggestive evidence of a harmful effect of elevated PFOA exposure on bone health, particularly measures of bone mineral density. Limited evidence supported possible negative effects of PFOA on skeletal size (height) and connective tissue disorders (osteoarthritis). No human epidemiological studies examined the relationship between PFOA and muscular disorders. No musculoskeletal health outcome epidemiology studies were previously reviewed in the 2016 HESD for PFOA {U.S. EPA, 2016, 3603279}.

Although relatively few studies have investigated musculoskeletal health outcomes related to PFOA exposure, some shared conclusions can be drawn. The observed associations in human epidemiological studies were primarily between increased PFOA exposure and decreased bone mineral density (consistently among various skeletal sites), bone mineral density relative to bone area, height in adolescence, osteoporosis, and osteoarthritis. These issues with bone density may correspond with the reports of reduced ossification and skeletal deformities in developmental animal models with gestational PFOA exposure (Section 3.3.1.2). Rarer outcomes, such as fracture, were not observed to be associated with PFOA 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.

Given the lack of available epidemiological and animal toxicity literature showing effects of PFOA on the musculoskeletal system, no studies or endpoints were identified for the derivation of PODs.

3.3.13 Gastrointestinal

3.3.13.1 Human Evidence

3.3.13.1.1 Introduction

PFOA 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 et al., 2020, 6315709}. Gastrointestinal outcomes only assessed in the context of immune system health, including ulcerative colitis and Crohn's disease, are discussed in the Immunosuppression section. However, some research suggests an overall immunosuppressive effect of PFOA 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 PFOA and gastrointestinal health outcomes {Dalsager et al. 2016, 3858505; Hammer et al., 2019, 8776815; Xu et al., 2020, 6315709; Timmermann et al., 2020, 6833710}. PFOA was measured in serum or blood, and the outcomes measured included diarrhea and vomiting, and IBD biomarkers zonulin and calprotectin. Dalsager et al. (2016, 3858505) measured PFOA in pregnant women in Denmark and collected self-reported health outcomes for their children (≤ 4 years). Hammer et al. (2019, 8776815) examined a subset of the general population in the Faroe Islands enrolled in the Children's Health and the Environment in the Faroes (CHEF) study. Xu et al. (2020, 6315709) examined child and adult residents of Ronneby, Sweden, exposed to PFAS in drinking water, as well as 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 PFOA from food packaging or preparation methods, increased PFOA absorption through the gastrointestinal tract, or reduced fecal excretion. Measuring PFOA and gastrointestinal outcomes concurrently was considered adequate in terms of exposure assessment timing. Given the long half-life of PFOA (median half-life = 2.7 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 et al. 2016, 3858505; Hammer et al., 2019, 8776815; Xu et al., 2020, 6315709} (Figure 113).

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 PFOA exposure levels. However, the concerns were considered relatively minor and likely to minimally impact interpretation of the results.

Concerns in the *low* confidence studies included potential for selection bias, including using unclear recruitment methods and, a convenience sample {Xu et al., 2020, 6315709}. Another concern was potential for outcome misclassification or underreporting due to inconsistent participation and adherence to the parent reporting mechanism {Dalsager et al. 2016, 3858505}. Another common reason for low confidence was a serious risk for residual confounding by SES (Hammer et al., 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.

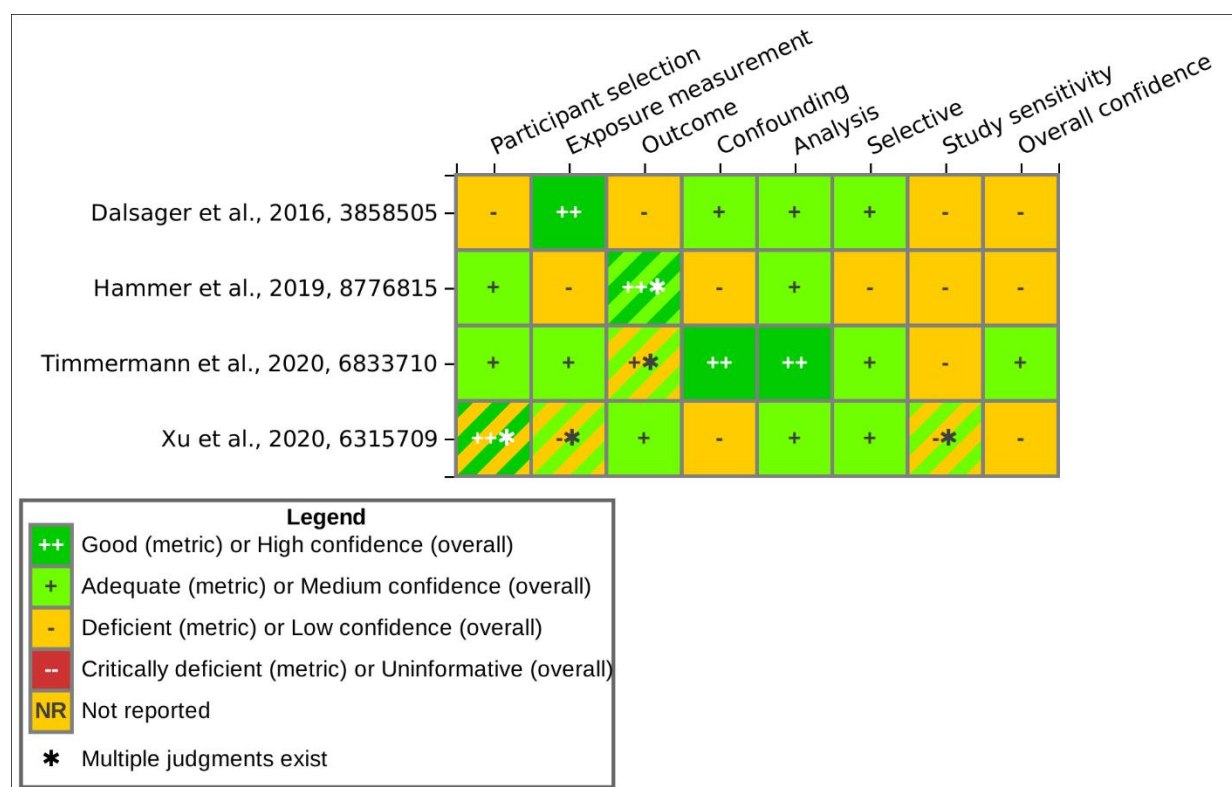


Figure 113. Summary of Study Evaluation for Epidemiology Studies of PFOA and Gastrointestinal Effects

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 PFOA.

Timmermann et al., 2020 (2020, 6833710) observed increased odds of diarrhea in very young children (up to 9 months old) in Guinea-Bissau. Dalsager et al. 2016 (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 PFOA. 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 are 2 studies from the most recent literature search conducted in 2020 and 1 key study from the 2016 PFOA HESD {EPA, 2016, 3603279} that investigated the association between PFOA and gastrointestinal effects. Study quality evaluations for these 3 studies are shown in Figure 114.

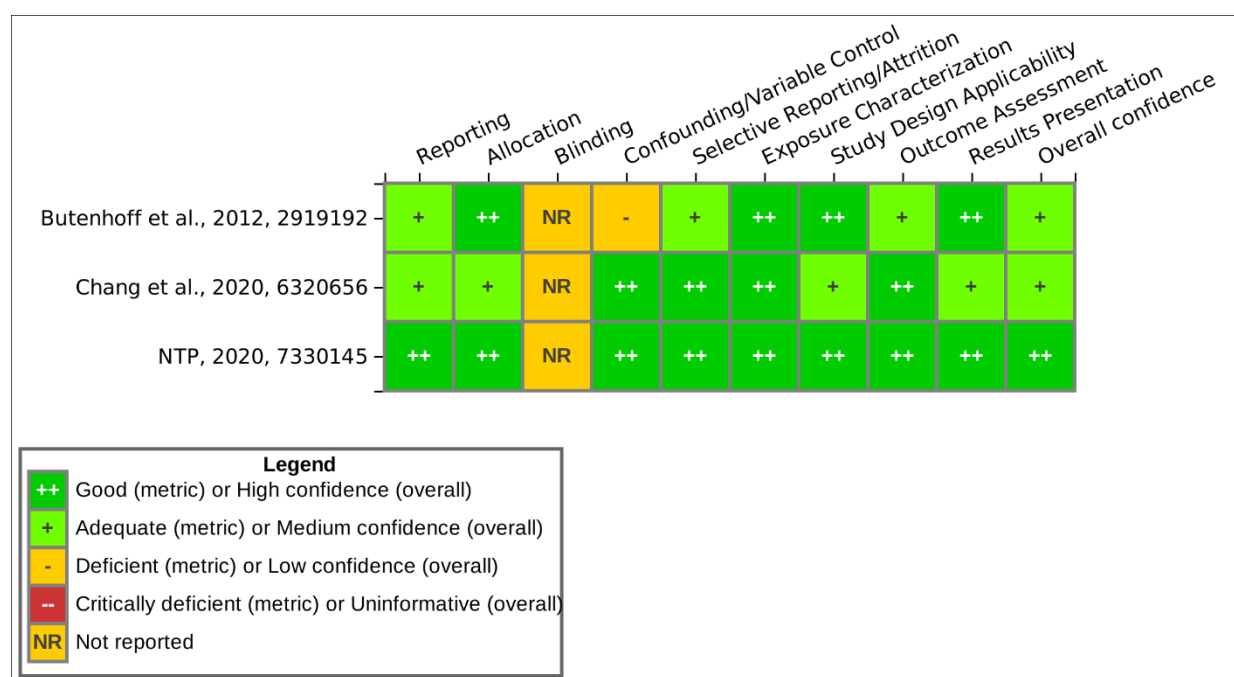


Figure 114. Summary of Study Evaluation for Toxicology Studies of PFOA and Gastrointestinal Effects

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

The only information available to assess the gastrointestinal tract is histopathological evaluations (Figure 115). In many cases, this was evaluated in the control and high-dose groups only.

Chronic studies in rats suggest that oral exposure to PFOA may increase the incidence of non-neoplastic lesions in the gastrointestinal tract {NTP, 2020, 7330145; Chang, 2020, 6320656}.

However, shorter durations may not elicit the response as noted in a study where no

histopathological findings were observed in the duodenum, jejunum, or ileum of the small intestine or the cecum, colon, or rectum of the large intestine of rats after 28 days. Likewise, no adverse effects were seen in the forestomach and glandular stomach or salivary gland {NTP, 2019, 5400977}.

NTP (2020, 7330145) used a matrix-type exposure paradigm whereby pregnant rats were administered PFOA on GD6 and exposure was continued in offspring postweaning for a total of 107 weeks. Dose groups for this report are referred to as “[perinatal exposure level (ppm)]/[postweaning exposure level (ppm)]” and ranged from 0/0–300/300 ppm in males and 0/0–300/1000 ppm in females (see further study design details in Section 3.3.1.2.1.2). At the 16-week interim evaluation, incidences of chronic active inflammation of the glandular stomach submucosa were increased in all male treated groups compared to the control; however, statistical significance was only achieved in the 0/300 ppm group. No significant differences were noted in groups with and without perinatal exposure and no effects were seen in females at interim sacrifice. At the 2-year evaluation, females of the 0/1000 and 300/1000 ppm groups exhibited increased incidences of ulcer, epithelial hyperplasia, and chronic active inflammation of the submucosa of the forestomach when compared to controls. In addition, a single case of squamous cell papilloma was noted in both exposure groups (NTP, 2020, 7330145).

In a dietary study, male and female rats fed 30 or 300 ppm PFOA for two years exhibited no stomach abnormalities during histopathological examination. In the salivary glands of male rats, significant increases in chronic sialadenitis were noted at 30 ppm (27%) and 300 ppm (30%). However, study authors reported this as being associated with antemortem viral infection. This effect was not observed in females (Butenhoff et al., 2012, 2919192).

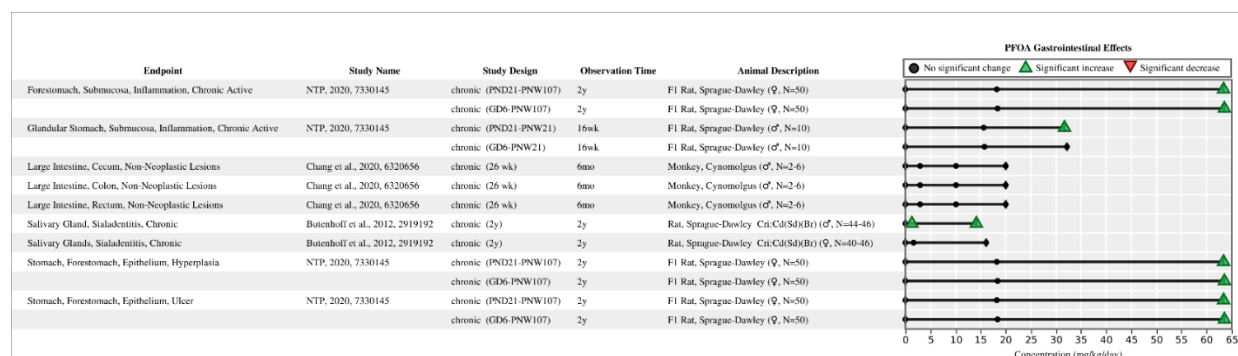


Figure 115. Gastrointestinal Effects in Rodents and Non-Human Primates Following Exposure to PFOA (logarithmic scale)

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

GD = gestation day; F1 = first generation; PND = postnatal day; PNW = postnatal week; mo = month; wk = week; y = year.

Archived colon tissues from the previously mentioned two-year dietary study in rats conducted by Butenhoff et al. (2012, 2919192) were subjected to pathology review by Chang et al. (2020, 6320656). Minimal neutrophilic infiltration was observed in 8/39 males and 4/34 females treated with PFOS compared to 0/36 and 2/33 male and female control animals, respectively Mild subacute inflammation was noted in 1/39 treated male rats with no incidences occurring in treated females or control animals. These incidences were not significant when compared to controls. In addition, signs of overt inflammation, including infiltration of inflammatory

leukocytes and tissue destruction and/or reaction were not observed. Therefore, these incidences were considered part of the normal mucosal immune system. Minimal to mild nematodiasis was observed in 6/50 male controls, 2/50 female controls, and 1/50 treated females. Study authors stated that it unknown whether PFOA contributed to the presence of the parasite in the treated group and noted that at the time of the original study, use of parasite-free animals was not common practice (Chang et al., 2020, 6320656).

In the same study, Chang et al. (2020, 6320656) examined archived cecum, colon, and rectum tissues of male cynomolgus monkeys administered gelatin capsules containing 0 (n = 6), 3 (n = 4), 10 (n = 6), or 30/20 (n = 2) mg/kg/day of PFOA for six months. Animals in the highest dose group received 30 mg/kg/day for the first 12 days; however, due to systemic toxicity, treatment halted and was resumed on day 22 at the reduced dose of 20 mg/kg/day. Isolated incidences of mild, brown pigment were noted in the cecum and colon and minimal eosinophil infiltrate was noted in the colon. These findings were not statistically significant and were considered to be normal background histomorphology. Isolated incidences of granulomatous lesions consistent with *Oesophagostomum* spp. were observed but were considered common in the intestinal tract of non-human primates at the time the study was conducted {Chang, 2020, 6320656}.

NTP conducted a 28-day study in which 10 or 100 mg/kg/day of PFOA were orally administered to male or female rats, respectively. No histopathological findings were noted in the duodenum, jejunum, or ileum of the small intestine or the cecum, colon, or rectum of the large intestine. Likewise, no adverse effects were seen in the forestomach and glandular stomach or salivary gland (NTP, 2019, 5400977).

3.3.13.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse gastrointestinal outcomes in the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 4 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to gastrointestinal effects. A summary of these studies is shown in Figure 116. Additional analysis on the mechanistic actions of PFOA on gastrointestinal health outcomes is ongoing and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	1	0	1	2
Cell Signaling Or Signal Transduction	1	0	0	1
Inflammation And Immune Response	0	0	1	1
Other	0	1	1	2
Grand Total	1	1	2	4

Figure 116. Summary of Mechanistic Studies of PFOA and Gastrointestinal Effects

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

3.3.13.4 Evidence Integration

In the 2016 HESD for PFOA, gastrointestinal outcomes from human epidemiological studies were only assessed in the context of immune system health, with limited evidence of associations with gastroenteritis. Overall, the available epidemiological evidence in this updated review does not support an inverse association between PFOA 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 PFOA on general IBD, vomiting, or diarrhea.

Evidence from animal models is also limited. The only significant non-neoplastic lesions observed were noted in the stomachs of male rats treated at 0/300 ppm and female rats treated at high doses (0/1000 ppm and 300/1000 ppm) in a 2-year feeding study {NTP, 2020, 7330145}. Additionally, lack of significant effects in rat colon and cynomolgus monkey cecum, colon, and rectum indicated no signs of ulcerative colitis {Chang, 2020, 6320656}.

Due to the limited evidence available from both epidemiological and animal toxicity studies, and because the incidence of adverse effects in animal studies were generally only evaluated and noted in the control and high dose groups, no studies or endpoints were considered for the derivation of PODs.

3.3.14 Dental

3.3.14.1 Human Evidence

3.3.14.1.1 Introduction

PFOA 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 PFOA 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 PFOA exposure and dental caries in children and adolescents {Puttige Ramesh, 2019, 5080517; Wiener, 2019, 5386081}. Dental caries 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. {Puttige Ramesh, 2019, 5080517} assessed data from 2,869 12–19-year-old adolescents included in the 1999–2012 NHANES and Wiener and Waters (2019, 5386081) examined data from 639 children ages 3–11 years in the 2013–2014 NHANES cycle. Therefore, no participant overlap is expected between these studies. Exposure to PFOA 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 PFOA and dental outcomes concurrently was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOA (median half-life = 2.7 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 117), 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.

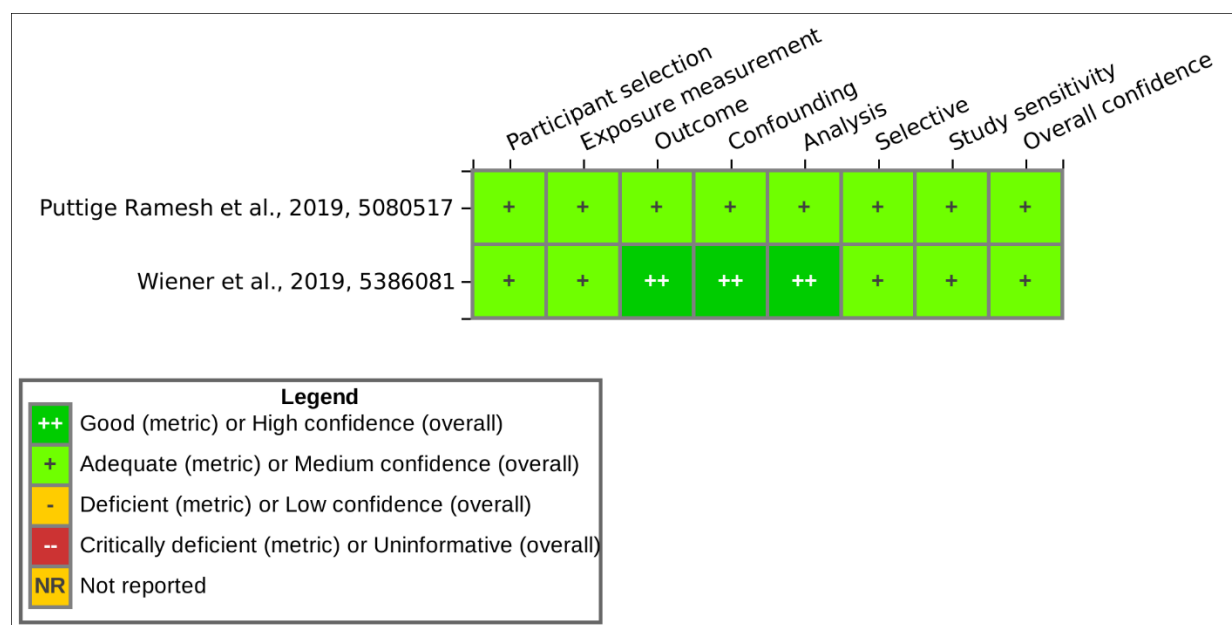


Figure 117. Summary of Study Evaluation for Epidemiology Studies of PFOA and Dental Effects

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. Both studies observed non-significantly increased odds of dental caries with increased PFOA exposure children and adolescents {Puttige Ramesh, 2019, 5080517; Wiener, 2019, 5386081}. Puttige Ramesh et al. (2019, 5080517) also observed increased odds of dental caries in the third highest quartile of exposures, but decreased odds in the second and highest quartiles compared to the lowest. 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). Wiener and Waters (2019, 5386081) adjusted the analysis 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. 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 PFOA exposure on dental outcomes in animals.

3.3.14.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse dental outcomes in the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are no studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to dental effects.

3.3.14.4 Evidence Integration

Overall, the available evidence in this review does not support an inverse association between PFOA and dental outcomes. Dental health outcomes were not previously reviewed in the 2016 HESD for PFOA {U.S. EPA, 2016, 3603279}. The present epidemiological review was limited by the availability of only two studies in humans. Only one outcome was examined (prevalence of dental caries), and both studies observed non-significantly increased odds of dental caries {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 that examine dental effects due to PFOA exposure.

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 PFOA and ocular effects {Zeeshan, 2020, 6315698}.

This cross-sectional study was conducted in Shenyang, China 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 PFOA serum concentrations among the 1202 study participants were 6.06 ng/ml (Q1 = 3.97 ng/ml, Q3 = 9.12 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, 2020, 6315698} was classified as *medium* confidence (Figure 118). 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.

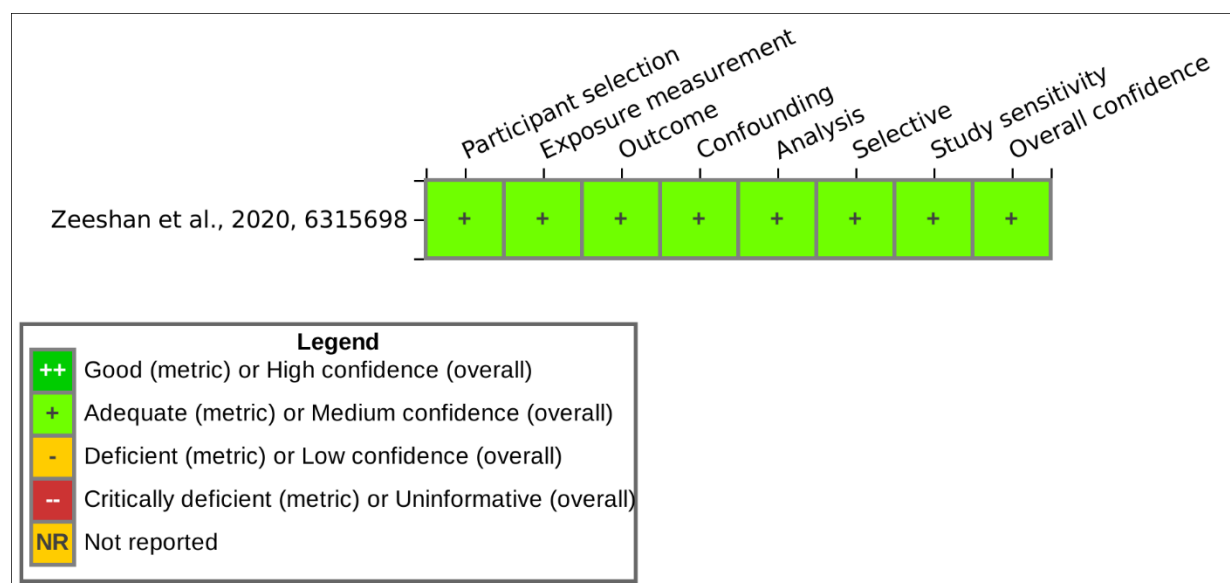


Figure 118. Summary of Study Evaluation for Epidemiology Studies of PFOA and Ocular Effects

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

3.3.15.1.3 Findings

{Zeeshan, 2020, 6315698} examined the effects of exposure to PFOA in adults aged 22–96 years, who had lived for at least 5 years in in Shenyang, China (Table C-24). Outcome examined included 1 ocular conditions, including VI, vitreous disorder, synechia, macular disorder, corneal pannus, and ACD, and combined eye disease (aggregating all 9 ocular conditions examined). A positive statistically significant association between VI and total serum PFOA was observed (OR: 1.80; 95% CI: 1.37, 2.37). When stratified by age, the association between combined eye disease and total serum PFOA was statistically significant for participants aged ≤65 years (OR:

1.25; 95% CI: 1.01, 1.56) but not for the older participants (OR: 1.19; 95% CI: 0.71, 1.98). No other associations were observed.

3.3.15.2 Animal Evidence

There are 2 studies from the most recent literature search conducted in 2020 that investigated the association between PFOA and ocular effects. Study quality evaluations for these 2 studies are shown in Figure 119.

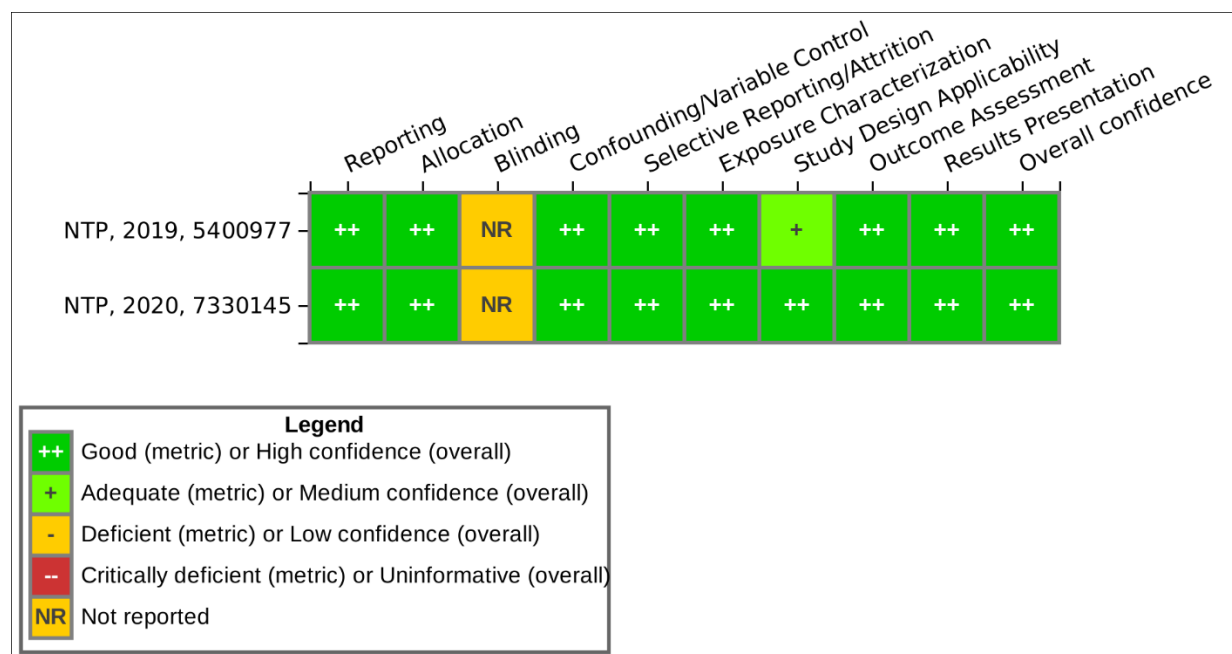


Figure 119. Summary of Study Evaluation for Toxicology Studies of PFOA and Ocular Effects

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

Eye irritation studies in rabbits suggest that PFOA acts as an ocular irritant {Gabriel, 1976, 4442370}; however, no adverse lesions were noted in eye tissues during histopathological examination in repeated-dose oral toxicity studies in rats. In a 28-day oral toxicity study where only control and high-dose groups were evaluated, no histopathological findings were noted in eyes of male rats treated with 10 mg/kg/day or female rats treated with 100 mg/kg/day {NTP, 2019, 5400977}. In a chronic exposure study, male and female Sprague-Dawley rats were fed diets containing PFOA for approximately two years (see further study design details in Section 3.3.1.2.1.2). Observation of gross abnormalities and histopathological examination of eye tissues were conducted in pups at 16 weeks and 2 years with no treatment related abnormalities noted {NTP, 2020, 7330145}.

3.3.15.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse ocular outcomes in the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are no studies from the most recent

literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to ocular effects.

3.3.15.4 Evidence Integration

In the 2016 Health Assessment for PFOA, no epidemiological evidence of an association between PFOA exposure and ocular health effects was examined. In this updated review, based on one study, there is suggestive evidence of an association between PFOA and VI and combined eye disease. However, 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 PFOA. Longitudinal studies are needed to ascertain causality between exposure to PFOA and ocular conditions. In addition, in the two available studies in animal models that assess ocular toxicity, there were no observed ocular effects with short-term or chronic PFOA exposure in male or female rats. Due to the limited evidence available, no studies or endpoints were identified 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 PFOA exposure. In the Puberty Cohort, a large sub-cohort of the DNBC in Denmark, Ernst et al. (2019, 5080529) examined the association between prenatal PFOA exposure and pubertal development in. 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. (2019, 5080529) was considered a *medium confidence* study (Figure 120), 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.

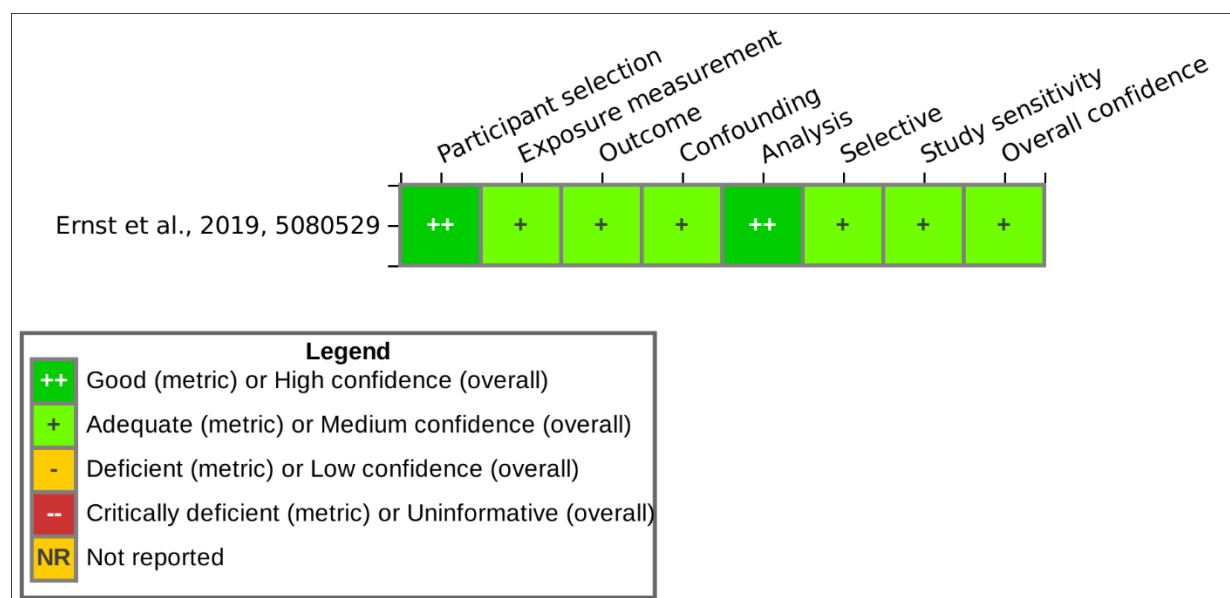


Figure 120. Summary of Study Evaluation for Epidemiology Studies of PFOA and Dermal Effects

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 associations between prenatal PFOA exposure and age at the occurrence of acne. Significant negative associations were observed for girls per doubling of PFOA (β : -5.16 ; 95% CI: $-8.50, -1.82$), and in the highest tertile of PFOA exposure compared to the lowest (β : -6.09 ; 95% CI: $-12.10, -1.70$) {Ernst, 2019, 5080529}. Associations in boys were negative and non-significant.

3.3.16.2 Animal Evidence

There are 2 studies from the most recent literature search conducted in 2020 that investigated the association between PFOA and dermal effects. Study quality evaluations for these 2 studies are shown in Figure 121.

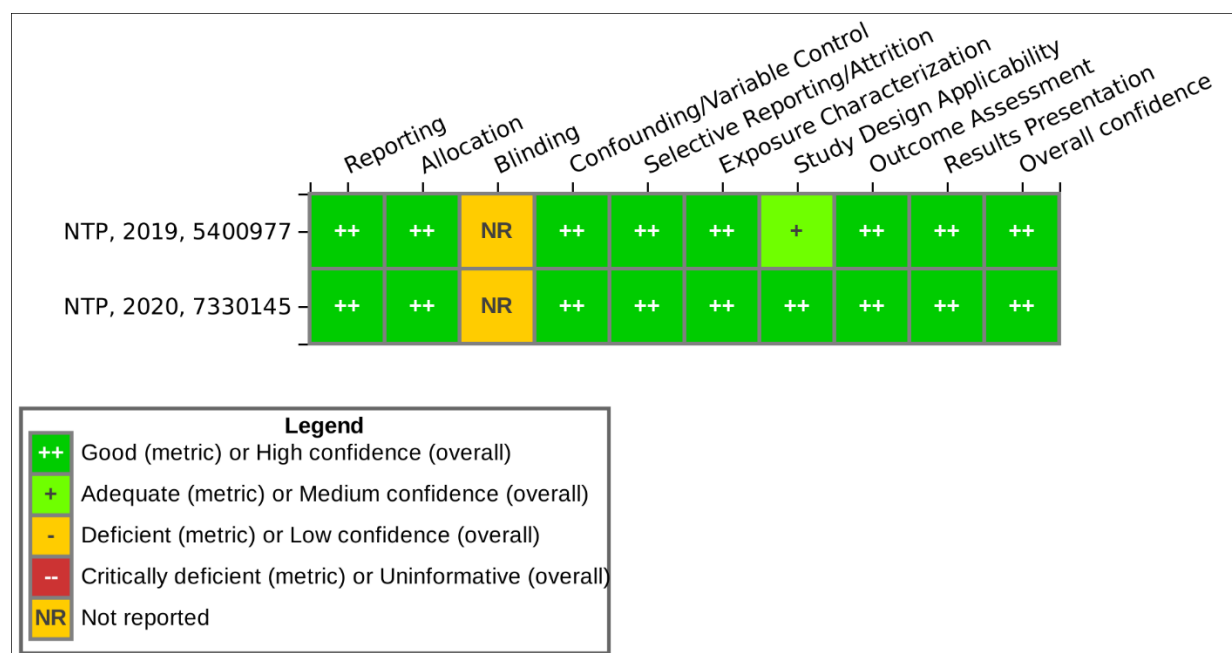


Figure 121. Summary of Study Evaluation for Toxicology Studies of PFOA and Dermal Effects

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

There is no evidence in the literature that oral PFOA exposure results in dermal toxicity in animal models. An NTP (2019, 5400977) study explored histopathology of the skin following 28 days of oral gavage of up to 10 mg/kg/day PFOA in male and up to 100 mg/kg/day PFOA in female Sprague Dawley rats. They observed no lesions of dermal tissue. Similarly, in a subsequent report, NTP (2020, 7330145) reported no lesions in dermal tissue in male or female Sprague Dawley rats that received PFOA via feed for 2 years (see study design details in Section 3.3.1.2.1.2).

3.3.16.3 Mechanistic Evidence

There was no mechanistic evidence linking PFOA exposure to adverse dermal outcomes in the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 2 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to dermal effects. A summary of these studies is shown in Figure 122. Additional analysis on the mechanistic actions of PFOA on dermal health outcomes is ongoing and is expected to be completed after the EPA SAB review.

Mechanistic Pathway	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	2	2
Extracellular Matrix Or Molecules	1	1
Inflammation And Immune Response	1	1
Oxidative Stress	2	2
Grand Total	2	2

Figure 122. Summary of Mechanistic Studies of PFOA and Dermal Effects

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

3.3.16.4 Evidence Integration

The 2016 HESD did not report on the association between oral PFOA exposure and dermal effects. In this updated review, one human epidemiological study and two animal toxicity studies examined the association between PFOA exposure and dermal endpoints. In the single epidemiological study examining prenatal PFOA exposure and dermal effects during puberty, Ernst (2019, 5080529) observed negative effects on age at the occurrence of acne, which were significant only in girls, suggesting earlier occurrences of acne with increasing PFOA exposure. In the two available studies in animal models that assess dermal toxicity, there were no observed dermal effects with short-term or chronic oral PFOA exposure in male or female rats.

Conclusions regarding PFOA exposure and resulting dermal effects are limited by the lack of studies examining the association. The evidence from a large Danish prospective cohort study supports a negative effect of PFOA exposure on age at occurrence of acne among girls. Dermal effects beyond acne are not currently represented in the epidemiological literature. There are also no reported biological consequences of oral PFOA exposure on dermal tissue in animal models. Further investigation is needed to fully characterize the relationship between PFOA and the range of dermal effects.

The available data do not support the skin as a target of oral PFOA toxicity no studies or endpoints were identified for the derivation of PODs.

3.3.17 Cancer

3.3.17.1 Human Evidence

3.3.17.1.1 Introduction

The 2016 HESD for PFOA {U.S. EPA, 2016, 3603279} concluded there was suggestive evidence of carcinogenic effects of PFOA for kidney and testicular cancer, based on two C8 Health Project studies and 2 occupational cohorts. Specifically, two studies involving participants in the C8 Health Project showed a positive association between PFOA levels (mean at enrollment 24 ng/mL) and kidney and testicular cancers {Barry, 2013, 2850946; Vieira, 2013, 2919154}. There is some overlap in the cases included in these studies. Similarly, as part of the C8 Health Project, the C8 Science Panel {C8 Science Panel, 2012, 1430770} concluded that a probable link existed

between PFOA exposure and testicular and kidney cancer. Two occupational cohorts in Minnesota and West Virginia (most recently updated prior to the 2016 draft, respectively, in Raleigh et al. (2014, 2850270) and Steenland and Woskie (2012, 2919168)) also examined cancer mortality. Raleigh et al. (2014, 2850270) reported no evidence of elevated risk for kidney cancer. In the West Virginia occupational cohort, Steenland and Woskie (2012, 2919168) observed significant elevated risk of kidney cancer in the highest exposure quartile (>2,384 ppm-years). However, each of these studies is limited by a small number of observed cases (six kidney cancer deaths, 16 incident kidney cancer cases, and five incidence testicular cancer cases in Raleigh et al. (2014, 2850270); 12 kidney cancer deaths and one testicular cancer death in Steenland and Woskie (2012, 2919168)). None of the general population studies reviewed for the 2016 Health Advisory examined kidney or testicular cancer, and no associations were found in the general population between mean serum PFOA levels up to 86.6 ng/mL and colorectal, breast, prostate, bladder, and liver cancer {Bonefeld-Jørgensen, 2014, 2851186; Eriksen, 2009, 2919344; Hardell, 2014, 2968084; Innes, 2014, 2850898}.

For this updated review, there are 13 studies (13 publications) identified since the 2016 document that investigated the association between PFOA and cancer (Table C-26). Two of the publications {Girardi, 2019, 6315730; Steenland, 2015, 2851015} were occupational studies and the remainder were conducted on the general population, with one in a high-exposure community (i.e., C8 population). Different study designs were also used including 3 cohort studies {Fry, 2017, 4181820; Girardi, 2019, 6315730; Steenland, 2015, 2851015;}, 3 case-control studies {Wielsoe, 2017, 38588479; Tsai, 2020, 6833693; Lin, 2020, 6835434}, 5 nested case-control studies {Mancini, 2019, 5381529; Ghisari, 2017, 3860243; Shearer, 2021, 7161466; Hurley, 2018, 5080646; Cohn, 2020, 5412451}, 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 {Wielsøe, 2017, 3858479}, Italy {Girardi, 2019, 6315730}, Taiwan {Tsai, 2020, 6833693}, and the United States {Fry, 2017, 4181820; Christensen, 2016, 3858533; Ducatman, 2015, 3859843; Steenland, 2015, 2851015; Shearer, 2021, 7161466; Hurley, 2018, 5080646; Cohn, 2020, 5412451}. All the studies measured PFOA in study subjects' blood components (i.e., serum or plasma) with one study measuring the levels in the maternal serum {Cohn, 2020, 5412451}. Cancers evaluated included bladder {Steenland, 2015, 2851015}, breast {Cohn, 2020, 5412451; Ghisari, 2017, 3860243; Hurley, 2018, 5080646; Mancini, 2019, 5381529; Tsai, 2020, 6833693; Wielsøe, 2017, 3858479}, colorectal {Steenland, 2015, 2851015}, germ cell tumors {Lin, 2020, 6835434}, liver {Girardi, 2019, 6315730}, lung {Girardi, 2019, 6315730}, lymphatic or hematopoietic tissue {Girardi, 2019, 6315730}, melanoma {Steenland, 2015, 2851015}, prostate {Steenland, 2015, 2851015; Ducatman, 2015, 3859843}, kidney {Shearer, 2021, 7161466}, and any cancer {Christensen, 2016, 3858533; Fry, 2017, 4181820; Girardi, 2019, 6315730}.

3.3.17.1.2 Study Quality

Of the 13 studies identified since the 2016 assessment (Figure 123), eight were considered *medium* confidence and five were *low* confidence {Christensen, 2016, 3858533; Girardi, 2019, 6315730; Lin, 2020, 6835434; Steenland, 2015, 2851015; Tsai, 2020, 6833693}. The main concerns with the *low* confidence studies were the possibility of outcome misclassification, confounding, or participation selection methods. Residual confounding was a concern mainly due to lack of appropriately addressing SES, which can be associated with both exposure and

cancer diagnosis. The two *low* confidence occupational studies {Girardi, 2019, 6315730; Steenland, 2015, 2851015} had several potential sources of bias including selection bias concern, outcome measurement limitations which may lead to survival bias, and poor/insufficient study sensitivity due to a low number of deaths. Girardi, 2019, 6315730 had the potential for residual confounding because of use of standardized mortality ratios (SMRs), which only account for gender, age, and calendar year. Confounders specific for cancer outcomes, besides age and gender, including factors such as smoking or socioeconomic factors were not addressed in the study and behavioral risk factors could have differed by outcome. Although PFOA 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 sizes.

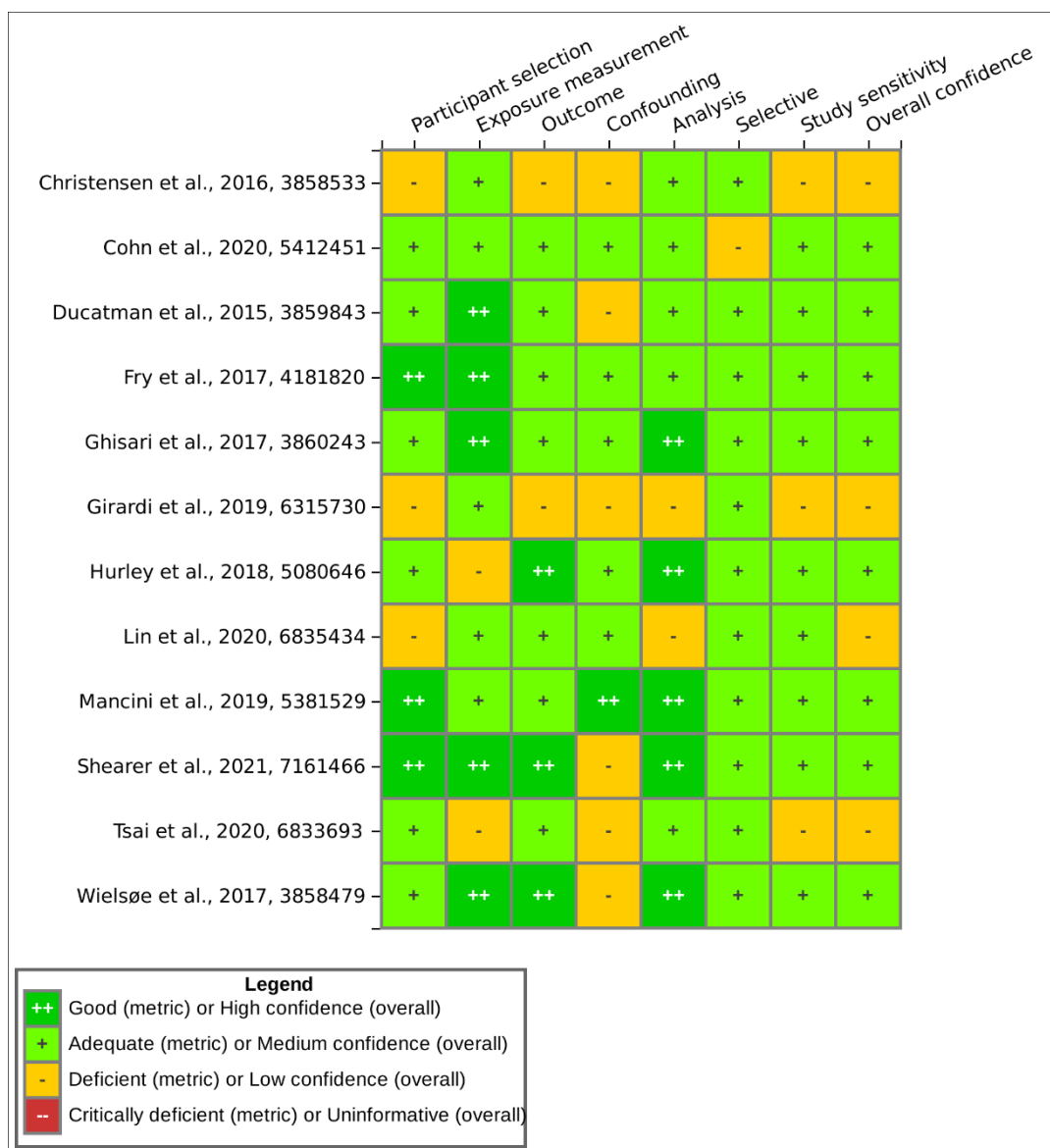


Figure 123. Summary of Study Evaluation for Epidemiology Studies of PFOA and Cancer Effects

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 PFOA 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 increase in cancer risk when evaluated on a per ng/mL increase in blood PFOA.

3.3.17.1.4 Findings from the General Adult Population

PFOA was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma (RCC)) {Shearer, 2021, 7161466}. This large *medium* confidence case-control study nested within the NCI's Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO) reported a statistically significant increase in risk of kidney cancer in highest exposure quartile and per doubling of PFOA concentration. Even after adjusting for other PFAS the association remained significant in analyses on a per doubling increase in PFOA. The increase in the highest exposure quartile remained and the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS), but it was no longer statistically significant.

Six of the general population studies published since the 2016 assessment examined breast cancer {Cohn, 2020, 5412451; Ghisari, 2017, 3860243; Hurley, 2018, 5080646; Mancini, 2019, 5381529; Tsai, 2020, 6833693; Wielsøe, 2017, 3858479} with mixed results. All studies were case-control studies (with some nested case-controls). Five studies were of *medium* confidence. One study was considered *low* confidence {Tsai, 2020, 6833693} because of concerns on temporality of exposure measurements and breast cancer development, lack of confirmation of control status via examination or medical records, and potential for residual confounding due to SES. Two nested case-control studies did not report an association between breast cancer and PFOA concentrations measured in maternal serum throughout pregnancy and 1-3 days after delivery {Cohn, 2020, 5412451; 75th percentile PFOA 0.6 ng/mL} or in in serum after case diagnosis and breast cancer {Hurley, 2018, 5080646; max concentration of 39.1 ng/mL}. Both studies were conducted in California and most breast cancer cases were obtained from the cancer registry. Two case-control studies from different areas using histologically hospital confirmed breast cancer cases observed increased risk of breast cancer {Tsai, 2020, 6833693; Wielsøe, 2017, 3858479}. Both studies observed significantly higher serum PFOA concentrations in breast cancer cases compared to controls, but nonsignificant when age adjusted {Wielsøe, 2017, 3858479}. Wielsøe, 2017, 3858479 (conducted in Greenland) observed a statistically significant increase in adjusted risk with increasing PFOA, either per ng/mL increase (OR=1.26; 95% CI: 1.01-1.58, p-value=0.039) or in the highest tertile compared to the lowest (OR=2.64; 95% CI: 1.17-5.97, p-value=0.019). Tsai, 2020, 6833693 (conducted in Taiwan) observed a statistically significant increase in risk of breast cancer only in the ≤50 year-old women (OR=1.14; 95% CI: 0.66, 1.96). Two nested case-control studies found associations between PFOA and breast cancer, but only in specific genotype or estrogen receptive groups of participants {Ghisari, 2017, 3860243; Mancini, 2019, 5381529}. Ghisari, 2017, 3860243 reported an increased risk for breast cancer identified from the cancer registry with increasing PFOA concentrations only in subjects 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 as pathologically confirmed from medical records after self-reported cancer diagnosis) varied by type of cancer with a statistically significant increase in estrogen receptor negative (ER-) and progesterone receptor negative (PR-) breast cancers in the second quartile only; increases observed in the other quartiles were not statistically significant nor was the trend found to be statistically significant. The sample size was small with 26 participants having ER- breast cancers and 57 having PR- breast cancers and the number per quartile was not provided. Tsai, 2020, 6833693 also reported an increase in risk in ER+ participants aged 50 years or younger and a decrease in risk for ER- breast cancers in participants aged 50 years or younger, but neither achieved statistical significance.

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., 20–49 years old) or older (i.e., 50–69 years old) men and concurrent mean serum PFOA concentration up to 46 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 cancers (i.e., incidence versus mortality) and obtained the information using different methods. Cancer mortality based on Public-use Linked Mortality Files was not associated with PFOA exposure in a *medium* confidence study on subjects over 60 years of age from NHANES 2003–2006 {Fry, 2017, 4181820; median PFOA concentration 23.7 ng/g lipid}. PFOA was associated with an increase in self-reported cancer incidence in a *low* confidence study on male anglers over 50 years {Christensen, 2016, 3858533}. Christensen (2016, 3858533) was considered *low* confidence due to the potential of self-selection because subjects were recruited from flyers and other methods and filled out an online survey including self-reported outcomes.

3.3.17.1.5 Findings from Occupational Studies

Two *low* confidence occupational studies examined cancer incidence {Steenland, 2015, 2851015} and mortality {Girardi, 2019, 6315730}. Issues of population selection, outcome measurement and low number of deaths reducing the sensitivity were noted. In a retrospective occupational cohort study based on the same DuPont cohort from West Virginia reported in the 2016 assessment {Steenland, 2012, 2919168}, Steenland, (2015, 2851015) observed no significant associations with incidence of cancers of the bladder, colorectal, prostate, and melanoma when compared to the general population (median serum levels in workers was 113 ng/mL in 2005 compared to 4 ng/mL in the general population). There was a modest evidence of positive non-significant trend for prostate cancer (across quartiles) and a statistically significant negative exposure-response trend for bladder cancers (p-value = 0.04).

Girardi, 2019, 6315730 conducted a retrospective cohort study at a factory in Italy where PFOA was produced from 1968–2014 and observed a statistically significant increases in liver cancer mortality, malignant neoplasms of the lymphatic and hematopoietic tissue, and in all malignant neoplasms with cumulative serum PFOA exposure of >16,956 ng/mL-years. There was no association observed with lung cancer in this occupational cohort. Mortality from cancers in this cohort was low and supplemental data provided mortality for other cancers including kidney, but no risk estimates were calculated.

3.3.17.2 Animal Evidence

There is 1 study from the most recent literature search conducted in 2020 and 1 key study from the 2016 PFOA HESD {U.S. EPA, 2016, 3603279} that investigated the association between PFOA and cancer effects. Study quality evaluations for these 2 studies are shown in Figure 124.

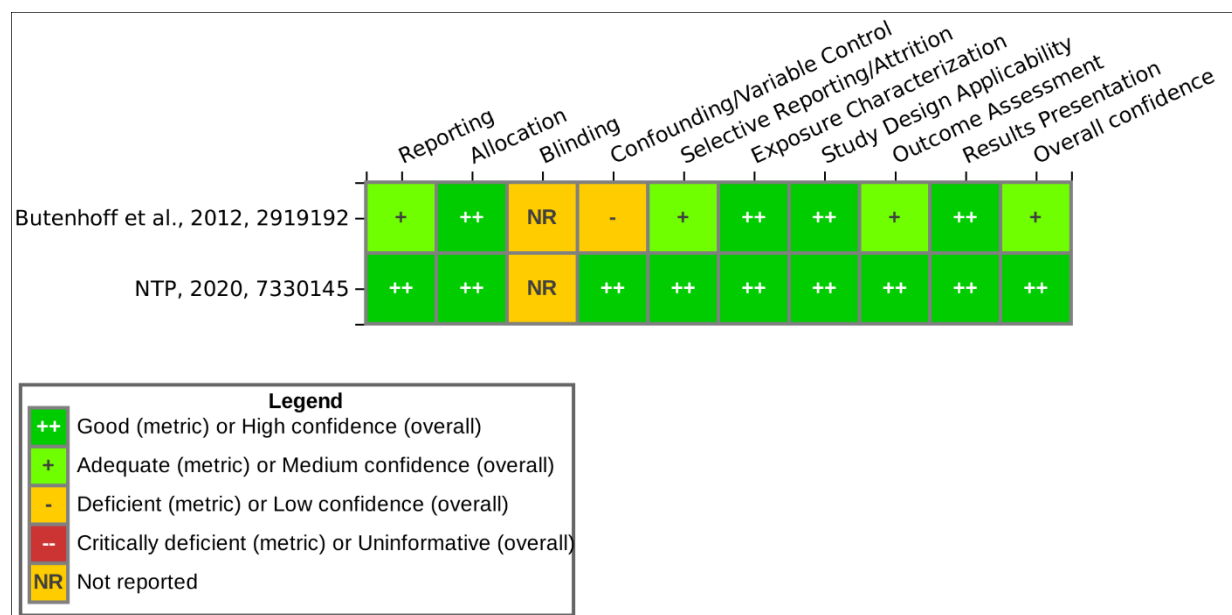


Figure 124. Summary of Study Evaluation for Toxicology Studies of PFOA and Cancer Effects

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

Three animal carcinogenicity studies indicate that PFOA exposure can lead to multiple types of neoplastic lesions including liver adenomas {Biegel et al. 2001, 673581; NTP 2020, 7330145} or carcinomas {NTP 2020, 7330145}, Leydig cell tumors (LCTs) {Biegel et al. 2001, 673581; Butenhoff, 2012, 2919192}, and pancreatic acinar cell tumors (PACTs) {Biegel et al. 2001, 673581; NTP 2020, 7330145} in male Sprague-Dawley rats. Neoplastic lesions were also observed in female Sprague-Dawley rats, but the incidence was not as high as observed in the males and often did not achieve statistical significance {Butenhoff et al. 2012, 2919192; NTP 2020, 7330145}. NTP {2020, 7330145} reported increased incidences of neoplastic lesions in female Sprague-Dawley rats, though these changes were not statistically significant or statistics could not be computed (liver neoplasms and PACTs) or there was uncertainty regarding the strength of response compared to controls (uterine adenocarcinomas). A fourth study {Filgo et al., 2015, 2851085} assessed hepatic tumor development in three strains of female mice after perinatal exposures to PFOA. This study is not further discussed here because the results were equivocal (i.e., few significant findings that did not display a dose-response relationship) and difficult to interpret due to small sample sizes (n=6-10 for some strains) and the lack of exposure postweaning.

In the three rat carcinogenicity studies {Biegel et al. 2001, 673581; Butenhoff et al. 2012, 2919192; NTP 2020, 7330145}, rats were fed diets containing similar concentrations of PFOA for approximately 2 years. Butenhoff et al. (2012, 2919192) analyzed a variety of tissues collected from male and female Sprague-Dawley rats fed diets containing 0, 30, or 300 ppm PFOA (equivalent to 1.3 and 14.2 mg/kg for males and 1.6 and 16.1 mg/kg for females) for 2 years. Similarly, Biegel et al. (2001, 673581) analyzed tissues collected from male Crl:CD® BR (CD) rats fed diets containing 0 or 300 ppm PFOA (equivalent to 13.6 mg/kg/day) for 24 months. Using a matrix-type exposure paradigm, NTP {2020, 7330145} administered PFOA in

feed to pregnant Sprague-Dawley (Hsd:Sprague Dawley® SD®) rats starting on GD6 and analyzed tissues of male and female offspring also fed postweaning diets containing PFOA for a total of 107 weeks. Dose groups for this report are referred to as “[perinatal exposure level]/[postweaning exposure level]” (e.g. 300/1,000; see further study design details in Section 3.3.1.2.1.2).

Liver adenomas were observed in the Biegel et al. study (2001, 673581) at an incidence of 10/76 (13%) at 13.6 mg/kg/day, compared to 2/80 (3%) in controls. Liver adenomas were also significantly increased in the NTP (2020, 7330145) in the 0/40, 0/80, and 300/80 ppm groups (Table 12). Both the 0/0 and 300/0 ppm control groups had no observed liver adenomas. Although no liver adenomas were observed in Butenhoff et al. (2012, 2919192), carcinomas were identified in the male controls, males in the low-dose group (1.3 mg/kg/day), and male and female rats in the high-dose group (14.2 and 16.1 mg/kg/day, respectively). NTP (2020, 7330145) reported increases in the incidence of hepatocellular carcinomas in the 300/80 ppm group. The differences in carcinoma incidences from controls were not statistically significant in either the Butenhoff et al. (2012, 2919192) or NTP (2020, 7330145) studies.

Table 12. Incidences of Liver Adenomas in Male Sprague-Dawley Rats as Reported by NTP (2020, 7330145)

Perinatal Dose	Postweaning Dose			
	0 ppm	20 ppm	40 ppm	80 ppm
0 ppm	0/50 (0%)	0/50 (0%)	7/50 (14%)*	11/50 (22%)*
300 ppm	0/50 (0%)	1/50 (2%)	5/50 (10%)	10/50 (20%)*

*Statistically significant compared to the respective control group (0/0 or 300/0 ppm) at $p \leq 0.05$.

Accompanying non-neoplastic/preneoplastic liver lesions were identified by Butenhoff et al. (2012, 2919192) in males and females at the 1- and 2-year sacrifices. An increased incidence of diffuse hepatomegalocytosis and hepatocellular necrosis occurred in the high-dose groups. At the 2-year sacrifice, hepatic cystoid degeneration (characterized by areas of multilocular microcysts in the liver parenchyma) was observed in males. Hyperplastic nodules in male livers were increased in the 14.2 mg/kg/day group. NTP similarly reported a variety of non-neoplastic and preneoplastic liver lesions in both male and female rats including increased incidences of liver necrosis and mixed-cell foci, hepatocyte hypertrophy, and focal inflammation. These lesions were more pronounced in males than females and were observed at both the 16-week interim and 107-week final necropsies. Refer to Section 3.3.3.2 for more detail.

Testicular LCTs were identified in both the Butenhoff et al. (2012, 2919192) and Biegel et al. (2001, 673581) studies. The tumor incidence reported by Butenhoff et al. (2012, 2919192) was 0/50 (0%), 2/50 (4%), and 7/50 (14%) for the 0, 1.3, and 14.2 mg/kg/day dose groups, respectively. The Biegel et al. study (2001, 673581) included one dose group (13.6 mg/kg/day); the tumor incidence was 8/76 (11%) compared to 0/80 (0%) in the control group. LCT incidence at similar dose levels was comparable between the two studies (11 and 14%). NTP (2020, 7330145) analyzed testicular tissue for LCTs but did not observe increased incidence due to PFOA treatment. The authors noted that this inconsistency with other carcinogenicity studies

could be a result of differences in exposure concentrations or stock of Sprague-Dawley rat (i.e. CD versus Hsd:Sprague Dawley).

PACTs were observed in both the NTP (2020, 7330145) and Biegel et al. (2001, 673581) studies. NTP (2020, 7330145) reported increased incidences of pancreatic acinar cell adenomas in males in all treatment groups compared to their respective controls (Table 13**Error! Reference source not found.**). NTP (2020, 7330145) observed increases in pancreatic acinar cell adenocarcinoma incidence in males in multiple dose groups and slight increases in the incidence of combined acinar cell adenoma or carcinoma in females from the 300/1,000 ppm dose group, though these increases did not reach statistical significance. In male rats, the incidence of PACTs in the Biegel et al. (2001, 673581) study was 8/76 (11%; 7 adenomas, 1 carcinoma) at 13.6 mg/kg/day while none were observed in the control animals. In a peer-reviewed pathological review of male pancreatic tissue collected by Butenhoff et al. (2012, 2919192), Caverly Rae et al. (2014, 5079680) identified 1/47 carcinomas in the 300 ppm group (compared to 0/46 in the control and 30 ppm groups) and no incidence of adenomas with any treatment. Pancreatic acinar hyperplasia was observed in males of the control, 1.3, and 14.2 mg/kg/day groups at incidences of 3/46 (7%), 1/46 (2%), and 10/47 (21%), respectively. Butenhoff et al. (2012, 2919192) also reported increased incidences of acinar atrophy in males (6/46 (13%), 9/46 (20%), and 11/49 (22%) in 0, 1.3, and 14.2 mg/kg/day dose groups, respectively), though this lesion was not discussed in the peer-reviewed pathology report {Caverly Rae et al., 2014, 5079680}. NTP (2020, 7330145) similarly reported increased incidences of acinus hyperplasia in males at incidence rates of 32/50 (64%), 37/50 (74%), 31/50 (62%) in the 0/20, 0/40, 0/80, and 27/50 (54%), 38/50 (76%), and 33/50 (66%) in the 300/20, 300/40, and 300/80 groups. The incidences in controls were 18/50 (36%) and 23/50 (46%) in the 0/0 and 300/0 groups, respectively. There were also low occurrences of acinus hyperplasia in the exposed female groups, though not as frequently observed as in males. However, the authors concluded that the incidence of pancreatic acinar cell neoplasms in males increased confidence that the occurrence in females was due to PFOA exposure.

Table 13. Incidences of Pancreatic Acinar Cell Adenocarcinomas in Male Sprague-Dawley Rats as Reported by NTP (2020, 7330145)

Perinatal Dose	Postweaning Dose			
	0 ppm	20 ppm	40 ppm	80 ppm
0 ppm	3/50 (6%)	28/50 (56%)*	26/50 (52%)*	32/50 (64%)*
300 ppm	7/50 (14%)	18/50 (36%)*	30/50 (60%)*	30/50 (60%)*

*Statistically significant compared to the respective control group (0/0 or 300/0 ppm) at $p \leq 0.05$.

NTP (2020, 7330145) observed increased incidences of uterine adenocarcinomas in female Sprague-Dawley rats during the extended evaluation (i.e., uterine tissue which included cervical, vaginal, and uterine tissue remnants). Incidence rates for this lesion are reported in Table 14. The accompanying incidences of uterine hyperplasia did not follow a dose-response relationship. Butenhoff et al. (2012, 2919192) identified mammary fibroadenomas and ovarian tubular adenomas in female rats, though there were no statistical differences in incidence rates between PFOA-treated dose groups and controls.

Table 14. Incidences of Uterine Adenocarcinomas in Female Sprague-Dawley Rats from the Standard and Extended Evaluations (Combined) as Reported by NTP (2020, 7330145)

Perinatal Dose	Postweaning Dose		
	0 ppm	300 ppm	1,000 ppm
0 ppm	1/50 (2%)	5/50 (10%)	8/50 (16%)*
150 ppm	–	3/50 (6%)	–
300 ppm	–	–	5/50 (10%)

*Statistically significant compared to the control group (0/0 ppm) at $p \leq 0.05$.

NTP concluded that under the exposure conditions presented, there was clear evidence of carcinogenic activity of PFOA in male Sprague Dawley rats based on increased incidences of hepatocellular neoplasms (predominately hepatocellular adenomas) and acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas {NTP, 2020, 7330145}. In females, NTP concluded there was some evidence of carcinogenic activity of PFOA based on increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms. The study authors also noted that the higher incidence of hepatocellular carcinomas and adenocarcinomas of the uterus may have been related to exposure.

3.3.17.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse cancer outcomes is discussed in Sections 3.1.2, 3.2.9, 3.3.1, 3.4.2, 3.4.3, 3.4.4, and 4.2 of the 2016 PFOA HESD {U.S. EPA, 2016, 3603279}. There are 27 studies from the most recent literature search conducted in 2020 that investigated the mechanisms of action of PFOA that lead to cancer effects. A summary of these studies is shown in Figure 125. Additional analysis on the mechanistic actions of PFOA on cancer health outcomes is ongoing 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	1	1	2
Cell Growth, Differentiation, Proliferation, Or Viability	2	0	19	19
Cell Signaling Or Signal Transduction	0	0	13	13
Extracellular Matrix Or Molecules	0	0	3	3
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	0	0	6	6
Hormone Function	0	1	3	4
Inflammation And Immune Response	0	0	1	1
Oxidative Stress	0	1	4	5
Xenobiotic Metabolism	0	1	1	2
Other	1	0	1	1
Not Specified (Review Article)	1	0	1	1
Grand Total	4	3	24	27

Figure 125. Summary of Mechanistic Studies of PFOA and Cancer Effects

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

3.3.17.4 Evidence Integration

In summary, one human epidemiological study identified since the 2016 assessment adds support to the previous evidence of an association between PFOA and kidney cancer {Shearer, 2021, 7161466}, No new epidemiological studies on testicular cancer were identified. It was noted in the 2016 assessment that an independent review of the epidemiological evidence for cancer based on 18 studies of occupational exposure to PFOA and general population exposure with or without co-exposure to PFOS (funded by 3M, but an independent assessment) {Chang, 2014, 2850282} found a lack of concordance between community exposures and occupational exposures one or two magnitudes higher than those for the general population. The discrepant findings across the study populations were described as likely due to chance, confounding, and/or bias {Chang, 2014, 2850282}. A recent critical review and meta-analysis of the literature concluded that there was an increased risk for kidney (16%) and testicular (3%) tumors for every 10 ng/mL increase in serum PFOA {Bartell and Vieira, 2021, 7643457}. Although the authors concluded that the associations were likely causal, they noted that the studies were limited and additional studies with larger cohorts could strengthen the conclusion.

The 2016 assessment indicated that there was no epidemiological support for an association between breast cancer and PFOA exposure in human populations. Although newer studies generally show no association, there is some evidence that PFOA may be related to breast cancer risk especially in participants with specific polymorphisms or for specific types of tumors

{Ghisari, 2017, 3860243; Mancini, 2019, 5381529}. Overall, the association between PFOA and breast cancer is unclear.

Two human occupational studies {Steenland, 2015, 2851015; Girardi, 2019, 6315730} support an increase in risk for liver cancer, malignant neoplasm of the lymphatic and hematopoietic tissue, as well as an increasing trend in prostate cancer that did not reach statistical significance. No associations were found for colorectal cancer in either the general population or occupational studies, or for lung cancer in occupational studies. These two *low* confidence occupational studies were the only source of evidence for associations between PFOA and cancer types other than kidney, testicular, and breast cancer.

In addition to the available epidemiological data, two multi-dose chronic cancer bioassays are available that investigate the relationship between dietary PFOA exposure and carcinogenicity in male and female rats {Butenhoff et al. 2012, 2919192; NTP 2020, 7330145}. Together, these two studies indicate that chronic PFOA exposure can result in increased incidences of different neoplastic lesions in multiple organ systems. In males, these include hepatocellular adenomas, LCTs, and PACTs. Results from a third single-dose chronic study in male rats provides supporting evidence for these neoplastic effects. Neoplastic lesions, including PACTs, hepatocellular adenomas/carcinomas, and uterine adenocarcinomas, were also observed in female rats, but the incidences were not as high as those observed in the males and often did not achieve statistical significance. In both males and females, there is evidence of non-neoplastic and/or preneoplastic lesions that accompany the observed neoplastic effects.

Results from human epidemiological studies are most consistent for kidney cancer in adults based on two C8 Health Project studies {Barry, 2013, 2850946; Vieira, 2013, 2919154}, an occupational mortality study {Steenland and Woskie, 2012, 2919168}, and a new nested case-control study {Shearer, 2021, 7161466}. Based on these findings, the endpoint of kidney cancer from Shearer et al. (2021, 7161466) was considered for the derivation of a POD. The study has exposure levels typical in the general population and low risk of bias. From the available animal studies in male rats, increased incidence of the endpoints of LCTs from Butenhoff et al. (2012, 2919192) and hepatocellular adenomas and PACTs from NTP (2020, 7330145) were also considered for the development 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 and compared to PODs derived from animal data when possible when the human data provided an association between PFOA and an adverse effect. 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., reproductive and endocrine 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., nervous, 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 (2018); 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.
Reduced immunoglobulin M (IgM) Response	Loveless et al., 2008, 988599; DeWitt et al., 2008, 1290826; Medium confidence	C57BL/6N mice (females), Crl:CD-1(ICR)BR mice (males)	Functional assessment indicative of immunosuppression. Immune effects were consistently observed across multiple studies

Endpoint	Study Reference and Confidence	Strain/Species/Sex	Notes
			including reduced spleen and thymus weights, altered immune cell populations, and decreased splenic and thymic cellularity.
Developmental Effects			
Decreased Birth Weight	Chu et al., 2020, 6315711; Govarts et al., 2016, 3230364; 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 Offspring Survival	Song et al., 2018, 5079725; Medium confidence	Kunming mice (F ₁ males and females)	Effect was consistently observed across multiple studies and species. Supported by the prenatal loss observed in Lau et al., 2006, 1276159 and Wolf et al., 2007, 1332672.
Decreased Fetal Body Weight	Li et al., 2018, 5084746; Medium confidence	Kunming mice (F ₁ males and females)	Effect was consistently observed across multiple studies and species: Lau et al., 2006, 1276159, Wolf et al., 2007, 1332672, Butenhoff et al., 2004, 1291063. Note that decreases in maternal body weight were not further considered because the decreased fetal body weight could be a potential confounder and was a more sensitive effect.
Developmental Scores for the Mammary Gland	Macon et al., 2011, 1276151; Medium confidence	CD-1 mice (F ₁ females)	Effect observed at low doses with no study NOAEL.
Delayed Time to Eye Opening	Lau et al., 2006, 1276159; Medium confidence	CD-1 mice (F ₁ males and females)	Effect also observed in Wolf et al., 2007, 1332672; however, those data were not amenable to BMD modeling (see C.13.2.).
Increased Placental Lesions	Blake et al., 2020, 6305864; Medium confidence	CD-1 mice (parental females)	Histopathological evidence of placental damage was selected over changes in placental

Endpoint	Study Reference and Confidence	Strain/Species/Sex	Notes
			weight observed in Blake et al. 2020, 6305864 and Jiang et al., 2020, 6320192.
Serum Lipid Effects			
Increased Total Cholesterol	Dong et al., 2019, 5080195; Medium confidence	Human (male and female)	BMD modeling performed by study authors.
Hepatic Effects			
Necrosis (focal, individual cell, both) in the Liver	Loveless et al., 2008, 7330145; Medium confidence, NTP, 2020, 7330145; High confidence	Crl:CD-1(ICR)BR mice (males), Sprague-Dawley rats (males)	Effect was accompanied by other liver lesions including cytoplasmic alteration and apoptosis. Females appear to be less sensitive. Necrotic liver cells were also observed in male mice in Crebelli, 2019, 5381564 and pregnant dams in Blake, 2020, 6305864. Effect is further supported by changes in serum ALT levels in animals and humans.
Endocrine Effects			
Increased TSH	NTP, 2019, 5400977; High confidence	Sprague-Dawley rat (females)	Effect observed at low doses in the female rat and is supported by suggestive evidence of increased TSH in the epidemiological literature. Female rats exhibited decreases in free T4 and total T4 but only in the high dose group. This effect was not considered for the male animals since the effect decreased in rats (NTP, 2019, 5400977) and increased in monkeys (Butenhoff et al., 2002 1276161). The changes in FT4 were more sensitive than TSH in males and are further considered below.
Decreased Free T4	NTP, 2019, 5400977; High confidence	Sprague-Dawley rats (male)	Effect was generally large in magnitude and consistently observed

Endpoint	Study Reference and Confidence	Strain/Species/Sex	Notes
			across multiple studies and species in the male. Decreases in free and total T4 are consistent with hypothyroxinemia in that a compensatory increase in TSH was not reported, nor was there evidence of thyroid gland histopathology. Decreased FT4 was also observed in the female rat, but it was limited to a high dose effect and TSH was more sensitive.
Reproductive Effects			
Reduced Number of Leydig Cells	Song et al., 2018, 5079725; Medium confidence	Kunming mice (male)	Effect observed at a later time point (PND70) and accompanied by decreased testosterone. Supported by evidence of decreased testosterone and altered sperm parameters observed in animals and humans.
Increased Length of Diestrus	Zhang et al., 2020, 6505878; Medium confidence	ICR mice (female)	Effect also observed in female rats {NTP, 2019, 5400977}, though at higher dose levels. Supported by evidence of altered ovarian physiology in mice and mixed evidence of reduced female fertility in humans.

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 SD from the control mean for continuous data or a BMR of 10% extra risk for dichotomous data. When severe or frank effects are modeled, a lower BMR can be adopted. For example,

developmental effects are frequently serious effects, and BMDs for these effects could employ a 5% BMR for dichotomous data or 0.5 SD for continuous data {U.S. EPA, 2012; U.S. EPA, 2020}. The BMRs selected for dose-response modeling of PFOA-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}.
Reduced immunoglobulin M (IgM) response	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 SD for continuous 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).
Decreased Fetal Body Weight, Decreased Survival	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

Endpoint	BMR	Rationale
		(2003, 1290574), U.S. EPA (2004, 198783), U.S. EPA (2012, 3114808).
Developmental Scores for the Mammary Gland, Delayed Time to Eye Opening	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 SD for continuous endpoints when biological information is not sufficient to identify the BMR. These endpoints of developmental score and time to eye opening are averaged across a dose group therefore a continuous response.
Increased Placental Lesions	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.
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		
Single and Focal Liver 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. Additionally, a 10% extra risk is consistent with the BMR used for constellation of liver lesions in the GenX chemicals toxicity assessment

Endpoint	BMR	Rationale
Endocrine Effects		
Increased TSH, Decreased Free T4	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.
Reproductive Effects		
Reduced Number of Leydig Cells, Increased Length of Diestrus	1 SD	No information is readily available that allows for determining a minimally biological significant response. The BMD Technical Guidance (U.S. EPA, 2012a) recommends a BMR based on 1 SD for continuous endpoints when biological information is not sufficient to identify the BMR.

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 to describe PFOA dosimetry in experimental animals 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 (Appendix E). 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 95% CIs. The application of the model outputs in the derivation of a human RfD is the focus of Section 4.1.2 of this document.

Table 17. PK Parameters from Wambaugh et al., 2013 Meta-Analysis of Literature Data for PFOA

Parameter	Units	CD1 Mouse (F) ^a	C57Bl/6 Mouse (F) ^a	Sprague-Dawley Rat (F) ^a	Sprague-Dawley Rat (M) ^a	Cynomolgus Monkey (M/F) ^a
Body Weight ^b (BW)	kg	0.02	0.02	0.20 (0.16–0.23)	0.24 (0.21–0.28)	7 (M), 4.5 (F)
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	290 (0.6–73,000)	340 (0.53–69,000)	1.7 (1.1–3.1)	1.1 (0.83–1.3)	230 (0.27–73,000)
Central Compartment Volume (V _{cc})	L/kg	0.18 (0.16–2.0)	0.17 (0.13–2.3)	0.14 (0.11–0.17)	0.15 (0.13–0.16)	0.4 (0.29–0.55)
Intercompartment Transfer Rate (k ₁₂)	1/h	0.012 (3.1×e ⁻¹⁰ –38,000)	0.35 (0.058–52)	0.098 (0.039–0.27)	0.028 (0.0096–0.08)	0.0011 (2.4×e ⁻¹⁰ –35,000)
Intercompartment Ratio (R _{V2:V21})	Unitless	1.07 (0.26–5.84)	53 (11–97)	9.2 (3.4–28)	8.4 (3.1–23)	0.98 (0.25–3.8)
Maximum Resorption Rate (T _{maxc})	μmol/h	4.91 (1.75–2.96)	2.7 (0.95–22)	1.1 (0.25–9.6)	190 (5.5–50,000)	3.9 (0.65–9,700)
Renal Resorption Affinity (K _r)	μmol	0.037 (0.0057–0.17)	0.12 (0.033–0.24)	1.1 (0.27–4.5)	0.092 (3.4×e ⁻⁴ –1.6)	0.043 (4.3×e ⁻⁵ –0.29)
Free Fraction	Unitless	0.011 (0.0026–0.051)	0.034 (0.014–0.17)	0.086 (0.031–0.23)	0.08 (0.03–0.22)	0.01 (0.0026–0.038)
Filtrate Flow Rate (Q _{filc})	Unitless	0.077 (0.015–0.58)	0.017 (0.01–0.081)	0.039 (0.014–0.13)	0.22 (0.011–58)	0.15 (0.02–24)
Filtrate Volume (V _{filc})	L/kg	0.00097 (3.34×e ⁻⁹ –7.21)	7.6×e ⁻⁵ (2.7×e ⁻¹⁰ –6.4)	2.6×e ⁻⁵ (2.9×e ⁻¹⁰ –28)	0.0082 (1.3×e ⁻⁸ –7.6)	0.0021 (3.3×e ⁻⁹ –6.9)

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 CD1 mouse were from Lou et al. (2009, 2919359), for the C57Bl/6 mouse were from DeWitt et al. (2008, 1290826), for the rat were from Kemper et al. (2003, 6302380), and for the monkey from Butenhoff et al. (2004, 3749227).^b Estimated average body weight for species used except with Kemper et al. (2003, 6302380) where individual rat weights were available and assumed to be constant.^c Cardiac outputs obtained from Davies and Morris (1993, 192570).

While this model provided parameters for all species of interest, 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 result in large ranges for some parameters. The wide CIs 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, PFOA 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 PFOA across all three species of interest, adequately predicted newer datasets published after publication, and was amendable to addition of a life stage component for predicting developmental study designs. For these important 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 PFOA concentration-time data in the species of interest, we compared model fits to in vivo datasets published following the 2016 HESD (Table 18 **Error! Reference source not found.**). For rats, this included Kudo et al. (2002, 2990271), Kim et al. (2016, 3749289), and Dzierlenga et al. (2020, 5916078). Model simulations demonstrated good agreement with available data for adult time-course PFOA PK predictions in the rat. For mice however, only one adult PFOA study was available for comparison {Fujii, 2015, 2816710} and only tracked PFOA concentrations through 24 hours. Therefore, only the original study used for parameter determination, Lou et al. (2009, 2919359), 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 in vivo studies when data were available.

Table 18. Model Predicted and Literature PK Parameter Comparisons for PFOA

	Male					Female				
	t1/2, α (days)	t1/2, β (days)	Vd, α (L/kg)	Vd, β (L/kg)	CL (L/d/kg)	t1/2, α (days)	t1/2, β (days)	Vd, α (L/kg)	Vd, β (L/kg)	CL (L/d/kg)
Rat										
Model	5.8	16.5	0.12	0.35	0.0147	0.16	2.84	0.16	2.81	0.686
Literature	1.64 ^a , 2.8 ^b	10.25 ^b	0.11 ^{a,c} , 0.15 ^{b,c}		0.047 ^a , 0.013 ^b	0.19 ^a , 0.028 ^b	0.22 ^b	0.17 ^{a,c} , 0.12 ^{b,c}		0.613 ^a , 0.81 ^b
Mouse										
Model	—	—	—	—	—	17.8	18.9	0.18	0.19	0.007
Literature	—	—	—	—	—	—	—	—	—	—

PK = pharmacokinetic; PFOA = perfluorooctanoic acid; $t_{1/2,\alpha}$ = initial-phase elimination half-life; $t_{1/2,\beta}$ = terminal-phase elimination half-life; $V_{d,\alpha}$ = volume of distribution during the initial phase; $V_{d,\beta}$ = volume of distribution during the terminal phase; CL = clearance.

^a Information obtained from Kim et al. (2016, 3749289).

^b Information obtained from Dzierlenga et al. (2020, 5916078).

^c Literature volumes of distribution represent central compartment volumes from a one-compartment^a or two-compartment^b model.

Because male mouse parameters are not available for PFOA, only female parameters are used for all PFOA modeling in mice. This assumption is addressed in Wambaugh et al. (2013, 2850932) and is based on a lack of evidence for sex-dependent PK differences in the mouse.

4.1.3.1.3 Life Stage Modeling

The Wambaugh et al. (2013, 2850932) model was modified to account for gestation, lactation, and post-weaning phases (Figure 126**Error! Reference source not found.**). Using the original model structure and published parameters, dams were dosed prior to conception and up to the date of parturition. Following parturition, a lactational phase involved PFOA transfer from the breastmilk to the suckling pup where the pup was modeled with a simple 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.

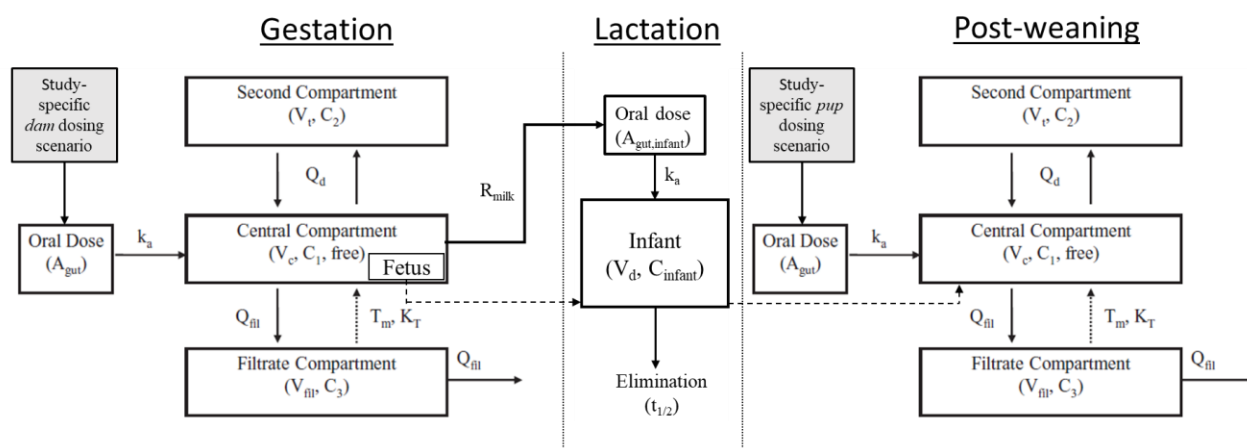


Figure 126. Model Structure for Life Stage Modeling

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 β -phase volumes of distribution between measured values in the literature and predicted volumes of distribution following extrapolation to relatively 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 {Dzierlenga, 2020, 5916078; Lou, 2009, 2919359} for the one compartment model to describe infant toxicokinetics. Finally, the Wambaugh et al. (2013, 2850932) model was not parametrized on 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 PFOA.

Table 19. Additional PK Parameters for Gestation/Lactation for PFOA

Parameter	Units	Rat	Mouse
Maternal Milk: Blood Partition Coefficient (P_{milk})	Unitless	0.11 ^{a,b}	0.32 ^e
Fetus:Mother Concentration Ratio (R_{fm})	Unitless	0.42 ^b	0.25 ^f
Elimination Half-Life ($t_{1/2}$)	Days	4.5 ^c	18.5 ^g
Volume of Distribution (V_d)	L/kg	0.11 ^d	0.2 ^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; PFOA = perfluorooctanoic acid.

^a Information obtained from Loccisano et al. (2013, 1326665) (derived from Hinderliter et al. (2005, 1332671)).

^b Information obtained from Hinderliter et al. (2005, 1332671).

^c Average of male/female half-lives reported in Dzierlenga et al. (2020, 5916078) and Kemper et al. (2003, 6302380).

^d Information obtained from Kim et al. (2016, 3749289).

^e Information obtained from Fujii et al. (2020, 6512379).

^f Information obtained from Blake et al. (2020, 6305864).

^g Information obtained from Lou et al. (2009, 2919359).

^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 to blood PFOA PC (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 PFOA and the species-specific milk

consumption rate during lactation (r_{milk}^i) for the i^{th} week of lactation. Milk rate consumptions are defined as:

- r_{milk}^0 , the starting milk consumption rate in kg milk per day (kg/d);
- r_{milk}^1 , the (average) milk consumption rate (kg/d) during the first week of lactation (and nursing);
- r_{milk}^2 , the (average) milk consumption rate (kg/d) during the second week of lactation; and
- r_{milk}^3 , 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_{milk}^i depending on the week of lactation.

Using this gestation/lactation model, we simulated two studies for PFOA exposure (one in mice and one 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 PFOA.

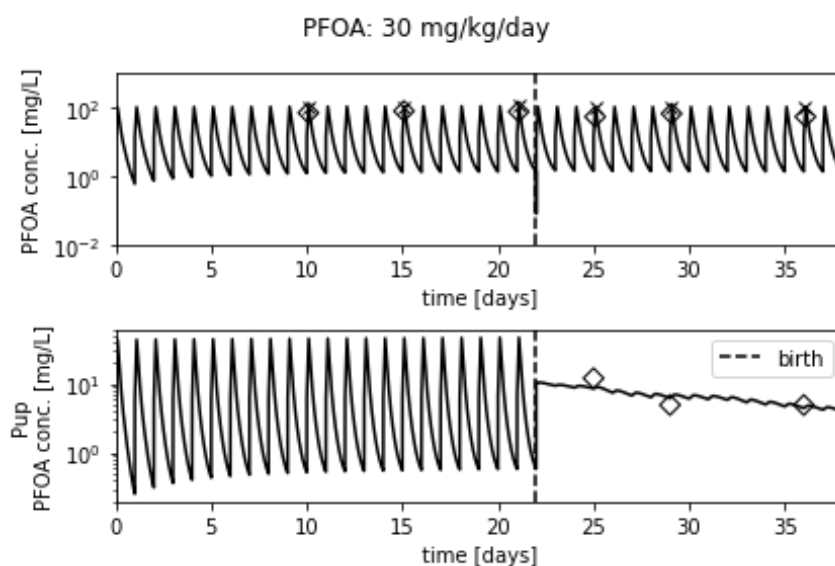


Figure 127. Gestation/Lactation Predictions of PFOA in the Rat

Top panel represents predicted dam concentrations with open diamonds (◊) representing the dam concentrations reported in Hinderliter et al. (2005, 1332671) and x's demonstrating the model-predicted value at that time. Bottom panel represents predicted pup concentrations with open diamonds (◊) representing the reported pup concentrations in Hinderliter et al. (2005, 1332671) where the source of PFOA exposure is from the breast milk. Vertical dashed line represents birth.

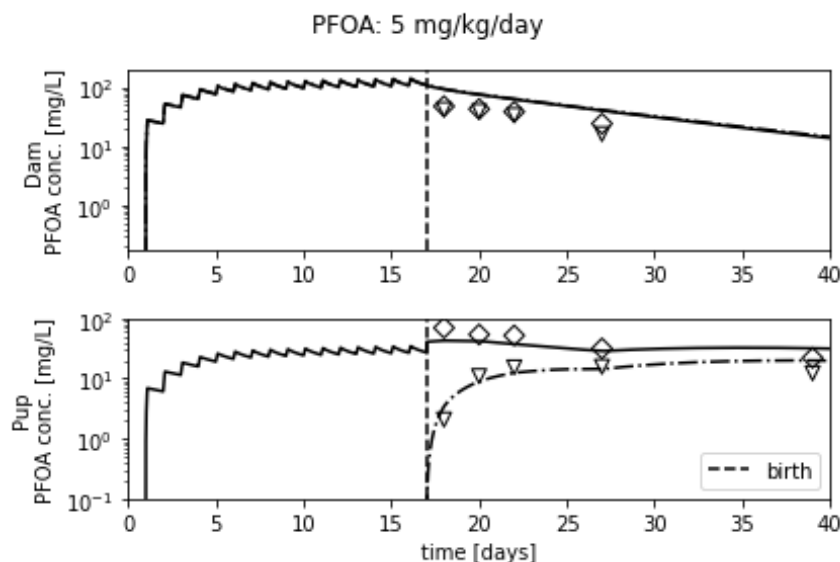


Figure 128. Gestation/Lactation Predictions of PFOA in the Mouse in a Cross-Fostering Study

Top panel represents predicted dam concentrations while bottom panel represents the predicted pup concentrations from White et al. (2009, 194811). Solid lines (—) represent a 5 mg/kg/day maternal dose paired with nursing pups that were exposed to PFOA in utero and open diamonds (◊) represent the reported dam and infant concentrations for this exposure scenario. Comparatively, dot-dashed lines (• —) represent the simulations from the cross-fostering study where dams were exposed to 5 mg/kg/day PFOA and pups born to the control dam were exposed through lactation. Open triangles (▽) represent the reported dam and infant concentrations for this cross-foster study.

Figure 127 demonstrates the model's ability to predict gestation/lactation study design in the rat for dams exposed to 30 mg/kg/day PFOA giving birth to pups who are exposed through lactation {Hinderliter, 2005, 1332671}. Comparatively, Figure 128 demonstrates model fits for PFOA exposure in mice from a cross-fostering study {White, 2009, 194811}. In each case, the original Wambaugh et al. (2013, 2850932) model with increasing maternal weight predicts dam concentrations in female rats and mice while the one compartmental lactational transfer model predicts infant concentrations for pups exposed both *in utero* and through lactation only.

While this model matches the selected PFOA developmental study data fairly well, there are several limitations to using this method. First, perinatal fetal concentrations assume instantaneous equilibration across the placenta and do not account for the possibility of active transporters mediating distribution to the fetus. In addition, clearance in the fetus 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, PFOA concentrations in 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 PFOA 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 area under the curve over the duration of the study (AUC, mg * day/L) or the blood concentration over the last 7 days ($C_{\text{last-7}}$, mg/L). In developmental studies dose-metrics were developed for the dam, the fetus (during gestation) and the pup (during lactation) for both time maximum blood concentrations 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 ($\text{AUC}_{\text{avg_dam_gest}}$), during lactation ($\text{AUC}_{\text{avg_dam_lact}}$), or combined gestation and lactation ($\text{AUC}_{\text{avg_dam_gest_lact}}$). In pups 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 ($C_{\text{avg_pup_gest}}$, $C_{\text{avg_pup_lact}}$, $C_{\text{avg_pup_gest_lact}}$ and $\text{AUC}_{\text{avg_pup_gest}}$, $\text{AUC}_{\text{avg_pup_lact}}$, $\text{AUC}_{\text{avg_pup_gest_lact}}$).

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 body weight during growth. In addition, previous modeling efforts suggest 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 as a suitable internal dosimetry target which provides support for using a simpler model that did not have individual tissue dosimetry.

For these reasons, EPA selected the model structure published by Verner et al. (2016, 3299692), which is a one compartment developmental models for humans. Several adjustments were undertaken to facilitate the application of the model to our use. First, the model was converted from acslX language to an R/MCSim framework. This allows the code to be more accessible to others by updating it to a contemporary modeling language. In addition, the modeling language conversion body weight curves for non-pregnant adults were revised based on U.S. Centers for Disease Control and Prevention (CDC) growth data for juveniles and values from EPA's *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 PFOA concentration in cord blood to maternal serum, and the ratio of PFOA 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 have 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.2.4. 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 (Section B.2). It was also used to simulate selected human 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 a human study, the steady state approximation was used to calculate a 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 a 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 PFOA Levels in Breastmilk and Maternal Serum or Plasma

Source	HERO ID	Milk to Maternal Plasma Ratio	New Study
Haug 2011	2577501	0.038	No
Seung-Kyu Kim 2011	2919258	0.025	No
Liu 2011	2919240	0.11	No
Cariou 2015 ^a	3859840	0.034	Yes
Sunmi Kim 2011 ^b	1424975	0.04	Yes
Verner 2016	3299692	0.058	–
Additional Studies	–	0.049	–

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.

^aMedian result based on the report of Pizzurro et al. (2019, 5387175).

^bMedian 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 were considered from an internal POD and a one-compartment approach was selected over a full PBPK model. Typically, PBPK models are preferred because they can provide individual tissue information and have a one-to-one correspondence with the biological system which 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 is typically scarcer. The decision to not use one of the PBPK models for PFOA 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 which 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 PFOA 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 PFOA in plasma.

Due to the previous issues in implementing PBPK models for PFAS, the known issues with the Loccisano model and the models based upon it, we decided that a one-compartment model was the best approach for this effort. Another justification for selecting a one-compartment model was that a one-compartment model is sufficient to predict blood (or serum/plasma) concentrations, and a major proportion of the PFOA in the body is found in serum/plasma due to albumin binding {Forsthuber, 2020, 6311640}. This makes serum/plasma a good biomarker for exposure and there were no other specific tissues that were considered essential to describe the dosimetry of PFOA.

We considered two one-compartment approaches for PFOA: the models of Verner et al. (2016, 3299692) and of Goeden et al. (2019, 5080506). As noted above, the Goeden et al. (2019, 5080506) model did not account for the decrease in concentration that occurs due to growth dilution which plays a substantial role in the PK of growing infants and children. Neglecting this factor will result in the overprediction of serum concentration in growing individuals. This left the Verner et al. (2016, 3299692) model as the best candidate for our purposes.

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 slow excretion and ongoing exposure. Additionally, some of the variability in measured half-life values may reflect 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 because this parameter requires knowledge of the total dose or exposure; V_d values are difficult to determine from environmental exposures and only one reported value is available (Thompson, 2010, 5082271). In the Verner et al. (2016, 3299692) model half-life, V_d , and hence clearance values are assumed to be constant across ages and sexes. The excretion of PFOA in children and infants is not well understood but could be different due to the ontogeny of renal transporters and age-dependent changes in overall renal function, and the amount of protein binding, especially in serum. It is even difficult to predict the overall direction of change in excretion (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 PFOA 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 one year, there was a constant relationship between maternal serum and breastmilk PFOS concentrations, and weaning was an immediate process with the infant transitioning from a fully breastmilk diet to the background exposure at one year.

4.1.4 Application of Pharmacokinetic Modeling for Animal-Human Extrapolation of PFOA Toxicological Endpoints and Dosimetric Interpretation of Epidemiological Endpoints

Table 21 displays the POD and estimated internal and HED PODs for immune, developmental, serum lipids, hepatic, endocrine and reproductive 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_{HED}s 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_{HED}s 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 (2018); Medium confidence	Human, male and female	BMDL ₅ , piecewise		1.7 x 10 ⁻⁴ (see appendix B.1 for BMD modeling details)	1.49 x 10 ⁻⁸
Decreased serum anti-diphtheria	Grandjean, (2012, 1248827);	Human, male and female	BMDL ₅ , piecewise		2.0 x 10 ⁻⁴	1.75 x 10 ⁻⁸

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)
antibody concentration in children	Grandjean, (2017, 3858518); Grandjean, (2017, 4239492); Budtz- Jørgensen (2018); Medium confidence				(see appendix B.1 for BMD modeling details)	
Decreased IgM response to SRBC	Dewitt et al., 2008, 1290826, Medium	Mouse, Female Study 1	BMDL _{1SD} , Polynomial 4		26.7 (Clast7; see appendix B.2.5 for BMD modeling details)	3.20 x 10 ⁻³
Decreased IgM response to SRBC	Dewitt et al., 2008, 1290826, Medium	Mouse, Female Study 2	BMDL _{1SD} , Hill		51.6 (Clast7; see appendix B.2.5 for BMD modeling details)	6.19 x 10 ⁻³
Decreased IgM response to SRBC	Loveless et al., 2008, 988599, Medium	Mouse, Male	BMDL _{1SD} , Exponential 3		90.7 (Clast7; see appendix B.2.8 for BMD modeling details)	1.09 x 10 ⁻²
Developmental Effects						
Decreased Birth Weight	Chu et al., 2020, 6315711, High Confidence	Human, male and female	BMDL _{5RD} , Hybrid		1.9 x 10 ⁻³ (see appendix B.1 for BMD modeling details)	1.00 x 10 ⁻⁶
	Govarts et al., 2016, 3230364, High Confidence	Human, male and female	BMDL _{5RD} , Hybrid		1.7 x 10 ⁻³ (see appendix B.1 for BMD modeling details)	4.62 x 10 ⁻⁸
	Sagiv et al., 2018, 4238410, High Confidence	Human, male and female	BMDL _{5RD} , Hybrid		9.0 x 10 ⁻³ (see appendix B.1 for BMD modeling details)	4.74 x 10 ⁻⁶
	Starling et al., 2017, 3858473, High Confidence	Human, male and female	BMDL _{5RD} , Hybrid		1.8 x 10 ⁻³ (see appendix B.1 for BMD modeling details)	9.47 x 10 ⁻⁷
	Wikström et al., 2020, 6311677, High Confidence	Human, male and female	BMDL _{5RD} , Hybrid		2.1 x 10 ⁻³ (see appendix B.1 for BMD modeling details)	1.10 x 10 ⁻⁶
	Song et al., 2018, 5079725;	Kunming Mice, F1 males and females	BMDL _{0.5SD} , Polynomial 3rd degree		8.9 (AUCavg_pup_g est; see appendix	4.59 x 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)
Decreased Fetal Body Weight	Medium confidence Li et al., 2018, 5084746; Medium confidence	Kunming Mice, F1 males and females	NOAEL ^a	1 mg/kg/day	B.2.12 for BMD modeling details) 8.5 (AUCavg_pup_g est; see appendix B.2.7 for modeling details)	1.50 x 10 ⁻³
Developmental Scores for the Mammary Gland	Macon et al., 2011, 1276151; Medium confidence	CD-1 Mice, F1 females	BMDL _{1SD} , Exponential 4		0.29 (AUCavg_pup_g est_lact; see appendix B.2.9 for BMD modeling details)	1.49 x 10 ⁻⁵
Delayed Time to Eye Opening	Lau et al., 2006, 1276159; Medium confidence	CD-1 Mice, F1 males and females	BMDL _{1SD} , Polynomial 2		9.4 (AUCavg_pup_g est_lact; see appendix B.2.6 for BMD modeling details)	1.67 x 10 ⁻³
Increased Placental Lesions	Blake et al., 2020, 6305864; Medium Confidence	CD-1 Mice, parental females	BMDL _{1SD} , Logistic		23 (AUCavg_dam_g est; see appendix B.2.6 for BMD modeling details)	3.19 x 10 ⁻³
Alterations in Serum Lipids						
Increased Total Cholesterol	Dong et al., 2019, 5080195; Medium confidence	Human, male and female	BMDL _{10RD} , Hybrid		2.41 x 10 ⁻² (see appendix B.1 for BMD modeling details)	6.72 x 10 ⁻⁷
Hepatic Effects						
Focal Necrosis	Loveless et al., 2008, 7330145; Medium confidence,	Crl:CD-1(ICR)BR Mice (males)	BMDL _{10RD} , Log-Probit		13.6 (Clast7; see appendix B.2.8 for BMD modeling details)	1.64 x 10 ⁻³
Necrosis	NTP, 2020, 7330145; High confidence	Sprague-Dawley Rats (males); Perinatal and Postweaning, Male Rats	BMDL _{10RD} , Log-logistic		26.9 (Clast7; see appendix B.2.11 for BMD modeling details)	3.23 x 10 ⁻³
Endocrine Effects						
Increased TSH	NTP, 2019, 5400977; High confidence	Sprague-Dawley Rats (females)	BMDL _{1SD} , Hill		1.67 (Clast7; see appendix B.2.10 for BMD modeling details)	2.00 x 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)
Decreased Free T4	NTP, 2019, 5400977; High confidence	Sprague- Dawley Rats (males)	LOAEL ^a	0.625 mg/kg/day	31.3 (Clast7; see appendix B.2.10 for BMD modeling details)	3.75 x 10 ⁻³
Reproductive Effects						
Reduced Number of Leydig Cells	Song et al., 2018, 5079725; Medium confidence	Kunming mice (male)	BMDL _{1SD} , Polynomial Degree 2		4.58 (AUCavg_pup_g est_lact; see appendix B.2.12 for BMD modeling details)	2.4 x 10 ⁻⁴
Increased Length of Diestrus	Zhang et al., 2020, 6505878; Medium confidence	ICR mice (female)	BMDL _{1SD} , Polynomial Degree 3		15.1 (Clast7; see appendix B.2.14 for BMD modeling details)	1.81 x 10 ⁻³

^a No models provided adequate fit; therefore, a NOAEL/LOAEL approach was selected.

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 chronic 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 the human and animal health outcomes, and because these endpoints represented the most sensitive effects after PFOA 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* {U.S. EPA, 2002, 88824} (Table 22).

Table 22. Uncertainty Factors for the Development of the Candidate Chronic RfD Values from EPA, 2002

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 PFOA contains numerous medium and high quality epidemiological and animal toxicological studies covering nearly all health systems, including numerous developmental toxicity and multigenerational reproductive toxicity studies.
UF _{TOT}	10	Composite UF _{TOT} = UF _A × UF _H × UF _S × UF _L × UF _D

An interspecies UF (UF_A) of 1 was applied to developmental and immunological effects observed in epidemiological studies because the dose response information from these studies are directly relevant to humans. There is no need to account for uncertainty in extrapolating from laboratory animals to humans.

An intraspecies UF (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 (lifestyle) 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 UF (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 lifestage; therefore, exposure during this time window can be considered more relevant than lifetime exposure {U.S. EPA, 1991, 732120}. According to the WHO/ International Programme on Chemical Safety (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 database UF (UF_D) of one was applied to account for deficiencies in the database for PFOA. 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	PODHED (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 (2018); Medium confidence	Human, male and female	1.49 x 10 ⁻⁸	1	10	1	1	1	10	1.5 x 10 ⁻⁹
Decreased serum anti-diphtheria antibody concentration in children	Grandjean, (2012, 1248827); Grandjean, (2017, 3858518); Grandjean, (2017, 4239492); Budtz-Jørgensen (2018); Medium confidence	Human, male and female	1.75 x 10 ⁻⁸	1	10	1	1	1	10	1.8 x 10 ⁻⁹
Developmental Effects										
Decreased Birth Weight	Chu et al., 2020, 6315711, High Confidence	Human, male and female	1.00 x 10 ⁻⁶	1	10	1	1	1	10	1.0 x 10 ⁻⁷
	Govarts et al., 2016, 3230364, High Confidence	Human, male and female	4.62 x 10 ⁻⁸	1	10	1	1	1	10	4.6 x 10 ⁻⁹
	Sagiv et al., 2018, 4238410, High Confidence	Human, male and female	4.74 x 10 ⁻⁶	1	10	1	1	1	10	4.7 x 10 ⁻⁷
	Starling et al., 2017, 3858473, High Confidence	Human, male and female	9.47 x 10 ⁻⁷	1	10	1	1	1	10	9.5 x 10 ⁻⁸
	Wikström et al., 2020, 6311677, High Confidence	Human, male and female	1.10 x 10 ⁻⁶	1	10	1	1	1	10	1.1 x 10 ⁻⁷

4.1.6 RfD Selection

The RfD selected for PFOA is 1.5×10^{-9} mg/kg-day based on the critical effect of decreased serum anti-tetanus 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 PFOA exposure could be explained by confounding across the PFAS. Exposure levels to PFOA were lower than PFOS (PFOA 4 ng/mL, PFOS 17 ng/mL), and there was a moderately high correlation between PFOA and PFOS, PFHxS, and PFNA (0.50, 0.53, 0.54, respectively). However, the authors assessed the possibility of confounding in a follow-up paper {Budtz-Jorgensen, 2018, 5083631} where estimates were adjusted for PFOS and there was no notable attenuation of the observed effects.

Another potential issue associated with the selection of decreased serum anti-tetanus or anti-diphtheria antibody concentrations in children for the RfD is the use of a 5% BMR and the clinical significance of that response. For tetanus and diphtheria, 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}. This may include neonates who depend on adequate maternal antibody transfer for immunity {Cook, 2001, 9642139}.

Though decreases in anti-tetanus antibody concentrations are not in themselves an adverse effect, they do prevent against tetanus infection, which is very rare in the United States due to high vaccination rates, but can cause life-threatening airway obstruction and/or irreversible or systemic toxin-mediated cardiac and neurologic complications {Cook, 2001, 9642139}. Between 2009-2017, the CDC was notified of 264 total cases of tetanus, including 2 cases of neonatal tetanus, 19 of which resulted in death. In addition to individuals who are not vaccinated, there are particular concerns of tetanus in individuals who received fewer than the recommended doses of tetanus for their age group. Lapse in coverage with appropriately timed booster doses may result in the reduction of antibody levels below the protective level of 0.15 IU/mL. In an analysis of individuals aged 6 years or more with serological samples from 1988-1994, 72.3% of Americans 6 years of age or older had fully protective levels of tetanus antibody (> 0.15 IU/mL) {McQuillan, 2002, 9642142}. This proportion greatly decreased in individuals 70 years of age and older; only 31.0% of individuals had antibody levels protective for tetanus. Notably, these results appear to be correlated with tetanus deaths in the United States. The 19 tetanus fatalities reported by the CDC between 2009-2017 were all in individuals above the age of 55, with the majority of fatalities in individuals from the 70+ age groups {Blain, 2020, 9642140}.

Additionally, it is unknown if PFOA exposure could impact antibody response to vaccinations other than tetanus and diphtheria. However, the animal toxicity literature provides evidence that PFOA induces general immune suppression (Section 3.3.4.2). 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 PFOA 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 PFOA exposure and serological vaccine responses in general. However, this updated review indicates an association between increased serum levels of PFOA 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 PFOA 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 noted non-significant associations between elevated levels of PFOA 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. Additionally, Abraham et al. (2020, 6506041) observed statistically significant correlations between adjusted tetanus, Hib, and diphtheria antibody levels and PFOA concentrations.

4.2 Cancer

4.2.1 Weight of Evidence

There is evidence of carcinogenic effects of oral PFOA exposure in both human epidemiological and animal toxicity studies. The evidence in epidemiological studies is primarily based on the incidence of kidney and testicular cancer, as well as potential incidence of breast cancer in genetically susceptible subpopulations. Other cancer types have been observed in humans, though the evidence for these is generally limited to *low* confidence studies. The evidence of carcinogenicity in animal models is provided in two chronic dietary studies in Sprague-Dawley rats which identified neoplastic lesions of the liver, pancreas, and testes.

As discussed in depth in the 2016 HESD {U.S. EPA, 2016, 3603729}, two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrollment 0.024 µg/mL) and kidney and testicular cancers {Barry, 2013, 2850946; Vieira, 2013, 2919154}. Vieira et al. (2013, 2919154) investigated the relationship between PFOA exposure and cancer among the residents living near the DuPont plant in Parkersburg, West Virginia. The adjusted ORs were increased for testicular cancer and for kidney cancer (OR: 5.1, 95% CI: 1.6, 15.6; n = 8 and OR: 1.7, 95% CI: 0.4, 3.3; n = 10, respectively) in the Little Hocking water district of Ohio and for kidney cancer (OR: 2.0, 95% CI: 1.3, 3.1; n = 23) in the Tupper Plains water district of Ohio. Barry et al. (2013, 2850946) extended this work and found significantly increased testicular cancer risk with an increase in the estimated cumulative PFOA serum level (HR: 1.34, 95% CI: 1.00, 1.79; n = 17). Increases, though nonsignificant, were also seen for kidney cancer (HR: 1.10, 95% CI: 0.98, 1.24; n = 105) and thyroid cancer (HR: 1.10, 95% CI: 0.95, 1.26; n = 86). As part of the C8 Health Project, the C8 Science Panel (2012, 9642155)

concluded that a probable link existed between PFOA exposure and testicular and kidney cancer {Steenland, 2020, 7161469}.

Eight *medium* confidence human epidemiological studies examining the carcinogenicity of PFOA have been published since the 2016 assessment, one of which adds support to the previous evidence of an association between PFOA and kidney cancer {Shearer, 2021, 7161466}. Six of those studies focused specifically on breast cancer risk. No new epidemiological studies on testicular cancer were identified. Shearer et al. (2021, 7161466) is a multi-center case-control study nested within the NCI's PLCO. This randomized clinical trial included all the participants of the screening arm of the PLCO trial who were newly diagnosed with histopathologically confirmed RCC during the follow-up period (n=326). The authors reported a statistically significant increase in risk of kidney cancer in highest exposure quartile and per doubling of PFOA concentration. After adjusting for other PFAS the association remained significant in analyses on a per doubling increase in PFOA. The increase in the highest exposure quartile remained and the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS), but it was no longer statistically significant. The analyses accounted for numerous confounders including BMI, smoking, history of hypertension, eGFR, previous freeze-thaw cycle, calendar and study year of blood draw, sex, race and ethnicity, study center.

Notably, a recent critical review and meta-analysis of the epidemiological literature concluded that there was an increased risk for kidney (16%) and testicular (3%) tumors for every 10 ng/mL increase in serum PFOA {Bartell and Vieira, 2021, 7643457}. Although the authors concluded that the associations were likely causal, they noted that there were a limited number of studies and additional studies with larger cohorts could strengthen the conclusion.

In addition to the available epidemiological data, two multi-dose chronic cancer bioassays are available that investigate the relationship between dietary PFOA exposure and carcinogenicity in male and female rats {Butenhoff et al. 2012, 2919192; NTP 2020, 7330145}. Increased incidence of neoplastic lesions were primarily observed in male rats from both studies, though results in females are supportive of potential carcinogenicity of PFOA. A third single-dose chronic cancer bioassay in male Sprague-Dawley rats also supports these results {Biegel et al., 2001, 673581}.

Testicular LCTs were identified in both the Butenhoff et al. (2012, 2919192) and Biegel et al. (2001, 673581) studies. The tumor incidence reported by Butenhoff et al. (2012, 2919192) was 0/50 (0%), 2/50 (4%), and 7/50 (14%) for the 0, 1.3, and 14.2 mg/kg/day dose groups, respectively. The Biegel et al. study (2001, 673581) included one dose group (13.6 mg/kg/day); the tumor incidence was 8/76 (11%) compared to 0/80 (0%) in the control group. LCT incidence at similar dose levels was comparable between the two studies (11 and 14%). In 2016 {U.S. EPA, 2016, 3603729}, EPA estimated a CSF of 0.07 (mg/kg/day)⁻¹ based on the incidence of LCTs identified by Butenhoff et al. (2012, 2919192).

PACTs were observed in both the NTP (2020, 7330145) and Biegel et al. (2001, 673581) studies. NTP (2020, 7330145) reported increased incidences of pancreatic acinar cell adenomas in males in all treatment groups compared to their respective controls (Table 13). This rare tumor type was also observed in female rats from the highest dose group, though these increases did not reach statistical significance. In male rats, the incidence of PACTs in the Biegel et al. (2001,

673581) study was 8/76 (11%; 7 adenomas, 1 carcinoma) at 13.6 mg/kg/day while none were observed in the control animals. NTP (2020, 7330145) also reported dose-dependent increases in the incidence of liver adenomas in male rats (Table 12), which were similarly observed by Biegel et al. (2001, 673581) at an incidence of 10/76 (13%) at 13.6 mg/kg/day, compared to 2/80 (3%) in controls. Overall, NTP concluded that under the exposure conditions of their report, there was *clear evidence* of carcinogenic activity of PFOA in male Sprague Dawley rats and *some evidence* of carcinogenic activity of PFOA in female Sprague Dawley rats based on the observed tumor types {NTP, 2020, 7330145}.

The report from NTP (2020, 7330145) also provides evidence that chronic exposure accompanied by perinatal exposure to PFOA does not increase cancer risk when compared to chronic exposure scenarios alone. There were no differences in the incidences of all tumor types examined across the treatment groups administered PFOA during both perinatal and postweaning periods compared with the postweaning-only treatment groups (see further study design details in Section 3.3.1.2.1.2). Age-dependent sensitivity to the carcinogenic effects of PFOA was previously only addressed in the study by Filgo et al. (2015, 2851085) in mice which is limited in terms of its gestational-only study design and small sample sizes.

Since publication of the 2016 HESD {U.S. EPA, 2016, 3603729}, the evidence supporting the carcinogenicity of PFOA has been strengthened. In particular, the evidence of kidney cancer from high-exposure community studies {Vieira et al., 2013; Barry et al., 2013} is now supported by evidence of RCC from a nested case-control study in the general population {Shearer et al., 2021, 7161466}. In animal models, the evidence of multi-site tumorigenesis reported in one chronic bioassay in rats {Butenhoff et al., 2012, 2919192} is now supported by evidence from a second chronic bioassay in rats similarly reporting multi-site tumorigenesis {NTP, 2020, 7330145}.

In summary, there is adequate evidence demonstrating carcinogenic potential in humans and one animal model. A plausible, though not definitively causal, association exists between human exposure to PFOA and kidney and testicular cancers in the general population and highly exposed populations. The evidence of carcinogenicity in animals is limited to two studies using the same strain of rat, however, results provide evidence of increased incidence of at least 3 tumor types (LCTs, PACTs, and hepatocellular adenomas) with chronic dietary exposure to PFOA. Importantly, site concordance is not always assumed between humans and animal models; agents observed to produce tumors may do so at the same or different sites in humans and animals {U.S. EPA, 2005, 6324329}.

EPA previously concluded that the induction of tumors is likely due to nongenotoxic mechanisms involving membrane receptor activation, perturbations of the endocrine system, and/or the process of DNA replication and cell division {U.S. EPA, 2016, 3603729}. An updated MOA analysis incorporating literature identified since 2016 is ongoing. Notably, other agencies have since published conclusions about the available evidence related to the MOA of PFOA. CalEPA's Office of Environmental Health Hazard Assessment concluded in their recent *Proposed Public Health Goals for PFOA and PFOS in Drinking Water* that PFOA "possesses several of the key characteristics of carcinogens, including the ability to induce oxidative stress, inflammation, and modulate receptor-mediated effects. Additionally, there is suggestive evidence that PFOA and PFOS are genotoxic, thus a genotoxic MOA for cancer remains plausible" {CalEPA, 2021, 9416932}. Moreover, IARC (2016, 3982387) concluded that there is moderate

evidence for many potential mechanisms for PFOA-induced toxicity (including PPAR α). Additionally, while EPA concluded that the liver tumors observed in the rat were not relevant to humans in the 2016 documents, the liver necrosis data in both mice and rats (see section 3.3.3.2.3) is compelling evidence that perhaps multiple MOAs are operative (e.g., cytotoxicity; Felter et al. (2018, 9642149).

Under EPA’s *Guidelines for Carcinogen Risk Assessment* {U.S. EPA, 2005, 6324329}, and based on the evidence of kidney and testicular cancer in humans and LCTs, PACTs, and hepatocellular adenomas in rats, PFOA is considered *Likely to Be Carcinogenic to Humans*.

4.2.2 CSF Development

In the 2016 HESD for PFOA, a CSF derived based on LCTs reported by Butenhoff et al. (2012, 2919192) was calculated to determine if a lifetime Health Advisory derived from the RfD would be protective for the cancer endpoint. The dose-response for the LCTs from Butenhoff et al. (2012) was modeled using EPA’s Benchmark Dose Software (BMDS) Version 2.3.1. The multistage cancer model predicted the dose at which a 4% increase in tumor incidence would occur. The 4% was chosen as the low-end of the observed response range within the Butenhoff et al. (2012) results. The CSF for PFOA of 0.07 (mg/kg/day)⁻¹ was derived from the BMDL₀₄ of 1.99 mg/kg/day after converting the animal BMDL to a HED using body weights to the ³/₄ power. The resultant 0.5 μ g/L value was greater than the lifetime Health Advisory (0.070 μ g/L) based on noncancer effects {U.S. EPA, 2016, 3982042}, indicating that the Health Advisory derived previously based on the developmental endpoint was protective for the cancer endpoint.

EPA has reevaluated the LCTs reported by Butenhoff et al. (2012, 2919192) in the current effort using the updated animal and human PK models described in 4.1.3. These modeling results are described in C.2.3. To duplicate the findings from the 2016 document, a BMR of 4% was chosen as the low end of the observed response range within the study results. EPA also derived CSFs for the tumor types identified by NTP that provide clear evidence of carcinogenic activity of PFOA in male Hsd:Sprague DawleySD rats: hepatocellular neoplasms (hepatocellular adenomas and carcinomas) and increased incidence of acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas {NTP, 2020, 7330145} (Table 24). A BMR of 10% was selected for these tumor types consistent with the BMD Technical Guidance (U.S. EPA, 2012a). These modeling results are described in C.2.11.5 and C.2.11.6, respectively.

Table 24. Cancer Slope Factors based on Animal Toxicological Data

Tumor Type	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD Internal dose ^a /Internal Dose Metric	POD _{HED}	CSF (BMR/POD _{HED})
Leydig Cell Adenomas in the Testes	Butenhoff et al., 2012, 2919192, Medium confidence	Male Sprague- Dawley	BMDL _{4RD} , Multistage Model 3	19276 (AUC (mg*d/L); see appendix C.2.3 for BMD modeling details)	3.20 x 10 ⁻³ mg/kg/day	12.2 (mg/kg/day) ⁻¹
Hepatocellular Adenomas or Carcinoma	NTP, 2020, 7330145, High confidence	F1 Male Sprague- Dawley Rats, Perinatal (300 PPM) and	BMDL _{10RD} , Multistage 2	88.6 (AUC _{avg,pup,tot} aL in mg/L; see appendix C.2.11.5 for	1.1 x 10 ⁻² mg/kg/day	9.4 (mg/kg/day) ⁻¹

Tumor Type	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	POD Internal dosea /Internal Dose Metric	POD _{HED}	CSF (BMR/POD _{HED})
Acinar Cell Adenoma	NTP, 2020, 7330145, High confidence	Postweaning Exposure F1 Male Sprague- Dawley Rats, Perinatal (300 PPM) and Postweaning Exposure	BMDL _{10RD} , Multistage 1	BMD modeling details) 15.7 (AUCavg,pup,tot aLin mg/L); see appendix C.2.11.6 for BMD modeling details)	1.9 x 10 ⁻² mg/kg/day	53 (mg/kg/day) ⁻¹

Finally, EPA calculated CSFs for RCC from {Shearer, 2021, 7161466} based on the method used by CalEPA (2021, 9416932). The underlying model involves a linear regression between PFOA exposure and cancer relative risk used to estimate the dose-response between PFOA and RCC risk. This was calculated using a weighted linear regression of the quartile specific RRs, with the weights defined as the inverse of the variance of each RR. Since the incidence of kidney cancer is relatively low and because the cases and controls were matched on age, the ORs represent a good approximation of the underlying RRs. The CSF is then calculated as the excess cancer risk associated with each ng/ml increase in serum PFOA (CSF_{serum}). The CSF_{serum} was calculated by first converting the linear regression model discussed above from the RR scale to the absolute risk scale. This was done assuming a baseline risk (R₀) of RCC or kidney cancer in an unexposed or lower exposure reference group. Since this is not available in a case-control study, the lifetime risk of RCC in U.S. males is used. The lifetime RCC risk was estimated by multiplying the lifetime risk of kidney cancer in U.S. males {American Cancer Society, 2020, 9642148} by the percentage of all kidney cancers that are the RCC subtype (90%). This gives an R₀ of 0.0202 × 90% = 0.0182. The CSF_{serum} was then calculated as either the product of the upper 95% CI or the central tendency of the dose-response slope and R₀. The results of the modeling and the CSFs derived are presented in Table 25.

Table 25. Cancer Slope Factors based on Epidemiological Data

Tumor Type	Study/ Confidence	Strain/ Species/Sex	POD Type/Model	Internal CSF - Increase in cancer risk per 1 ng/mL serum increase	CSF - Increase in cancer risk per 1 ng/(kg*d) increase in dose
Renal cell carcinoma (RCC)	Shearer et al. (2021) 7161466; <i>Medium</i> confidence	Human, male and female 55- 74 years	CSF serum in adults (per ng/mL of serum PFOA); central tendency slope	1.78 x 10 ⁻⁶ (ng/mL) ⁻¹ (modeling approach described above)	0.01483 (ng/kg/day) ⁻¹
Renal cell carcinoma (RCC)	Shearer et al. (2021) 7161466; <i>Medium</i> confidence	Human, male and female 55- 74 years	CSF serum in adults (per ng/mL of serum PFOA); upper limit of the 95% CI	3.52 x 10 ⁻⁶ (ng/mL) ⁻¹ (modeling approach described above)	0.0352 (ng/kg/day) ⁻¹

The PFOA bioassay data from NTP (2020, 7330145) provides evidence that chronic exposure accompanied by perinatal exposure to PFOA does not increase cancer risk when compared to chronic exposure scenarios alone. There were no differences in the incidences of all tumor types examined across the treatment groups administered PFOA during both perinatal and postweaning periods compared with the postweaning-only treatment groups (see further study design details in Section 3.3.1.2.1.2 and results in 3.3.17.2). Therefore, age-dependent adjustment factors were not applied to the PFOA CSFs.

Overall, these results indicate that PFOA is a more potent carcinogen than described in the 2016 HESD.

5.0 Relative Source Contribution Derivation

5.1 Relative Source Contribution

5.1.1 Background

EPA applies an RSC when calculating the MCLG to provide a margin of safety that an individual's total exposure from a contaminant (i.e., PFOA) 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 PFOA, 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 percent. In the case of MCLG development, this means that 20 percent of the exposure equal to the RfD is allocated to drinking water and the remaining 80 percent is reserved for other potential sources, such as diet, air, consumer products, etc. This 20-percent 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 percent based on the available data, allowing the remaining 20 percent for other potential sources {U.S. EPA, 2000, 19428}. Applying a lower RSC (e.g., 20 percent) 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 percent 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 percent for the final Health Advisory for PFOA {U.S. EPA, 2016, 3982042}, based on the physical properties of PFOA 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; TCEQ, 2016, 5975349}.

Alternatively, several states have applied an RSC for PFOA 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, 9418094; WA DOH, 2020, 9418278}, and New York applied an RSC of 60% {NYSDOH, 2018, 6984171}.

5.1.2 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).

5.1.2.1 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 PFOA and seven other frequently measured PFAS (perfluorooctanesulfonate (PFOS), perfluorobutanoic acid (PFBA), PFBS, PFDA, PFHxA, PFHxS, and PFNA). Machine learning approaches were used during the literature scoping and title/abstract screening to prioritize exposure pathways of interest by automated tagging and to select studies for inclusion using an iterative predictive screening model. Title/Abstract screening for the PECO criteria identified 486 studies for full text screening; only 6 studies fully addressed the protocol requirements {Wu, 2014, 2533322; Makey, 2017, 3860102; Byrne, 2017, 4165183; Kim, 2019, 5080673; Balk, 2019, 5918617; Poothong, 2019, 5080584}. The extraction of exposure measurement data and study characteristics from each included study was performed in

DistillerSR software. Exposure intake calculations were used to estimate a percentage of occupant serum concentrations that could be attributed to indoor exposure pathways other than drinking water and diet. The included studies were evaluated using an approach modified from EPA's *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 (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., PFOA was measured in sera or a non-indoor environmental medium). Because the combination of PFOA 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}.

5.1.2.2 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 that were published between 2003–2020. ICF's *litstream*TM software was used to conduct title-abstract (TiAb) and full-text screening, and to extract relevant primary data into a comprehensive evidence database. Parameters of interest included: sampling dates and locations (focused on locations in the United States, Canada, and Europe), numbers of collection sites and participants, analytical methods, limits of detection and detection frequencies, and occurrence statistics.

Detailed data on PFAS occurrence in high-priority household and environmental media from 210 studies were extracted, as well as limited data on human matrices from 422 additional papers. 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 PFOA 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.

5.1.3 Summary of Potential PFOA Sources

PFOA is a synthetic, fully fluorinated, organic acid that is used in many types of consumer products and in the production of fluoropolymers {U.S. EPA, 2016, 3982042}. PFOA has been used in flame repellants, cosmetics, paints, polishes, and processing aids used in the manufacture of nonstick coatings on cookware. It is one of a large group of perfluoroalkyl substances that are used in consumer and industrial products to improve their resistance to stains, grease, and water. 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}. Despite the United States phase out of production, EPA has found widespread PFOA contamination in water, sediments, and soils. Exposure to PFOA can occur through food, fish and shellfish, water, house dust, and contact with consumer products.

5.1.3.1 Dietary Sources

Ingestion of food is a potentially significant source of exposure to PFOA and is often claimed to be the dominant source of exposure 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 is typically estimated to be about 2-3 ng/kg/day {Gleason, 2017, 5024840}. 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 analytical methods with poor sensitivity, relatively few food items with detectable levels, and levels that can vary greatly depending on sources or location {Gleason, 2017, 5024840}.

There is currently no comprehensive, nationwide Total Diet Study (TDS) for PFOA that can be used to draw conclusions about the occurrence and potential risk of PFOA 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, collected for the TDS. 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, but not including PFOA: fish sticks (PFOS and PFNA), canned tuna (PFOS and PFDA), and protein powder (PFOS). PFOA was not detected in any of the food samples analyzed in FDA TDS samples of produce, meats, dairy and grain products in 2019 or 2021 {FDA, 2021, 9419076}. In a 2018 focused study near a PFAS production plant in the Fayetteville, North Carolina area, PFOA was detected in several produce samples (cabbage, collard greens, kale, mustard greens, swiss chard, and lettuce) {FDA, 2018, 9419064}. In bottled water, PFOA was below the lower limit of quantification (LOQ; 4 ng/L) in all (30) analyzed samples 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 general levels of PFAS in the U.S. food supply {FDA, 2021, 9419076}. In a 2010 study, PFOA was detected in food samples collected from

five grocery stores in Texas {Schecter, 2010, 729962}; based on the results from this study and on dietary intakes from the 2007 U.S. Department of Agriculture food availability data set, the estimated daily exposure to PFOA per capita was 60 ng/day {U.S. EPA, 2016, 3982042}.

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 LOQ, lower bound dietary exposure estimates were obtained by assigning zero to values below LOD/LOQ. Median chronic dietary exposures of PFOA for children and adults were estimated as 0.30 and 0.18 ng/kg body weight per day, respectively. The most important contributor was “Fish and other seafood,” followed by “Eggs and egg products,” “Meat and meat products,” and “Fruit and fruit products.” “Vegetables and vegetable products” and “Drinking water” were also found to be important contributors to dietary PFOA exposure. It is unclear whether or not 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 {Poonthong, 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 PFOA, dietary intake was by far the greatest contributor to aggregate exposure (contributing 92% of total estimated PFOA intake), but intake from ingestion of house dust represented the dominant pathway for some of the top 20% most highly exposed individuals. On average, measured serum concentrations of PFOA were similar to modeled concentrations based on intakes. It is notable that while the authors reported significant positive correlations between PFOA concentrations in serum and estimated intakes based on surface dust and vacuum cleaner bag dust samples, correlations with estimated dietary intakes were not significant, which the authors attributed to temporal variations in dietary intakes over several 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 PFOA approximately three times higher from solid food than from liquids.

5.1.3.1.1 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 fluorotelomer alcohol (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, California.

Under FDA rules, there are dozens of PFAS chemicals still approved for food contact materials. In 2018, 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 {Schneider, 2017, 3981864; Yuan, 2016, 3859226; Zabaleta, 2020, 6505866}. The data from the cited 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}. Schneider 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 the Schreder & Dickman (2018, 9419077) report, inorganic fluoride was the analyte for the initial analysis. Fifty six percent of the dessert and bread wrappers were positive for fluoride, 38 percent of the

sandwich and burger wrappers, and 20 percent of the paper-board containers. None of the 30 (hot/cold) paper beverage cups tested positive in contrast to 16 percent of beverage containers (milk/juice) made from other materials. Generally, food contact papers had higher fluoride detection frequencies than food contact paperboard. Twenty fast food packaging samples of the 407 total samples were selected for more extensive PFAS specific analysis. PFOA, PFHxA, and PFBS were among the PFAS with the highest detection rates; PFOA was detected in 6/20 samples.

An analysis of popcorn bags, snack bags, and sandwich bags purchased in 2018 from international vendors and grocery stores in the United States found little evidence of PFOA, with only two popcorn bags with content above the limit of quantitation of 5.11 ng per gram of paper {Monge Brenes, 2019, 5080553}. The authors presented these results as evidence of a reduction in PFOA 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 PFOA in the 2 bags from the United States, levels typically at about 4 ng/g in those from several European countries, and levels around 50 ng/g in bags from China.

Yuan et al. (2016, 3859226) analyzed 25 food contact materials purchased in Columbus, Ohio for PFAS as compared with 69 products purchased in China. The primary PFAS substances detected were consistently the C6 to C14 telomer alcohols. In food packaging materials from China, of the 15 detected perfluorinated carboxylic acids, PFOA was the most frequently detected (90%) and was detected with the highest median concentration (1.72 ng/g). In contrast to the products from China, the primary analyte from U.S. paper food contact products other than popcorn bags was the 6:2 telomer alcohol. The authors also report a migration efficiency of PFOA from paper bowl packaging into food stimulants of 1.58 percent. This is a relatively low efficiency compared to several of the FTOHs which the authors reported to migrate with greater than 90 percent efficiency.

Zabaleta et al. (2020, 6505866) looked at PFAS in 25 paper- and paperboard packaging materials primarily collected in Spain. Except in the single microwave popcorn bag collected from China, none of the perfluorocarboxylic acids (C3, C6, C7, C8, C 9, C10), including PFOA, were 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 C3 to C10 perfluorinated carboxylates. Zabaleta et al. (2020, 6505866) also monitored migration of the PFAS carboxylates (C6 to C10) from packaging materials into cereal, rice, or milk. For each PFAS studied the percent migration to milk exceeded that to rice with the lowest percent migration being that to cereal. Percent migration to foods decreased as the carbon chain length increased (C6 to C10) after a 6-month period. The migration percentage of PFOA into cereal, rice, and milk powder products over 6 months ranged from 1.4-5.6 percent.

5.1.3.1.2 Fish and Shellfish

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. PFOA has been detected in freshwater fish fillet samples collected during several national studies in rivers and the Great

Lakes; however, PFOA is reported at a lower frequency and at lower levels compared to other PFAS, including PFOS (Table 26 **Error! Reference source not found.**).

Table 26. Summary of EPA national freshwater fish tissue monitoring results for PFOA

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	No PFOA detections reported.
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	PFOA detected in 4 percent of samples. Maximum detected concentration 0.27 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	PFOA detected in 12 percent of samples. Maximum detected concentration 0.97 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	PFOA detected in 14 percent of samples. Maximum detected concentration 1.93 ng/g.

In addition, there are several available studies that assess PFAS concentrations in fish, shellfish, and other aquatic species. In 2015, Penland et al. (2020, 6512132) measured PFAS concentrations in invertebrates and vertebrates along the Yadkin – Pee Dee River in North Carolina and South Carolina. PFOA was detected in whole body tissues of unionid mussels (7.41 ng/g wet weight) and aquatic insects (10.68 ng/g wet weight), but was not detected in Asian clam, snails, or crayfish. PFOA was measured in muscle tissue of 2/11 sampled fish species: the channel catfish (21.19 ng/g wet weight) and notchlip redhorse (45.66 ng/g wet weight).

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. Samples were analyzed for 16 PFAS, including PFOA. Brown crab and shrimps had the highest average concentrations of PFOA (0.78 and 0.43 ng/g ww, respectively). PFOA was also detected in farmed fish including eel and trout, and marine fish species including cod, haddock, and sole. However, the PFAS with generally the highest percent detection and average concentration in all sample types was PFOS.

In summary, PFOA has been detected in fish and shellfish samples from freshwater and marine fish and shellfish, as well as in both farmed and wild-caught samples. While most of the data were collected from freshwater fish samples, recent studies suggest ingestion of many types of fish and shellfish can be a potential source of exposure to PFOA. However, in contrast to PFOS, PFOA concentrations in biotic media tend to be low, or below detection levels, highlighting the lower overall bioaccumulation potential for this chemical, based on its physical-chemical properties, including a shorter perfluorinated chain length, and a carboxylate head group. In addition, trophic biomagnification is rarely observed in aquatic food webs with PFOA.

5.1.3.2 Water

5.1.3.2.1 Ambient Water

PFOA is one of the dominant PFAS compounds detected in ambient water, along with PFOS {Ahrens, 2011, 2657780; Benskin, 2012, 1274133; Dinglasan-Panlilio, 2014, 2545254; Nakayama, 2007, 2901973; Remucal, 2019, 5413103; Zareitalabad, 2013, 5080561}. Most of the current, published PFOA occurrence studies have focused on a handful of broad geographic regions, many times targeting sites with known manufacturing or industrial uses of PFASs, such as the Great Lakes, the Cape Fear River, and waterbodies near Decatur, Alabama {Boulanger, 2004, 1289983; Cochran, 2015, 9416545; Hansen, 2002, 1424808; Konwick, 2008, 1291088; Nakayama, 2007, 2901973; 3M Company, 2000, 9419083}. PFOA concentrations in surface waters range over seven orders of magnitude, generally in pg/L to ng/L concentrations, but sometimes reaching µg/L levels {Zareitalabad, 2013, 5080561}. Figure 129 (adapted from Jarvis et al., 2021, 9416544, for PFOA) shows the distribution of the minimum and maximum PFOA concentrations (ng/L) measured in surface waters for each state or waterbody (excluding the Great Lakes) with reported data in the publicly available literature.

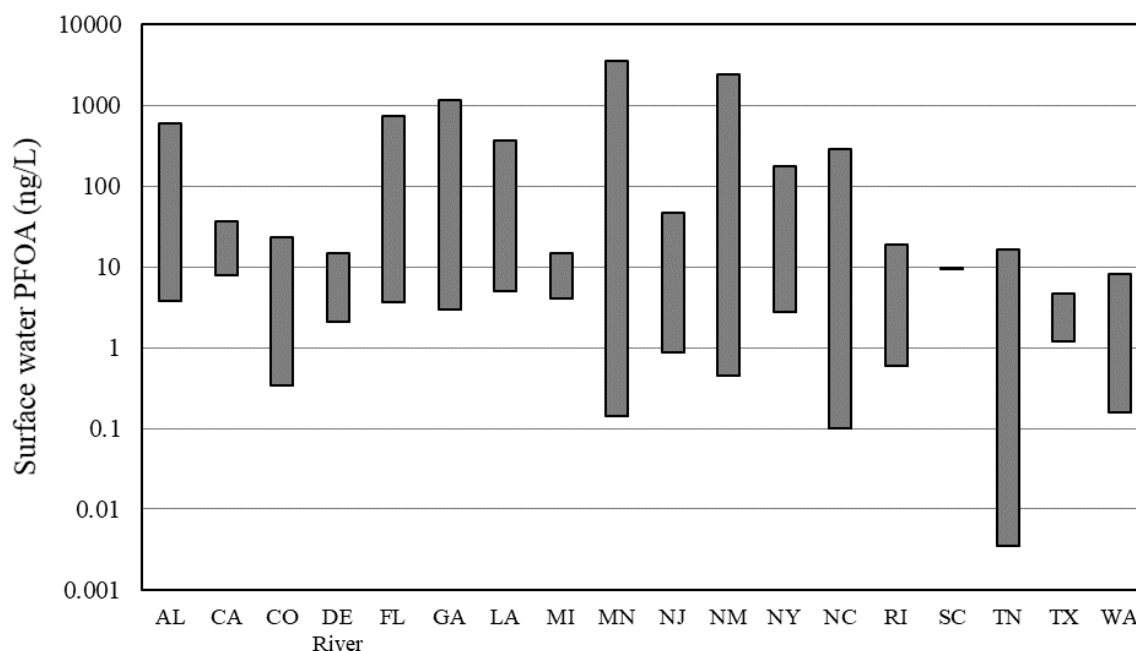


Figure 129. Distribution of Available PFOA Concentrations in Surface Waters for States/Waterbodies (excludes Great Lakes)

PFOA concentrations in surface water tend to increase with levels of urbanization. Across the Great Lakes region, PFOA was higher in the downstream lakes of Erie and Ontario and lower in the upstream lakes of Superior, Michigan, and Huron {Remucal, 2019, 5413103}. Similarly, Zhang et al. (2016, 3470830) observed measured PFOA concentrations in urban areas (urban average PFOA concentration = 10.17 ng/L; n = 20) to be more than three time greater than concentrations in rural areas (rural average PFOA concentration = 2.95 ng/L; n = 17) within New Jersey, New York, and Rhode Island. Temporal variation of PFOA in surface waters remains largely unknown due to data limitations.

5.1.3.2.2 Drinking Water

Ingestion of drinking water is a potentially significant source of exposure to PFOA. However, inhalation and dermal absorption (e.g., while showering, bathing, etc.) are not expected to be significant sources of exposure from contaminated drinking water {Gleason, 2017, 5024840}. Serum PFOA concentrations are known to be elevated among individuals living in communities with drinking water contaminated from environmental discharges. As documented in Gleason et al. (2017, 5024840), estimates of the relative importance of drinking water with respect to total exposure are highly dependent on the assumed concentration of PFOA 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 PFOA, East et al. (2021, 9416543) estimated that drinking water contributes 10% of total intake for adults and 9% for children.

EPA's UCMR3 required PWS monitoring for six PFAS: PFOS, PFOA, PFNA, PFHxS, perfluoroheptanoic acid (PFHpA), and PFBS. Under UCMR 3, PFOA was found in 2.48 percent of systems, at a median concentration of 0.03 µg/L and a maximum concentration of 0.349 µg/L

{U.S. EPA, 2019, 9419085}. EPA found that 4.0 percent 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. The 4.0 percent 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}. Data from more recent state monitoring efforts demonstrate occurrence in multiple geographic locations consistent with UCMR 3 monitoring results.

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. PFOA was reported in source water at 76 percent of systems, with a median concentration of 6.32 ng/L and maximum concentration of 112 ng/L. Similarly, in treated drinking water, PFOA was detected in 76 percent of systems, with a median concentration of 4.15 ng/L and maximum concentration of 104 ng/L.

5.1.3.3 Consumer Product Uses

A targeted analysis of 29 U.S. and Canadian cosmetic products with high fluorine content {Whitehead, 2021, 9416542} found high concentrations of fluorotelomer alcohols (FTOH), including 8:2 FTOH, commonly present in the formulations. A fraction of 8:2 FTOH is believed to undergo metabolic transformation into PFOA. In addition to direct contact with personal care products, products and articles (and the use of these) may be sources in the indoor environment that manifest as measured occurrence in house dust and indoor air. An earlier investigation of consumer exposure to PFOA by Trudel et al. (2008, 214241) used mechanistic modeling together with information on product-use habits to estimate oral and dermal exposures clothes, carpet, upholstery, and food contact materials. Noting that PFOA may be contained as a contaminant in older and in new products, the authors estimated exposure via both mill-treated and home-treated carpets. The authors concluded that contact with consumer products is not a significant contributor to total exposure, but that since PFOA may be a contaminant in even new products, consumer exposure may continue to occur, particularly via both mill-treated and home-treated carpets. The authors also point out that carpet and other textiles are likely to be continuing sources of PFOA in house dust. In contrast, in an analysis of 116 articles of commerce from the United States, U.S. EPA (2009, 1290922) identified carpets and related products as potentially the most significant source of PFCAAs out of 13 total product categories analyzed. PFOA was detected in all 13 product types. Other important indoor sources of PFCAAs include floor wax/sealant and home textiles, upholstery, and apparel. In a similar analysis of 52 European products collected between 2014–2016, Borg and Ivansson (2017, 9416541) reported that PFOA was the most commonly detected PFAS and was detected in all samples except those that did not contain any detectable levels of PFAS. Notably, the authors specifically targeted products that were known or suspected to contain PFAS in their analyses.

Liu et al. (2014, 2324799) investigated trends in PFAS content of household goods between 2007 and 2011. They reported that while PFOA concentrations displayed an overall downward trend with significant reductions observed in nearly all product categories, PFOA was still detected in many products. Kotthoff et al. (2015, 2850246) similarly reported broad detection of PFOA in a 2010 sampling effort that collected 115 European consumer products, including carpets, leather, outdoor materials, cooking materials, and others. PFOA was detected in all but one sample type, often at the highest median concentration compared to other PFCAAs. FTOHs

were frequently detected at the highest median concentration overall. The products with the highest concentrations of total PFAS included ski wax (median concentration of 15.5 $\mu\text{g/kg}$ PFOA), leather products (median concentration of 12.4 $\mu\text{g/m}^2$), and outdoor materials (median concentration of 6 $\mu\text{g/m}^2$ PFOA). PFOA has also been detected in textile samples of outdoor apparel from Europe and Asia {Gremmel et al., 2016, 3858525; van der Veen et al., 2020, 6316195}. PFOA was detected in jackets ranging from concentrations of 0.02–4.59 $\mu\text{g/m}^2$ {Gremmel, 2016, 3858525}. Interestingly, the level of almost all individual PFAS, including PFOA, and total PFAS increased when the textiles were subjected to weathering (i.e., increased ultraviolet (UV) radiation, temperature, and humidity for 300 hours to mimic the average lifespan of outdoor apparel) {van der Veen, 2020, 6316195}.

5.1.3.4 Indoor Dust

Several studies suggest that PFOA and its precursors in indoor air and/or house dust may be an important exposure source for some individuals {Shoeib, 2011, 1082300; Schlummer, 2013, 2552131; Gebbink, 2015, 2850068; Poonthong, 2020, 6311690}. PFOA is generally a dominant ionic PFAS constituent in indoor air and 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 130 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).

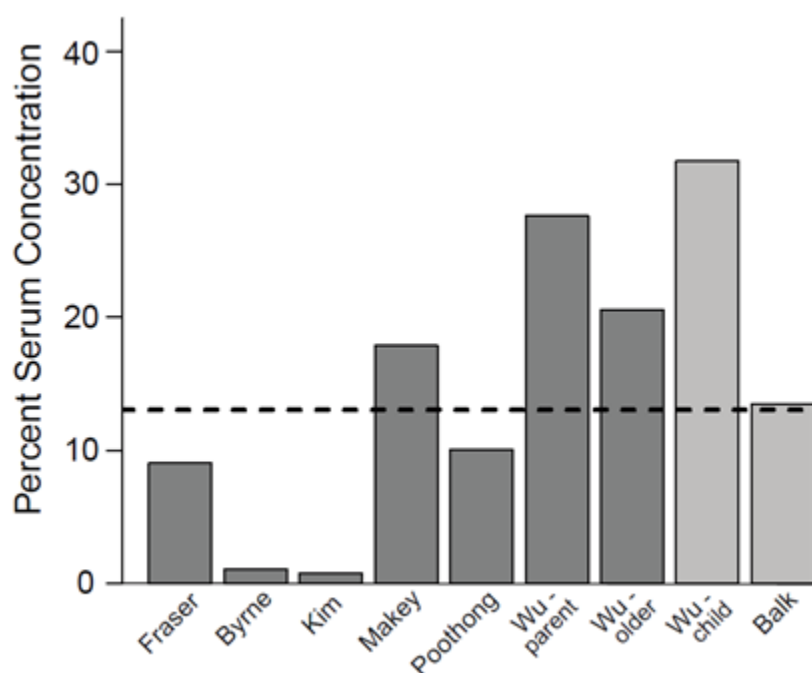


Figure 130. Percent Serum Concentration Attributed to Exposure from House Dust Ingestion and Dermal Contact for PFOA

^aBlack dashed line indicates the weighted mean of all studies, weighted by sample size.

^bDark gray shading indicates adult populations and light gray shading indicates child populations.

PFOA was found at the second highest levels (mean concentration of 1.98 ng/g) of 15 PFAS measured in dust samples taken from households in Seoul, South Korea {Kim, 2019, 5080673}. PFOA 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 only a borderline weak-to-moderate relationship between PFOA concentrations in dust and serum. Similarly, PFOA was measured at the highest concentrations (geometric mean concentrations ranging from 41.4–45.0 ng/g) and frequencies (ranging from 89–91% detected) in dust sampled from Californian households, but concentrations of PFOA in dust were only marginally correlated with serum from older adults and not with serum of parents with young children {Wu, 2014, 2533322}. Makey et al. (2017, 3860102) measured PFOA and PFOA precursors in dust and similarly found weak correlations between concentrations in dust and serum PFOA concentrations in pregnant Canadian participants. One study in Alaska Natives found no correlation between dust and serum PFOA concentrations {Byrne, 2017, 4165183}.

Using data curated from scientific literature published between 2011 and 2017 to estimate aggregate exposure to PFOA, East et al. (2021, 9416543) estimated that dust ingestion contributes 8% of total intake for adults and 46% for children. Gebbink et al. (2015, 2850068) similarly estimated that 2.4–7.6% of direct PFOA 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}. Poothong and colleagues (2020, 6311690) looked closely at data from individual participants and found that while dust contributed to about 4% of the total PFOA intake, 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 there are high detection rates of PFOA in dust, dust concentrations are often not correlated with serum concentrations, indicating that dust is likely only a major source of PFOA for certain individuals or age groups.

5.1.3.5 *Ambient Air*

Perfluoroalkyl chemicals have been found in ambient air globally, with the highest concentrations observed or expected in urban areas and nearest to industrial facilities, areas where AFFF firefighting foams are used, wastewater treatment plants, waste incinerators, and landfills {Ahrens, 2011, 2325317}. Perfluorinated acids were measured in Albany, New York air samples (gas mean concentration of 3.16 pg/m³ and particulate phase mean concentration of 2.03 pg/m³) {Kim, 2007, 1289790}. In Minneapolis, Minnesota, PFOA in the particulate phase ranged from 1.6–5.1 pg/m³ and from 1.7–16.1 pg/m³ in the gas phase (MPCA, 2008, 9419086). Even remote areas far from urban centers have previously reported PFOA concentrations in air samples: PFOA has been detected in Resolute Bay, Nunavut, Canada (Stock, 2007, 1289794), as well as other Arctic environments {Butt, 2010, 1291056}.

PFOA is not listed as a hazardous air pollutant under the Clean Air Act. However, two states (New York and Michigan) have set enforceable air emissions limits. Ambient air is a possible source of exposure to PFOA for the general population; however, the contribution of air to total exposure is likely low. For example, De Silva et al. {2021, 7542691} estimated that <1% of PFOA exposure to humans in the United States is from inhalation.

5.1.3.6 Other Possible Exposure Sources

PFOA 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 increase 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 NNHANES has measured blood serum concentrations of several PFAS in the general U.S. population since 1999. For PFOA and PFOS, these two compounds 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 of PFOA and PFOS 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 HFPO 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.

5.1.4 Recommended RSC

EPA used the Exposure Decision Tree methodology to derive the RSC for this MCLG {U.S. EPA, 2000, 19428}. Findings from studies on populations in the United States, Canada, and Western Europe suggest that diet is the major contributor to total PFOA exposure among adults, 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 PFOA drinking water RSC for the U.S. general population based on an all-women's prospective national cohort, i.e. The Nurses' Health Study. PFOA 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. Using this approach, the RSC for PFOA was

estimated to be 12% with a 95% probability interval of 11–14%. 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 PFOA, they identified dietary ingestion as the major contributor to total intake among adults, and incidental dust ingestion and dietary ingestion as the major contributors for 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 PFOA exposure from drinking water at 4.2 and 1.2 ng/day, or approximately 10 and 9% of total intake, for adults and children, respectively. Estimates of both percent contribution from water and total intake were about half of that reported in an earlier study using similar methods {Lorber, 2011, 2914150}.
- Gebbink et al. (2015, 2850068) estimated the relative contributions of the major exposure media to total direct and indirect PFOA 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 of diet, dust, water, and air to total exposures. Only data for samples collected in North America, Europe, Korea, and Japan were included in the evaluation. The authors point out that both the blood serum concentrations and the temporal trends of PFASs in the United States, Europe, and Japan are similar. The data for direct and indirect contributors to serum PFOA are presented graphically in the published paper. They are consistent with the following exposure patterns for the combination of direct and indirect (precursor) exposures in adults:
 - Low-exposure scenario: diet (~50%) > air (~25%) > dust (~15%) > water (~10%);
 - Intermediate-exposure scenario: diet (~45%) > dust (~35%) > water (~10%) ≈ air (~10%); and
 - High-exposure scenario: dust (~65%) > diet (~20%) > water (10%) > air (~5%).
 - As the environmental level increases, so does the contribution of precursors to total exposure, increasing from about 15% to 30% to 60% as the exposure increases from low to high.

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 often in Europe 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 PFOA and its precursors, it is difficult to assess current relative exposures to the general population.
- Some of the data are several years old and may not accurately reflect current exposures.

Additionally, there is a lack of data on other routes of exposure:

- 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 PFOA in water that becomes incorporated in solid foods during home preparation and cooking or that is present in commercial beverages.
- Transformation of PFOA precursors that decay or are metabolized to PFOA is a route that is rarely evaluated in dietary studies yet can contribute to total exposure. Air and dust can be vehicles for derivatives that metabolically degrade to PFOA.

In summary, based on the physical properties, detected levels, and available exposure information for PFOA, food, indoor dust, and drinking water are potentially significant sources for the general population. Following the Exposure Decision Tree in EPA's 2000 Methodology {U.S. EPA, 2000, 19428}, significant potential sources other than drinking 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 percent (0.20) for PFOA.

6.0 References

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Appendix A. 2019 Literature Search Strategies

A.1 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.

Additionally, the National Toxicology Program (NTP) website was searched for study tables and individual animal data from PFOA and PFOS toxicity studies with reports in preparation that could provide relevant health effects information. Three sets of study tables were identified and included as relevant: 1.) 28-day PFOS study table and individual animal data, 2.) 28-day PFOA study table and individual animal data and 3.) technical report pathology tables and curves for a two-year carcinogenicity study for PFOA. Although peer-reviewed NTP technical reports for the 28-day toxicity and 2-year carcinogenicity studies are not yet available, this information was included in this literature search because these data have undergone standard NTP quality assurance/control processing, peer review and are publicly available.

1 **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="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 "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-	4/10/2019: 2,191 results

Database	Search String	Results
	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 perfluoroo*[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"+"pfoa"+"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 \(2018a\)](#) fit multivariate models of PFOA measured at age five years (after four vaccinations), 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 PFOA 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 PFOS ($p = 0.76$; see [Budtz-Jørgensen and Grandjean \(2018a\)](#) Table 3), or for the model that did adjust for PFOS ($p = 0.69$), but the piecewise model tended to show slightly better fit values due to greater flexibility.

[Budtz-Jørgensen and Grandjean \(2018a\)](#) 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 0.52 and 0.16 ng/mL based on the piecewise linear model (Table 1, [Budtz-Jørgensen and Grandjean \(2018a\)](#)). extended this analysis to control for PFOS concentrations and reported the corresponding BMD_{05} and $BMDL_{05}$ of 0.67 and 0.17 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 et al., 2018](#)). 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 PFOA 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%	0.52	0.16	0.67	0.17^a

^a Value 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 PFOA concentrations in the study population which was reported as having a geometric mean of 4.06 ng/mL with a 27th–75th percentile range of 3.33 ng/mL to 4.96 ng/mL ([Grandjean et al. \(2012\)](#); 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 model is slightly higher than the BMD₅ estimate from the models with just PFOA, but the BMDL₅ estimates are very close. 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 PFOA (Pearson correlation of 0.50; [Grandjean et al. \(2012\)](#); Table 2), can impact the β and the $se(\beta)$ for PFOA 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 (0.16 ng/mL vs. 0.17 ng/mL) and EPA advanced the derivation of the cRfD based on results that control for PFOS because this model fit well overall.

For immunotoxicity related to tetanus, associated with PFOa, the POD is based on a BMR of 5% and a BMDL₅ of 0.17 ng/mL.

B.1.2 Modeling Results for Decreased Diphtheria Antibody Concentrations

[Budtz-Jørgensen and Grandjean \(2018a\)](#) fit multivariate models of PFOA 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 PFOA, 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 PFOA exposure without adjustment for PFOS ($p = 0.86$; see [Budtz-Jørgensen and Grandjean \(2018a\)](#) Table 3), or for the model that did adjust for PFOS and PFOA ($p = 0.92$), but the piecewise model tended to show slightly better fit values due to greater flexibility.

[Budtz-Jørgensen and Grandjean \(2018a\)](#) showed that the $BMD = \log_2(1-BMR)/\beta$ and used a BMR of 5% to estimate the corresponding BMD₅ and BMDL₅ of 0.48 and 0.17 ng/mL, respectively, based on the piecewise linear model (Table 1). [Budtz-Jørgensen and Grandjean \(2018a\)](#) extended this to further control for PFOA concentrations and reported corresponding BMD₅ and BMDL₅ of 1.06 ng/mL and 0.20 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, 9642066}. 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 PFOA 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%	0.48	0.17	1.06	0.20 ^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 PFOA concentrations in the study population which was reported as having a geometric mean of 4.06 ng/mL with a 27th–75th percentile range of 3.33 ng/mL to 4.96 ng/mL ([Grandjean et al. \(2012\)](#); 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 almost 2-fold higher than the BMD₅ estimate from the model with just PFOA, but the BMDL₅ is much closer. Including an additional PFAS in the model that is highly correlated with PFOA (Pearson correlations of 0.50; [Grandjean et al. \(2012\)](#); Table 2), can impact the β and the $se(\beta)$ for PFOA 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 (0.17 ng/mL vs. 0.20 ng/mL) and EPA advanced the derivation of the cRfD based on results that control for PFOS because this model fit well overall and these results provide the best available POD for PFOA and diphtheria.

For immunotoxicity related to diphtheria, associated with PFOA, the POD is based on a BMR of 5% and a BMDL₅ of 0.20 ng/mL.

B.1.3 Modeling Results for Decreased Birthweight

Five high confidence studies [Chu et al. (2020) {6315711}, Govarts et al. (2016) {3230364}, Sagiv et al. (2018) {4238410}, Starling et al. (2017) {3858473}, and Wikström et al. (2020) {6311677}] reported decreased birth weight in infants whose mothers were exposed to PFOA. These candidate studies offer a variety of PFOA 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. The samples in the Govarts et al. (2016) {3230364} study were collected from umbilical cords.

All five 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 on 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/natality.html>) provides vital statistics for babies born in the United States. There were 3,791,712 all live births in the United States in 2018 according to final natality data. The mean and standard deviation 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., 2,500 g, or 5.5 lb.). The full natality data for the United States data on birth weight was used as it is more relevant for deriving toxicity values for the US general public than the study-specific birth weight 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. America's Children and the Environment (ACE) Biomonitoring on Perfluorochemicals report (<https://www.epa.gov/americanchildrenenvironment/ace-biomonitoring-perfluorochemicals-pfcs#B6>) provides the median blood serum levels of PFOA of 1.0 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 five available epidemiological studies.

Chu et al. (2020) {6315711} reported a β coefficient of -73.6 g (95% CI: $-133.2, 33.4$) per $\ln(\text{ng/mL})$ increase for the association between birth weight and maternal PFOA 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 (1.5 ng/mL) and 25th–75th percentile range (1.0–2.6 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:

$$\mu = \ln(q_{50}) = \ln(1.5) = 0.43 \quad (1)$$

$$\sigma = \ln(q_{75}/q_{25})/1.349 = \ln(2.6/1.0)/1.349 = 0.75 \quad (2)$$

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 -45.2 g (95% CI: $-77.6, -12.8$) 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 et al., 1995 {8632216}; Reyes and Manalich, 2005 {1065677}; Tian et al., 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 approach¹². 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 3169.2 g. Given the median exposure of 1.0 ng/mL from ACE Biomonitoring on Perfluorochemicals report as \bar{x} , the mean birth weight in the US as \bar{y} and the re-expressed β as m term, the intercept b can be estimated as:

$$b = \bar{y} - m\bar{x} = 3261.6 \text{ g} - \left(-45.2 \text{ g}\left(\frac{\text{ng}}{\text{mL}}\right)^{-1}\right) 1.0 \frac{\text{ng}}{\text{mL}} = 3306.8 \text{ g} \quad (3)$$

The BMD was calculated by rearranging the equation $y = mx + b$ and solving for x , using 3306.8 g for the b term and -45.2 for the m term. Doing so results in a value of 3.0 ng/mL:

$$x = (y - b)/m = (3169.2 \text{ g} - 3306.8 \text{ g})/(-45.2 \text{ g}\left(\frac{\text{ng}}{\text{mL}}\right)^{-1}) = 3.0 \text{ ng/mL}$$

To calculate the BMDL, the method is essentially the same except that the lower limit (LL) on the β coefficient (-77.6) is used for the m term. However, Chu et al. (2020) {6315711} reported a two-sided 95% CI 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:

¹² While the explicit application of the hybrid approach is not commonly used in IRIS dose/concentration/exposure-response analyses, the more commonly used SD-definition of the BMR for continuous data is simply one specific application of the hybrid approach. The SD-definition of the BMR assumes that the cut-off for adversity is the 1.4th percentile of a normally distributed response and that shifting the mean of that distribution by one standard deviation approximates an extra risk of 10%.

$$SE = \frac{Upper\ Limit - Lower\ Limit}{3.92} = \frac{-12.8\ g(\frac{ng}{mL})^{-1} - (-77.6\ g(\frac{ng}{mL})^{-1})}{3.92} = 16.5\ g(\frac{ng}{mL})^{-1}$$

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

$$\begin{aligned} 95\% \text{ one-sided } LL &= \beta - 1.645(SE(\beta)) = -45.2\ g(\frac{ng}{mL})^{-1} - 1.645\left(16.5\ g(\frac{ng}{mL})^{-1}\right) \\ &= -72.4\ g(\frac{ng}{mL})^{-1} \end{aligned}$$

Using this value for the m term results in a BMDL value of 1.9 ng/mL maternal serum concentration.

Govarts et al. (2016) {3230364} reported a β coefficient of $-34.5\ g$ (95% CI: $-129.0, 60.0$) per IQR increase in Z-score of $\ln(\text{ng/mL})$ PFOA exposures, corresponding to a β coefficient of $-53.4\ g$ (95% CI: $-199.5, 92.8$) per $\ln(\text{ng/mL})$ increase, for the association between birth weight and PFOA concentrations in umbilical cord plasma samples in a United States cohort. Given the reported study-specific median ($1.5\ \text{ng/mL}$) and 25th–75th percentile range (1.1 – $2.1\ \text{ng/mL}$) of the exposure, EPA estimated the mean (0.42) and standard deviation (0.48) of the log normally distributed exposure. The re-expressed β coefficient is $-20.7\ g$ (95% CI: $-77.5, 36.1$) per ng/mL , and the intercept b is $3282.4\ g$. A BMD of $5.5\ \text{ng/mL}$ is calculated from Govarts et al. (2016) {3230364} 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 re-expressed β coefficient from the re-expressed lower limit on the 95% two-sided CI of $-77.5\ g$ per ng/mL . Using the lower limit value ($-68.4\ g$ per ng/mL), a BMDL of $1.7\ \text{ng/mL}$ is calculated.

Sagiv et al. (2018) {4238410} reported a β coefficient of $-18.5\ g$ (95% CI: $-45.4, 8.3$) per IQR increase in PFOA (ng/mL), corresponding to a β coefficient of $-4.9\ g$ (95% CI: $-11.9, 2.2$) per ng/mL increase, for the association between birth weight and maternal PFOA serum concentrations in a United States cohort. The intercept b is $3,266.5\ g$ based on the β coefficient of $-4.9\ g$ per ng/mL and the corresponding 95% one-sided lower limit for the β coefficient is $-10.8\ g$ per ng/mL . A BMD of $20.0\ \text{ng/mL}$ and a BMDL of $9.0\ \text{ng/mL}$ are calculated using the same approach as above.

Starling et al. (2017) {3858473} reported a β coefficient of $-51.4\ g$ (95% CI: $-97.2, -5.7$) per $\ln(\text{ng/mL})$ for the association between birth weight and maternal PFOA serum concentrations in a United States cohort. Given the reported study-specific median ($1.1\ \text{ng/mL}$) and 25th–75th percentile range (0.7 – $1.6\ \text{ng/mL}$) of the exposure, EPA estimated the mean (0.10) and standard deviation (0.61) of the log-normally distributed exposure. The re-expressed β coefficient is $-45.0\ g$ (95% CI: $-85.1, -5.0$) per ng/mL and the intercept b is $3306.6\ g$. The 95% one-sided lower limit for the re-expressed β coefficient is $-78.6\ g$ per ng/mL . The values of the BMD and BMDL are $3.1\ \text{ng/mL}$ and $1.8\ \text{ng/mL}$, respectively.

Wikström et al. (2020) {6311677} reported a β coefficient of $-68.0\ g$ (95% CI: $-112.0, -24.0$) per $\ln(\text{ng/mL})$ for the association between birth weight and maternal PFOA serum concentrations

in a Swedish cohort. Given the reported study-specific median (1.6 ng/mL) and 25th–75th percentile ranges (1.1–2.3 ng/mL) of the exposure, EPA estimated the mean (0.48) and standard deviation (0.54) of the log normally distributed exposure. The re-expressed β coefficient is –41.0 g (95% CI: –67.5, –14.5) per ng/mL and the intercept b is 3,302.6 g. The 95% one-sided lower limit for the re-expressed β coefficient is –63.3 g per ng/mL. The values of the BMD and BMDL are 3.3 ng/mL and 2.1 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 PFOA exposure in the US population (i.e., 8.27% is not the tail probability of 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:

$$P(0) = \frac{1}{\sigma_c \sqrt{2\pi}} \int_{-\infty}^{2,500} e^{-\frac{(x-b)^2}{2\sigma_c^2}} dx = \frac{1}{590.7 \sqrt{2\pi}} \int_{-\infty}^{2,500} e^{-\frac{(x-b)^2}{2 \cdot 590.7^2}} dx$$

$$b = \bar{y} - m\bar{x} = 3261.6 - (\beta_{re-expressed} * 1.0 \frac{ng}{mL})$$

In this alternative approach, $P(0)$ is 9.86% if there is no background exposure ($\bar{x} = 0$). By using the median of serum PFNA concentrations (1.0 ng/mL) from ACE Biomonitoring on Perfluorochemicals report as background exposure (\bar{x}), the tail probability using this alternative approach was study-specific and ranged from 8.60%–9.72%. 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 1.7 ng/mL to 12.5 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 PFOA, the individual study POD selected from the available epidemiologic literature is 1.7 ng/mL PFOA concentration in maternal serum or umbilical cord plasma samples.

Table B-3. BMDs and BMDLs for Effect of PFOA on Decreased Birth Weight using Percentage (8.27%) of Live Births Falling Below the Public Health Definition of Low Birth Weight or Alternative Study-specific Tail Probability

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%)		Alternative Tail Probability ^b		
									BMD (ng/mL)	BMDL (ng/mL)	P(0)	BMD (ng/mL)	BMDL (ng/mL)
Chu et al. (2020)	3 rd	1.5 (1.0–2.6)	(0.43, 0.75)	-73.6 (-126.4, -20.9)	-45.2 (-77.6, -12.8)	3306.8	16.5	-72.4	3.0	1.9	8.60%	3.2	2.0
Govarts et al. (2016)	B	1.5 (1.1–2.1)	(0.42, 0.48)	-34.5 (-129.0, 60.0)	-20.7 (-77.5, 36.1)	3282.4	29.0	-68.4	5.5	1.7*	9.27%	6.7	2.0
Sagiv et al. (2018)	1 st	5.8 (4.1–7.9)	(1.76, 0.49)	-18.5 (-45.4, 8.3)	-4.9 (-11.9, 2.2)	3266.5	3.6	-10.8	20.0	9.0	9.72%	27.6	12.5
Starling et al. (2017)	2 nd –3 rd	1.1 (0.7–1.6)	(0.1, 0.61)	-51.4 (-97.2, -5.7)	-45.0 (-85.1, -5.0)	3306.6	20.4	-78.6	3.1	1.8	8.60%	3.3	1.9
Wikström et al. (2020)	1 st –2 nd	1.6 (1.1–2.3)	(0.48, 0.54)	-68.0 (-112.0, -24.0)	-41.0 (-67.5, -14.5)	3302.6	13.5	-63.3	3.3	2.1	8.71%	3.5	2.3

*Smallest BMDL using the five 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) and cord blood samples collected at birth (B).^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 US population.

ACE Biomonitoring on Perfluorochemicals report also provides the median blood serum levels of PFOA of 5 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 PFOA concentrations (1.78 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 Govarts et al. (2016) {3230364}, 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 PFOA on Decreased Birth Weight by Background Exposure using Percentage (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)
Govarts et al. (2016)	1.00	3282.4	5.5	1.7	9.27%	6.7	2.0
	1.78	3298.5	6.2	1.9	8.82%	6.9	2.1
	5.00	3365.3	9.5	2.9	7.15%	8.0	2.4

^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 PFOA 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 PFOA 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 10.5 and 5.6 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 PFOA 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.1.5 Modeling Results for Cancers

This updated review indicated that there is an increase in risk for kidney or renal cell carcinoma (RCC) and testicular cancers and PFOA exposure (Shearer, 2021, 7161466; Chang, 2014, 2850282; Bartell and Vieira, 2021, 7643457). Although newer studies generally show no

association, there is some evidence that PFOA may be related to breast cancer risk especially in participants with specific polymorphisms or specific types of tumors {Ghisari, 2017, 3860243; Mancini, 2019, 5381529}. Two occupational studies {Steenland, 2015, 2851015; Girardi, 2019, 6315730} support an increase in risk for liver cancer, malignant neoplasm of the lymphatic and hematopoietic tissue, as well as an increasing trend in prostate cancer that did not reach statistical significance. No associations were found for colorectal cancer in either the general population or occupational studies, or for lung cancer in occupational studies.

Results are most consistent for kidney cancer in adults based on two C8 Health Project studies {Barry, 2013, 2850946; Vieira, 2013, 2919154} and an occupational mortality study {Steenland and Woskie, 2012, 2919168} from the 2016 HA and a new nested case-control study {Shearer, 2021, 7161466}.

For dose-response modelling, {Shearer 2021, 7161466} was selected as the key study. Considerations included study population (general population versus occupational or high-exposed populations), statistical power and study quality.

Shearer et al. (2021, 7161466) is a multi-center case-control study nested within the National Cancer Institute's (NCI) Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO). The PLCO is a randomized clinical trial of the use of serum biomarkers for cancer screening. The cases in this study {Shearer 2021, 7161466} included all the participants of the screening arm of the PLCO trial who were newly diagnosed with RCC during the follow-up period (N = 326). All cases were histopathologically confirmed. Controls were selected from among participants of the PLCO trial screening arm who had never had RCC. Controls were individually matched to the RCC cases by age at enrollment, sex, race/ethnicity, study center, and year of blood draw. PFOA concentrations were measured in the baseline serum samples collected between 1993 and 2002. Median PFOA levels in controls was 5.0 ng/mL, comparable with 4.8 ng/mL in adults 60 and over from NHHANES 1999-2000. The analyses accounted for numerous confounders including BMI, smoking, history of hypertension, estimated glomerular filtration rate, previous freeze-thaw cycle, calendar and study year of blood draw, sex, race and ethnicity, study center. Socio-economic status was not explicitly considered in the analyses.

There was a statistically significant increase in odds of RCC per doubling of PFOA (OR = 1.71, 95% CI: 1.23, 2.37) and in the highest versus slowest quartile (OR = 2.63, 95% CI: 1.33, 5.2). Although non-significant elevated risks were observed in the second and third quartiles, there was a statistically significant increasing trend with increasing PFOA exposure across quartiles (p-trend = 0.007). Statistically significant increased odds of RCC were observed in participants ages 55–59 years, and in men and in women, separately (Table C-26).

B.1.5.1 Cancer Slope Factor (CSF) Calculations

The methods used to calculate CSFs based on data from {Shearer, 2021, 7161466} is based on that used by US EPA for its CSF calculation for TCE {U.S. EPA, 2011, 9642147} and arsenic (need reference).

The underlying model involves a linear regression between PFOA exposure and cancer relative risk used to estimate the dose-response between PFOA and RCC risk. This was calculated using a weighted linear regression of the quartile specific RRs, with the weights defined as the inverse of the variance of each RR. Since the incidence of kidney cancer is relatively low and because

the cases and controls were matched on age, the ORs represent a good approximation of the underlying RRs.

The CSF is then calculated as the excess cancer risk associated with each ng/ml increase in serum PFOA (CSF_{serum}). The CSF_{serum} was calculated by first converting the linear regression model discussed above from the RR scale to the absolute risk scale. This was done assuming a baseline risk (R_0) of RCC or kidney cancer in an unexposed or lower exposure reference group. Since this is not available in a case-control study, the lifetime risk of RCC in US males is used. The lifetime RCC risk was estimated by multiplying the lifetime risk of kidney cancer in US males {American Cancer Society, 2020, 9642148} by the percentage of all kidney cancers that are the RCC subtype (90%). This gives an R_0 of $0.0202 \times 90\% = 0.0182$. The CSF_{serum} was then calculated as the product of the upper 95% CL of the dose-response slope and R_0 . The estimated CSF_{serum} is $0.00178 \text{ (ng/kg-day)}^{-1}$.

B.2 Toxicology Studies

B.2.1 Blake, 2020, 6305864

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Blake (2020, 6305864) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses placental lesions at GD17.5 in P_0 female CD-1 mice.

B.2.1.1 Placental Lesions at Gestation Day 17.5

Increased incidence of placental lesions at GD17.5 was observed in P_0 female CD-1 mice. Dichotomous models were used to fit dose-response data. Benchmark responses (BMR) of 5% and 10% extra risk were chosen. The doses and response data used for the modeling are listed in Table B-5. The AUC normalized per day during gestation ($AUC_{\text{avg,dam,gst}}$) and maximum maternal concentration during gestation ($C_{\text{max,dam}}$) were both considered and shown below because placental lesions 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.

Table B-5. Dose-Response Modeling Data for Placental Lesions at GD17.5 in P_0 Female CD-1 Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose		Number per Group	Incidence
	$AUC_{\text{avg,dam,gst}}$ (mg/L)	$C_{\text{max,dam}}$ (mg/L)		
0	0	0	41	1
1	26.7	45.1	32	3
5	75.6	128.9	40	27

The benchmark dose (BMD) modeling results for placental lesions at GD17.5 for $AUC_{\text{avg,dam,gst}}$ are summarized in Table B-6 and Figure B-1. The best fitting model was the Logistic 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 Logistic 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% change ($BMDL_{10}$) from the selected Logistic model for $AUC_{avg,dam,gest}$ is 22.7 mg/L.

Table B-6. Summary of Benchmark Dose Modeling Results for Placental Lesions at GD17.5 for AUC_{avg,dam,gest} in P₀ Female CD-1 Mice Following Exposure to PFOA

Model ^a	Goodness of Fit		Scaled Residual			BMD ₅ (mg/L)	BMDL ₅ (mg/L)	BMD ₁₀ (mg/L)	BMDL ₁₀ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD ₅	Dose Group near BMD ₁₀	Control Dose Group					
Dichotomous Hill	– ^b	87.8	4.0×e ^{–5}	4.0×e ^{–5}	1.2×e ^{–4}	23.9	13.1	29.8	19.0	EPA selected the Logistic model. The Multistage Degree 2, Logistic and Probit 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	– ^c	85.8	–4.9×e ^{–4}	–4.9×e ^{–4}	1.6×e ^{–4}	23.8	12.3	30.0	18.4	
Log-Logistic	– ^c	85.8	–1.9×e ^{–8}	–1.9×e ^{–8}	–4.9×e ^{–8}	23.7	13.1	30.1	19.0	
Multistage Degree 2	0.410	84.5	–0.7	–0.7	0.2	16.8	8.2	24.1	15.1	
Multistage Degree 1	0.009	91.7	0.3	0.3	0.3	4.7	3.5	9.6	7.1	
Weibull	– ^c	85.8	1.4×e ^{–6}	1.4×e ^{–6}	–3.7×e ^{–6}	23.2	11.3	30.6	17.8	
Logistic	0.874	83.8	–0.1	–0.1	0.1	20.5	13.8	30.2	22.7	
Log-Probit	– ^c	85.8	4.5×e ^{–7}	4.5×e ^{–7}	1.2×e ^{–6}	24.2	14.7	29.6	19.8	
Probit	0.620	84.0	–0.3	–0.3	0.4	18.1	12.3	27.2	20.5	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD₅ = dose level corresponding to a 5% change; BMDL₅ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change; BMD₁₀ = dose level corresponding to a 10% change; BMDL₁₀ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% change.

^aSelected model in bold.

^bDegrees of freedom are negative (Goodness of fit test cannot be calculated).

^cDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

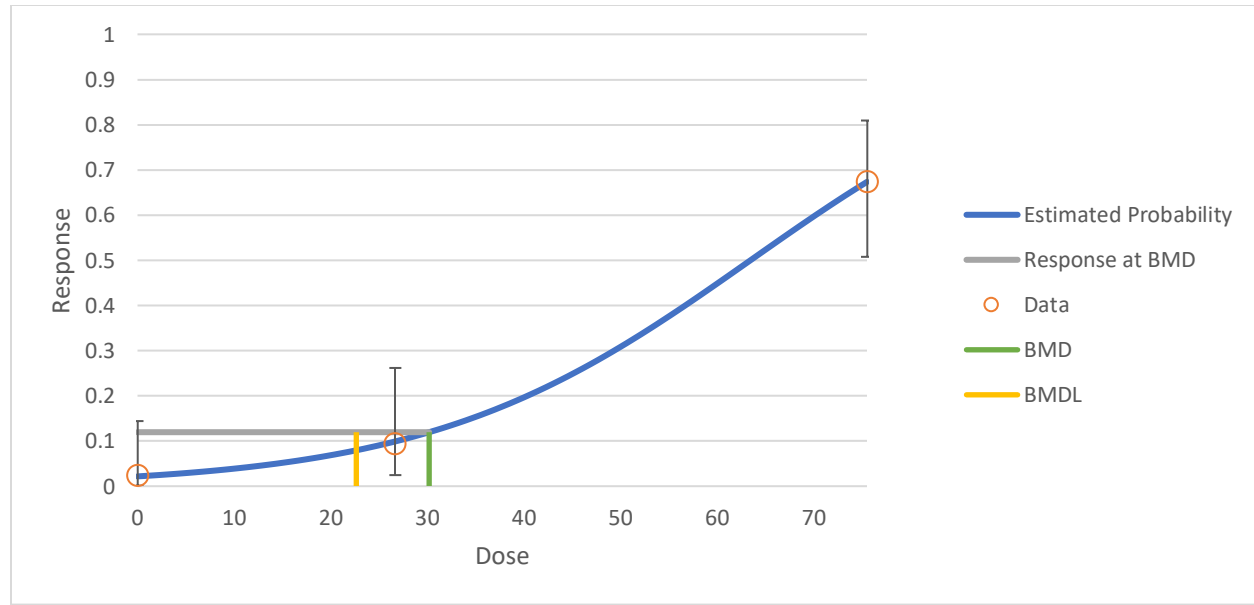


Figure B-1. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Logistic Model for Placental Lesions at GD17.5 for $AUC_{avg,dam,gest}$ in P₀ Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The benchmark dose (BMD) modeling results for placental lesions at GD17.5 for $C_{max,dam}$ are summarized in Table B-7 and Figure B-2. The best fitting model was the Logistic 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 Logistic 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% change ($BMDL_{10}$) from the selected Logistic model for $C_{max,dam}$ is 38.5 mg/L.

Table B-7. Summary of Benchmark Dose Modeling Results for Placental Lesions at GD17.5 for C_{max,dam} in P₀ Female CD-1 Mice Following Exposure to PFOA

Model ^a	Goodness of Fit		Scaled Residual			BMD ₅ (mg/L)	BMDL ₅ (mg/L)	BMD ₁₀ (mg/L)	BMDL ₁₀ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD ₅	Dose Group near BMD ₁₀	Control Dose Group					
Dichotomous Hill	– ^b	87.8	2.3×e ^{–5}	2.3×e ^{–5}	1.5×e ^{–5}	40.6	22.0	50.2	32.0	EPA selected the Logistic model. The Multistage Degree 2, Logistic, and Probit model 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	– ^c	85.8	8.6×e ^{–8}	8.6×e ^{–8}	–1.2×e ^{–7}	40.2	20.7	50.8	31.0	
Log-Logistic	– ^c	85.8	–7.7×e ^{–5}	–7.7×e ^{–5}	6.4×e ^{–4}	40.0	22.0	50.9	32.0	
Multistage Degree 2	0.426	84.4	–0.7	–0.7	0.2	28.6	13.9	41.0	25.5	
Multistage Degree 1	0.010	91.6	0.3	0.3	0.3	8.0	5.9	16.4	12.1	
Weibull	– ^c	85.8	5.0×e ^{–6}	5.0×e ^{–6}	–9.5×e ^{–6}	39.2	18.9	51.8	29.9	
Logistic	0.888	83.8	–0.1	–0.1	0.1	34.9	23.5	51.4	38.5	
Log-Probit	– ^c	85.8	5.5×e ^{–6}	5.5×e ^{–6}	–1.5×e ^{–6}	40.9	24.7	50.0	33.3	
Probit	0.635	84.0	–0.3	–0.3	0.3	30.8	20.9	46.2	34.9	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD₅ = dose level corresponding to a 5% change; BMDL₅ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change; BMD₁₀ = dose level corresponding to a 10% change; BMDL₁₀ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% change.

^aSelected model in bold.

^bDegrees of freedom are negative (Goodness of fit test cannot be calculated).

^cDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

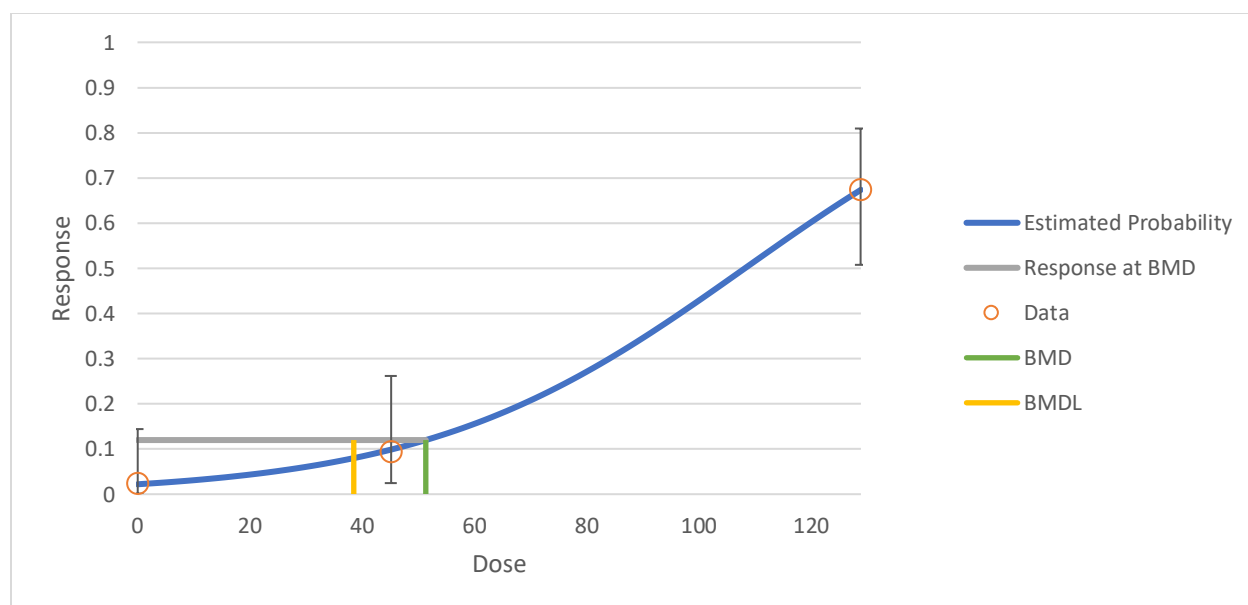


Figure B-2. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Logistic Model for Placental Lesions at GD17.5 for C_{max,dam} in P₀ Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.2 Butenhoff, 2004, 1291063

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Butenhoff (2004, 1291063) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses absolute body weight in P₀ male Sprague-Dawley rats and absolute body weight in F₁ male Sprague-Dawley rats.

B.2.2.1 Absolute Body Weight in P₀ Male Sprague-Dawley Rats

Decreased mean response of absolute body weight was observed in P₀ 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-8. The average concentration over 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 resulting in decreased body weight.

Table B-8. Dose-Response Modeling Data for Absolute Body Weight in P₀ Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	30	581 ± 40

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
1	61.7	30	575 ± 48
3	137.2	30	542 ± 47
10	220.3	30	513 ± 54
30	230.3	29	432 ± 64

^aData are presented as mean ± standard deviation.

The benchmark dose (BMD) modeling results for absolute body weight are summarized in Table B-9. No models provided an adequate fit, therefore a NOAEL approach was taken for this endpoint.

Table B-9. Summary of Benchmark Dose Modeling Results for Absolute Body Weight in P₀ Male Sprague-Dawley Rats Following Exposure to PFOA (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	1635.1	1.786	-1.5	104.8	86.3	No models had adequate fit (p-values greater than 0.1)
Exponential 3	<0.0001	1624.2	3.616	0.5	182.2	143.9	
Exponential 4	<0.0001	1635.1	1.786	-1.5	104.8	86.3	
Exponential 5	0.010	1607.5	0.928	1.4	217.9	215.8	
Hill	0.005	1608.8	0.611	1.5	218.6	215.3	
Polynomial Degree 4	<0.0001	1620.5	3.570	0.1	178.9	144.1	
Polynomial Degree 3	<0.0001	1623.6	-0.812	0.1	174.2	140.5	
Polynomial Degree 2	<0.0001	1623.3	-0.014	-0.3	157.8	133.3	
Power	0.006	1608.3	0.004	1.6	220.1	215.7	
Linear	<0.0001	1633.2	1.627	-1.5	108.8	91.4	

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.2.2.2 Absolute Body Weight in F₁ Male Sprague Dawley Rats

Decreased mean response of absolute body weight was observed in F₁ 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-10. The AUC normalized per day over entire study (AUC_{avg,pup,total}) 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 body weight.

Table B-10. Dose-Response Modeling Data for Absolute Body Weight in F1 Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	30	560 ± 60
1	29.8	29	527 ± 55
3	59.5	30	524 ± 48
10	98.8	30	499 ± 64
30	129.6	29	438 ± 42

^aData are presented as mean ± standard deviation.

The BMD modeling results for absolute body weight are summarized in Table B-11 and Figure B-3. The best fitting model was the Polynomial Degree 4 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Polynomial Degree 4 model had the lowest AIC. The BMDL_{1SD} from the selected Polynomial Degree 4 model is 70.5 mg/L.

Table B-11. Summary of Benchmark Dose Modeling Results for Absolute Body Weight in F1 Male Sprague-Dawley Rats Following Exposure to PFOA (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	0.022	1613.8	1.2	-0.3	64.5	51.9	EPA selected the Polynomial Degree 4 model. The Polynomial Degree 3 and 4 had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 4 model had the lowest AIC.
Exponential 3	0.055	1612.0	0.9	1.5	98.4	67.2	
Exponential 4	0.022	1613.8	1.2	-0.3	64.5	51.9	
Exponential 5	0.055	1612.0	0.9	1.5	98.4	67.2	
Hill	0.018	1613.7	1.0	1.4	96.9	66.7	
Polynomial Degree 4	0.410	1607.0	0.7	0.8	93.4	70.5	
Polynomial Degree 3	0.148	1610.0	1.0	0.8	91.4	69.0	
Polynomial Degree 2	0.071	1611.5	1.4	0.8	87.7	65.2	
Power	0.062	1611.7	0.9	1.4	97.2	66.2	
Linear	0.033	1612.9	1.1	-0.2	66.7	54.9	

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.

^aSelected model in bold.

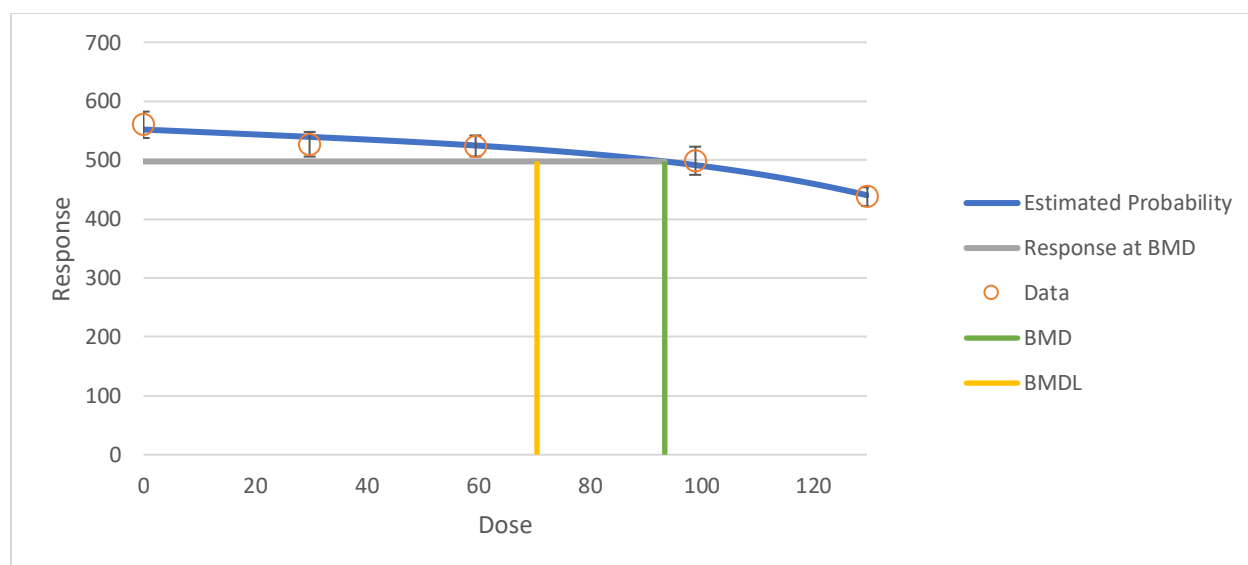


Figure B-3. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 4 Model for Absolute Body Weight in F1 Male Sprague-Dawley Rats Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.3 Butenhoff, 2012, 2919192

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Butenhoff (2012, 2919192) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses Leydig cell adenomas in the testes in male Sprague-Dawley Crl:COBS@CD(SD)BR rats.

B.2.3.1 Leydig Cell Adenomas in the Testes

Increased incidence of Leydig cell adenomas in the testes was observed in male Sprague-Dawley Crl:COBS@CD(SD)BR rats. Dichotomous models were used to fit dose-response data. Benchmark responses (BMR) of 4% and 10% change in the response were chosen. The 4% change was chosen because it is the low end of the observed response within the study and the 10% change was chosen because it is the recommended standard reporting level for comparison across chemicals per EPA's *Benchmark Dose Technical Guidance* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-12. The AUC for duration of the study (AUC) was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of Leydig cell adenomas.

Table B-12. Dose-Response Modeling Data for Leydig Cell Adenomas in the Testes in Male Sprague-Dawley Crl:COBS@CD(SD)BR Rats Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L/day)	Number per Group	Incidence
0	0	50	0

Administered Dose (mg/kg/day)	Internal Dose (mg/L/day)	Number per Group	Incidence
1.3	27,794.7	50	2
14.2	122,076.3	50	7

The benchmark dose (BMD) modeling results for Leydig cell adenomas in the testes are summarized in Table B-13 and Figure B-4. The best fitting model was the Multistage Degree 1 model based on adequate p-values (greater than 0.1), the benchmark dose lower limits (BMDLs) were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 1 model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a 4% change in the response (BMDL₄) from the selected Multistage Degree 1 model is 49,744.5 mg/L/day.

Table B-13. Summary of Benchmark Dose Modeling Results for Leydig Cell Adenomas in the Testes in Male Sprague-Dawley Crl:COBS@CD(SD)BR Rats Following Exposure to PFOA

Model ^a	Goodness of Fit		Scaled Residual			BMD ₄ (mg/L/day)	BMDL ₄ (mg/L/day)	BMD ₁₀ (mg/L/day)	BMDL ₁₀ (mg/L/day)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD ₄	Dose Group near BMD ₁₀	Control Dose Group					
Multistage Degree 2	0.977	59.3	0.2	-0.1	-8.7×e ⁻⁴	31,873.8	19,275.2	82,265.4	49,745.1	EPA selected the Multistage Degree 1 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 1 model had the lowest AIC.
Multistage Degree 1	0.977	59.3	0.2	-0.1	-8.7×e⁻⁴	31,873.8	19,276.1	82,265.4	49,744.5	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD₄ = dose level corresponding to a 4% change in the response; BMDL₄ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 4% change in the response; BMD₁₀ = dose level corresponding to a 10% change in the response; BMDL₁₀ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% change in the response.

^aSelected model in bold.

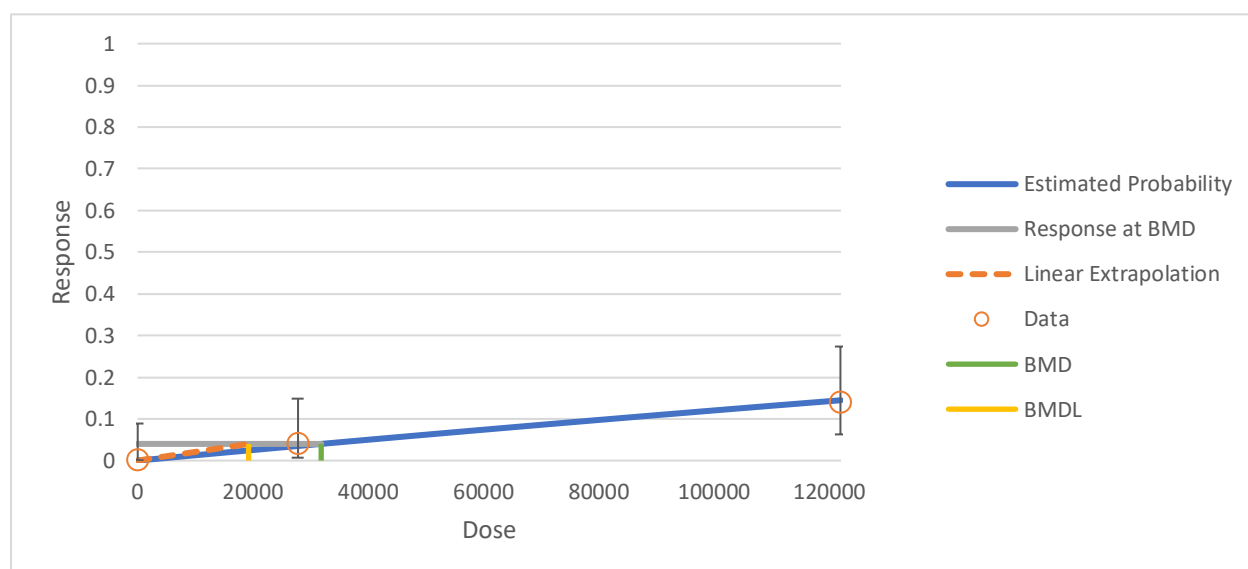


Figure B-4. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Leydig Cell Adenomas in the Testes in Male Sprague-Dawley Crl:COBS@CD(SD)BR Rats Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.4 Chen, 2017, 3981369

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Chen (2017, 3981369) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses number of corpora lutea in P₀ female Kunming mice observed at GD13.

B.2.4.1 Number of Corpora Lutea

Decreased mean response of number of corpora lutea was observed in P₀ female Kunming mice at GD13. Continuous models were used to fit dose-response data. Benchmark responses (BMR) of a change in the mean equal to 0.5 and 1 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table B-14. The AUC normalized per day during gestation (AUC_{avg,dam,gest}) was selected for this model rather than alternate metrics such as C_{max} because the AUC is expected to better correlate with an effect leading to decrease number of corpora lutea.

Table B-14. Dose-Response Modeling Data for Number of Corpora Lutea in P₀ Female Kunming Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (number of corpora lutea) ^a
0	0	6	7.4 ± 2.7 ^b
2.5	62.1	6	5.5 ± 1.2
5	83.3	6	4.5 ± 1.2
10	101.0	6	3.3 ± 1.7

^aData are presented as mean \pm standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for number of corpora lutea are summarized in Table B-15 and Figure A. The best fitting model was the Linear 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 Linear 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 Linear model is 29.9 mg/L.

Table B-15. Summary of Benchmark Dose Modeling Results for Number of Corpora Lutea in P₀ Female Kunming Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	BMD _{1SD} (mg/L)	BMDL _{1SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.5SD}	Dose Group near BMD _{1SD}	Control Dose Group					
Exponential 2	0.522	99.7	−0.21	0.73	−0.21	18.0	11.2	38.5	23.7	EPA selected the Linear model. All models, except Hill, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Linear model had the lowest AIC.
Exponential 3	0.816	100.4	−0.11	−0.11	0.01	41.2	12.6	59.1	26.5	
Exponential 4	0.522	99.7	−0.21	0.73	−0.21	18.0	11.2	38.5	23.7	
Exponential 5	0.816	100.4	−0.11	−0.11	0.01	41.2	12.6	59.1	26.6	
Hill	— ^b	102.4	−0.07	−0.07	0.01	37.9	9.8	58.1	23.4	
Polynomial Degree 3	0.952	100.4	0.03	0.03	0.00	32.3	15.5	56.8	31.1	
Polynomial Degree 2	0.905	100.4	−0.06	−0.06	0.01	36.1	15.5	57.7	31.0	
Power	0.887	100.4	−0.07	−0.07	0.01	37.9	15.5	58.2	31.0	
Linear	0.730	99.0	−0.24	0.50	−0.24	21.7	14.9	43.4	29.9	

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; 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).

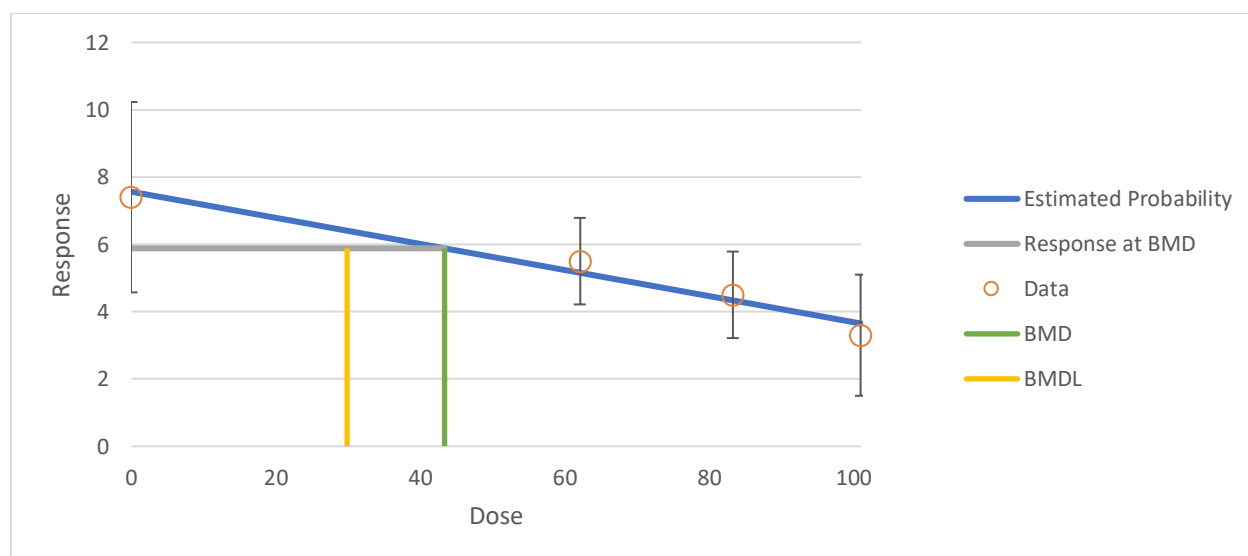


Figure B-5. Plot of Mean Response by Dose with Fitted Curve for the Selected Linear Model for Number of Corpora Lutea in P₀ Female Kunming Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.5 Dewitt, 2008, 1290826

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Dewitt (2008, 1290826) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses serum sheep red blood cells (SRBC)-specific IgM antibody titers in female C57BL/6N mice (Study I) and serum sheep red blood cells (SRBC)-specific IgM antibody titers in female C57BL/6N mice (Study II).

B.2.5.1 Serum Sheep Red Blood Cells-specific IgM antibody titers in Female C57BL/6N Mice (Study I)

Decreased mean response of SRBC-specific IgM antibody titers was observed in female C57BL/6N mice (Study I). 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-16. The $C_{7,avg}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of effects leading to decreased response of SRBC-specific IgM antibody titers.

Table B-16. Dose-Response Modeling Data for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study I) Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (log ₂ to reach 0.5 OD) ^a
0	0	8	8.0 ± 0.3 ^b
3.75	113.4	8	7.1 ± 0.6
7.5	180.9	8	6.8 ± 0.3
15	209.6	8	6.1 ± 0.8
30	242.8	8	5.6 ± 0.8

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for serum SRBC-specific IgM antibody titers are summarized in Table B-17 and Figure B-6. The best fitting model was the Polynomial Degree 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 Polynomial Degree 4 model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to one standard deviation from the control mean (BMDL_{1SD}) from the selected Polynomial Degree 4 model is 26.7 mg/L.

Table B-17. Summary of Benchmark Dose Modeling Results for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study I) Following Exposure to PFOA (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	0.0602	74.0	-0.2	-0.2	29.4	20.5	EPA selected the Polynomial Degree 4 model. Polynomial Degree 3 and Polynomial Degree 4 had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 4 model had the lowest AIC.
Exponential 3	0.0537	74.5	0.01	0.01	53.5	23.0	
Exponential 4	0.0603	74.0	-0.2	-0.2	29.3	20.5	
Exponential 5	0.0155	76.5	0.01	0.01	53.7	23.0	
Hill	0.0178	76.3	0.003	0.003	51.2	25.2	
Polynomial Degree 4	0.208	71.2	0.08	0.08	44.8	26.7	
Polynomial Degree 3	0.179	71.6	0.06	0.06	46.5	26.0	
Polynomial Degree 2	0.0703	74.0	0.03	0.03	48.3	25.1	
Power	0.0610	74.2	0.01	0.01	51.5	24.6	
Linear	0.0901	73.1	-0.2	-0.2	32.8	23.2	

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.

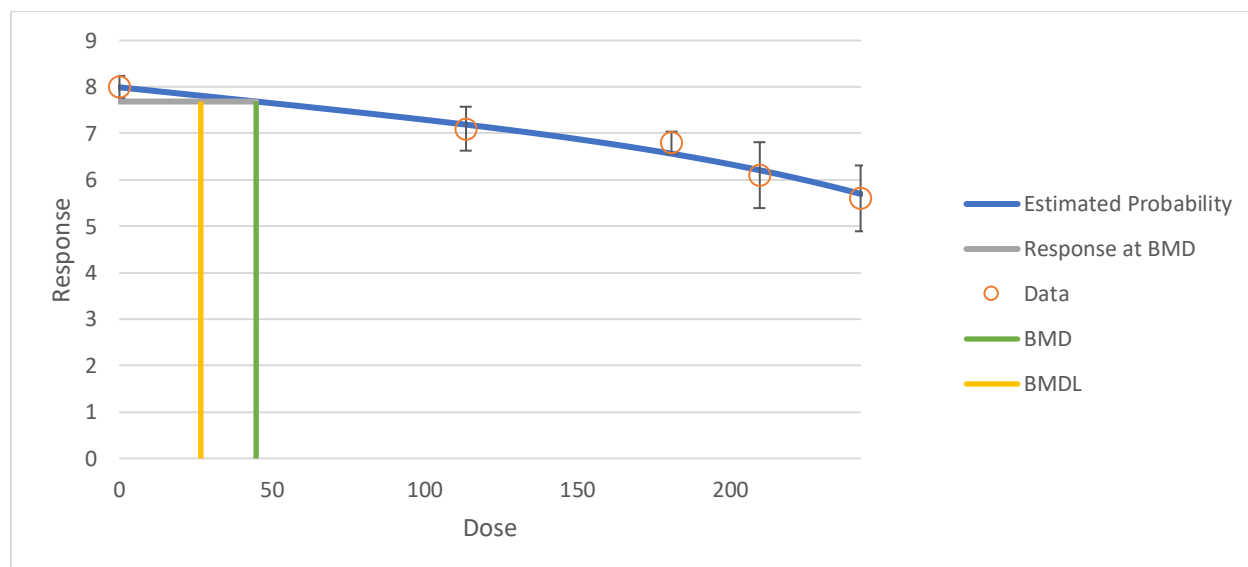


Figure B-6. Plot of Mean Response by Dose with Fitted Curve for the Polynomial Degree 4 Model for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study I) Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.5.2 Serum Sheep Red Blood Cells-specific IgM antibody titers in Female C57BL/6N Mice (Study II)

Decreased mean response of serum SRBC-specific IgM antibody titers was observed in female C57BL/6N mice (Study II). 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-18. The $C_{7,avg}$ was selected for this model.

Table B-18. Dose-Response Modeling Data for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study II) Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (log ₂ to reach 0.5 OD) ^a
0	0	8	7.9 ± 0.3 ^b
3.75	29.8	8	8.0 ± 0.3
7.5	58.9	8	7.8 ± 0.3
15	113.4	8	7.4 ± 0.3
30	180.9	8	7.3 ± 0.3

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for serum SRBC-specific IgM antibody titers are summarized in Table B-19 and Figure B-7. 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 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 Hill model is 51.6 mg/L.

Table B-19. Summary of Benchmark Dose Modeling Results for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study II) Following Exposure to PFOA (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	0.175	20.1	0.5	-0.9	68.6	46.9	EPA selected the Hill model. All models, except Exponential 3, Exponential 4, and Power, 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	0.095	21.9	0.3	-0.7	77.7	47.6	
Exponential 4	0.084	22.1	0.5	-1.0	68.1	36.8	
Exponential 5	0.369	20.0	-0.2	-0.5	76.2	51.5	
Hill	0.412	19.8	-0.1	-0.5	72.1	51.6	
Polynomial Degree 4	0.175	20.1	0.5	-0.9	70.2	48.5	
Polynomial Degree 3	0.175	20.1	0.5	-0.9	70.2	48.5	
Polynomial Degree 2	0.175	20.1	0.5	-0.9	70.2	48.9	
Power	0.090	22.0	0.3	-0.7	77.9	49.2	
Linear	0.175	20.1	0.5	-0.9	70.2	48.6	

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.

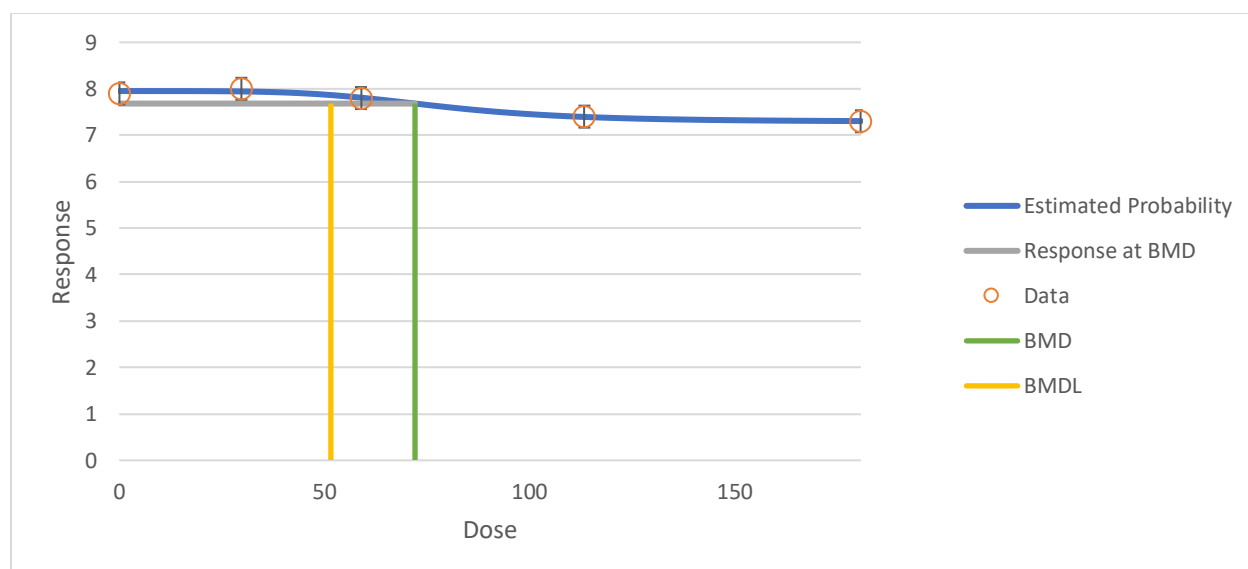


Figure B-7. Plot of Mean Response by Dose with Fitted Curve for the Selected Hill Model for Serum Sheep Red Blood Cells-specific IgM Antibody Titers in Female C57BL/6N Mice (Study II) Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.6 Lau, 2006, 1276159

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Lau (2006, 1276159) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses prenatal loss (% live per litter) and maternal body weight change in P₀ female CD-1 mice, and fetal body weight and time to eye opening in F₁ male and female CD-1 mice.

B.2.6.1 Prenatal Loss (% live per litter)

Increased mean response of prenatal loss 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 0.5 standard deviations from the control mean was chosen. The doses and response data used for the modeling are listed in Table B-20. 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 prenatal loss 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.

Table B-20. Dose-Response Modeling Data for Prenatal Loss in P₀ Female CD-1 Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose		Number per group	Mean Response (% live per litter) ^a
	AUC _{avg,dam,gest} (mg/L)	C _{max,dam} (mg/L)		
0	0	0	42	4.1 ± 9.1 ^b

Administered Dose (mg/kg/day)	Internal Dose		Number per group	Mean Response (% live per litter) ^a
	AUC _{avg,dam,gest} (mg/L)	C _{max,dam} (mg/L)		
1	33.9	62.0	15	1.0 ± 2.7
3	74.9	114.9	16	7.4 ± 10
5	91.6	135.9	20	2.4 ± 3.6
10	112.6	177.4	14	7.7 ± 12.3
20	139.7	252.9	5	25.9 ± 26.2

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for prenatal loss using AUC_{avg,dam,gest} and C_{max,dam} are summarized in Table B-21 and Table B-22, respectively. The data was non-monotonic, and no models provided an adequate fit (constant variance models did not have adequate fit for the variance model and nonconstant variance models did not have adequate fit), therefore a no-observed-adverse-effect level (NOAEL) approach was taken for this endpoint.

Table B-21. Summary of Benchmark Dose Modeling Results for Prenatal Loss using AUC_{avg,dam,gest} in P₀ Female CD-1 Mice Following Exposure to PFOA (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	0.014	838.7	-1.13	2.5	93.5	79.1	No models had adequate fit for the constant variance model (p-values were less than 0.05).
Exponential 3	0.214	832.7	0.24	0.3	117.9	99.8	
Exponential 4	<0.0001	849.4	-2.16	1.0	96.3	66.8	
Exponential 5	0.111	834.7	0.21	0.2	117.1	102.1	
Hill	0.247	832.4	0.03	0.2	114.3	103.3	
Polynomial Degree 5	0.260	830.8	-0.80	0.8	105.2	94.5	
Polynomial Degree 4	0.130	832.8	-1.67	0.9	100.6	90.1	
Polynomial Degree 3	0.021	837.8	-1.98	1.1	95.4	83.8	
Polynomial Degree 2	0.003	842.0	-2.22	1.2	90.8	74.3	
Power	0.221	832.7	0.21	0.2	117.1	102.0	
Linear	<0.001	847.4	-2.16	1.0	96.3	58.5	

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.

Table B-22. Summary of Benchmark Dose Modeling Results for Prenatal Loss using C_{\max_dam} in P₀ Female CD-1 Mice Following Exposure to PFOA (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	851.9	-9999	-0.715	-9999	0	No models had adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	812.8	0.581	0.263	195.9	163.6	
Exponential 4	<0.0001	837.0	2.86	-0.838	1468	961.0	
Exponential 5	<0.0001	814.1	0.471	0.254	189.6	161.5	
Hill	<0.0001	807.3	-9999	0.721	65535	0	
Polynomial Degree 5	<0.0001	812.9	1.43	-0.133	457.4	253.0	
Polynomial Degree 4	<0.0001	813.1	1.52	-0.121	538.5	300.9	
Polynomial Degree 3	<0.0001	814.8	1.74	-0.130	631.0	615.3	
Polynomial Degree 2	<0.0001	822.0	2.18	-0.140	1269	270.3	
Power	<0.0001	817.0	1.45	0.162	541.2	356.9	
Linear	<0.0001	834.0	2.95	-0.152	7908	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.

B.2.6.2 Maternal Body Weight Change

Decreased mean response of maternal body weight change was observed in P₀ female CD-1 mice. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to one standard deviation from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (USEPA, 2012, 1239433). 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}) 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 body weight.

Table B-23. Dose-Response Modeling Data for Maternal Body Weight Change in P₀ Female CD-1 Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	45	22 ± 6.7 ^b
1	33.9	17	23 ± 4.1

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
3	74.9	17	27 ± 4.1
5	91.6	27	21 ± 5.2
10	112.6	26	17 ± 5.1
20	139.7	42	5 ± 13.0
40	185.0	9	-4 ± 3.0

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for maternal body weight are summarized in Table B-24. The data was non-monotonic, and no models provided an adequate fit (constant variance models did not have adequate fit for the variance model and nonconstant variance models did not have adequate fit), therefore a NOAEL approach was taken for this endpoint.

Table B-24. Summary of Benchmark Dose Modeling Results for Maternal Body Weight Change in P₀ Female CD-1 Mice Following Exposure to PFOA (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	1362.9	3.190	-2.1	89.0	67.2	No models had adequate fit for the constant variance model (p-values were less than 0.05) and none of the non-constant variance models had adequate fit.
Exponential 3	0.076	1282.7	-0.071	-0.9	117.6	110.2	
Exponential 4	<0.0001	1362.9	3.190	-2.1	89.0	67.2	
Exponential 5	0.076	1282.7	-0.071	-0.9	117.6	110.2	
Hill	0.110	1282.2	-0.004	-0.9	117.0	109.7	
Polynomial Degree 6	<0.0001	1301.5	0.928	-1.4	113.0	100.6	
Polynomial Degree 5	<0.0001	1301.5	0.928	-1.4	113.0	100.6	
Polynomial Degree 4	<0.0001	1301.5	0.928	-1.4	113.0	100.6	
Polynomial Degree 3	<0.0001	1301.5	0.928	-1.4	113.0	100.6	
Polynomial Degree 2	<0.0001	1301.2	1.993	-2.4	98.5	91.6	
Power	<0.0001	1300.7	1.051	-1.5	111.0	100.6	
Linear	<0.0001	1344.2	4.302	-3.0	78.5	66.1	

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.

B.2.6.3 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. A BMR of a change in the mean equal to

0.5 standard deviations and a BMR of a 5% decrease in pup weight were chosen. The doses and response data used for the modeling are listed in Table B-25. The AUC normalized per day during gestation ($AUC_{avg,pup,gst}$) 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 fetal body weight.

Table B-25. Dose-Response Modeling Data for Fetal Body Weight in F₁ Male and Female CD-1 Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	42	1.1 ± 0.1 ^b
1	8.5	15	1.0 ± 0.1
3	18.7	16	1.0 ± 0.2
5	22.9	20	1.0 ± 0.2
10	28.1	14	1.0 ± 0.2
20	34.9	5	0.9 ± 0.2

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for fetal body weight are summarized in Table B-26 and Figure B-8. The best fitting model was the Polynomial Degree 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 Polynomial Degree 4 model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change (BMDL₅) from the selected Polynomial Degree 4 model is 16.3 mg/L.

Table B-26. Summary of Benchmark Dose Modeling Results for Fetal Body Weight Change in F₁ Male and Female CD-1 Mice Following Exposure to PFOA (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	0.243	-93.6	-1.5	1.1	0.1	33.2	17.2	22.2	11.8	EPA selected the Polynomial Degree 4 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 4 model had the lowest AIC.
Exponential 3	0.485	-94.6	-0.2	-0.2	0.7	30.5	24.8	28.6	20.9	
Exponential 4	0.243	-93.6	-1.5	1.1	0.1	33.2	17.2	22.2	11.8	
Exponential 5	0.485	-94.6	-0.2	-0.2	0.7	30.5	24.8	28.6	20.9	
Hill	0.293	-92.6	-0.2	-0.2	0.7	30.5	24.7	28.7	21.5	
Polynomial Degree 5	0.635	-96.5	0.1	0.1	0.5	29.6	22.5	27.3	16.6	
Polynomial Degree 4	0.713	-98.1	0.2	0.2	0.5	29.2	22.1	26.5	16.3	
Polynomial Degree 3	0.464	-95.5	0.2	0.8	0.3	28.8	21.1	25.3	15.0	
Polynomial Degree 2	0.337	-94.5	0.2	0.9	0.2	29.2	19.1	24.0	13.2	
Power	0.484	-94.6	-0.2	-0.2	0.7	30.5	24.7	28.7	20.5	
Linear	0.246	-93.6	-1.5	1.1	0.1	32.7	17.5	22.1	12.1	

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; BMD₅ = dose level corresponding to a 5% change; 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; BMDL₅ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change.

^aSelected model in bold.

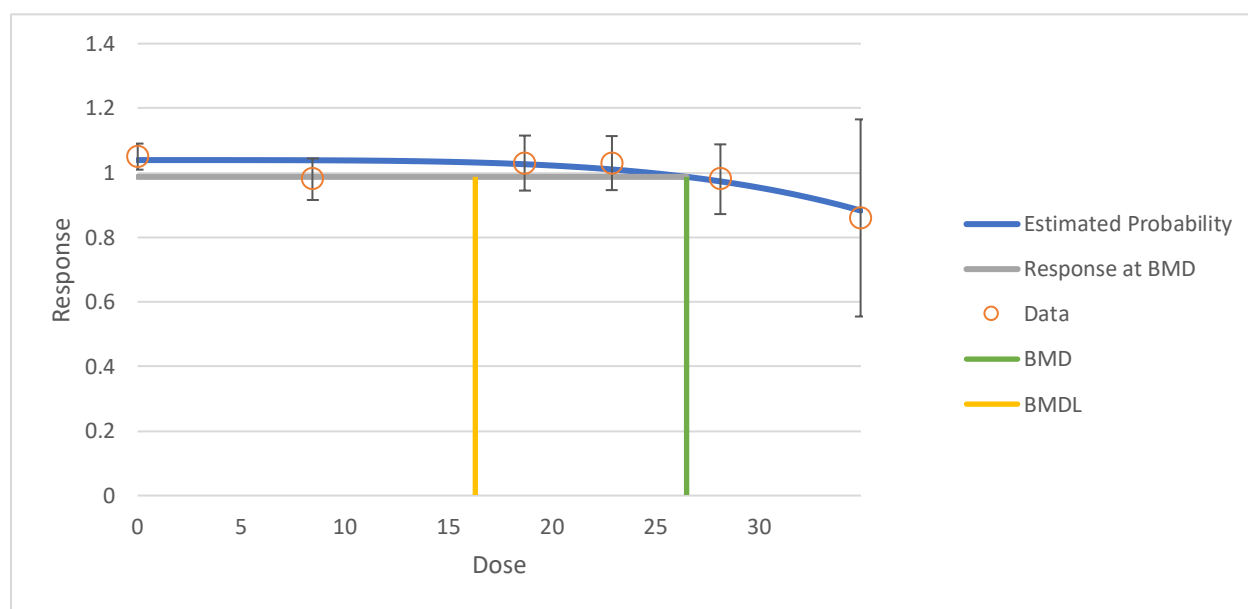


Figure B-8. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 4 Model for Fetal Body Weight in F₁ Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.6.4 Time to Eye Opening

Decreased mean response of time to eye opening 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 and 1 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table B-27. The AUC normalized per day during gestation ($AUC_{avg,pup,gest}$), AUC normalized per day during lactation ($AUC_{avg,pup,lact}$), 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 time of eye opening 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 time to eye opening could be due to exposure during the gestation or lactation lifestages.

Table B-27. Dose-Response Modeling Data for Time to Eye Opening in F₁ Male and Female CD-1 Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose				Number per group	Mean Response (days) ^a
	$AUC_{avg,dam,gest}$ (mg/L)	$AUC_{avg,dam,lact}$ (mg/L)	$C_{max,pup,gest}$ (mg/L)	$C_{max,pup,lact}$ (mg/L)		
0	0	0	0	0	22	14.8 ± 0.5 ^b
1	8.8	12.7	16.0	22.3	8	15.2 ± 0.6
3	19.1	19.3	28.8	37.5	8	15.5 ± 0.3
5	23.2	20.6	34.0	41.5	17	16.0 ± 0.8
10	28.3	22.5	44.4	47.5	13	17.2 ± 1.1

20	35.1	25.2	63.3	56.7	3	17.9 ± 1.4
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^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

For $AUC_{avg,pup,gest}$, the benchmark dose (BMD) modeling results for time to eye opening are summarized in Table B-28 and Figure B-9. The best fitting model was the Polynomial Degree 2 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 Polynomial Degree 2 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 Polynomial Degree 2 model is 9.4 mg/L.

Table B-28. Summary of Benchmark Dose Modeling Results for Time to Eye Opening using $AUC_{avg,pup,gest}$ in F1 Male and Female CD-1 mice Following Exposure to PFOA (nonconstant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	BMD _{1SD} (mg/L)	BMDL _{1SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.5SD}	Dose Group near BMD _{1SD}	Control Dose Group					
Exponential 2	0.006	159.1	-0.525	0.511	0.511	3.5	2.8	7.0	5.5	EPA selected the Polynomial Degree 2 model. All models, except Exponential 2, 4, and 5, and Linear, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 2 model had the lowest AIC.
Exponential 3	0.144	152.1	-0.583	1.313	-0.457	11.6	6.9	15.4	10.7	
Exponential 4	0.002	161.9	-0.605	0.457	0.457	3.4	2.6	6.7	5.3	
Exponential 5	0.067	154.0	-0.514	1.377	-0.532	12.0	7.5	15.8	11.3	
Hill	0.105	153.1	0.556	0.556	-1.092	16.8	15.6	19.2	12.0	
Polynomial Degree 5	0.102	153.2	0.780	0.780	-0.194	8.5	4.2	13.9	8.3	
Polynomial Degree 4	0.102	153.2	0.801	0.801	-0.192	8.5	4.3	13.9	8.5	
Polynomial Degree 3	0.198	151.3	-0.649	0.987	-0.246	9.6	5.0	14.4	9.5	
Polynomial Degree 2	0.184	150.8	1.067	1.067	-0.150	9.5	5.4	13.5	9.4	
Power	0.145	152.0	-0.516	1.377	-0.530	12.0	7.2	15.8	11.0	
Linear	0.004	159.9	-0.605	0.495	0.495	3.4	2.6	6.8	5.3	

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 deviation(s) from the control mean; BMDL_{0.5SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation(s) 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 deviation from the control mean.

^aSelected model in bold.

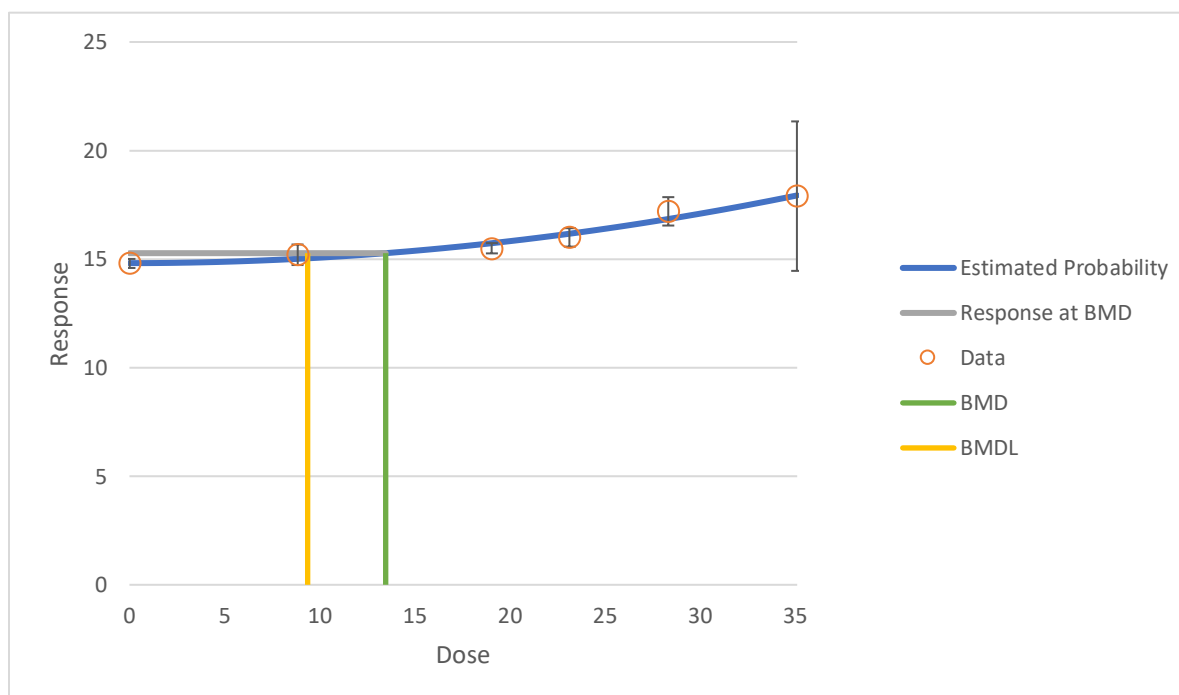


Figure B-9. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 2 Model for Time to Eye Opening in F₁ Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $AUC_{avg,pup,lact}$, the benchmark dose (BMD) modeling results for time to eye opening are summarized in Table B-29 and Figure B-10. The best fitting model was the Polynomial Degree 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 Polynomial Degree 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 change in the mean equal to one standard deviation from the control mean ($BMDL_{1SD}$) from the selected Polynomial Degree 5 model is 12.0 mg/L.

Table B-29. Summary of Benchmark Dose Modeling Results for Time to Eye Opening using $AUC_{avg,pup,lact}$ in F1 Male and Female CD-1 mice Following Exposure to PFOA (nonconstant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	BMD _{1SD} (mg/L)	BMDL _{1SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.5SD}	Dose Group near BMD _{1SD}	Control Dose Group					
Exponential 2	<0.0001	172.8	-1.7	0.4	0.4	3.2	2.7	6.4	4.9	EPA selected the Polynomial Degree 5 model. The Hill and Polynomial Degree 5 models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 5 model had the lowest AIC.
Exponential 3	0.047	154.6	-0.9	-0.5	-0.5	15.2	14.8	17.3	16.9	
Exponential 4	<0.0001	175.4	0.4	0.4	0.4	3.1	2.4	6.2	4.8	
Exponential 5	0.020	156.5	-0.8	1.5	-0.6	15.6	15.2	17.6	17.1	
Hill	0.106	153.1	0.6	0.6	-1.1	18.5	17.5	19.3	18.8	
Polynomial Degree 5	0.100	152.4	-1.2	-1.2	-0.1	13.2	6.8	16.1	12.0	
Polynomial Degree 4	0.054	153.9	1.1	1.1	-0.1	13.2	7.6	15.7	12.1	
Polynomial Degree 3	0.018	156.5	0.4	0.4	0.2	11.1	7.8	14.0	11.6	
Polynomial Degree 2	0.001	162.2	-0.6	-0.6	0.4	7.9	5.9	11.2	9.4	
Power	0.050	154.5	-0.9	1.5	-0.6	15.4	14.0	17.4	16.5	
Linear	<0.0001	173.4	0.4	0.4	0.4	3.1	2.4	6.2	4.8	

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; 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.

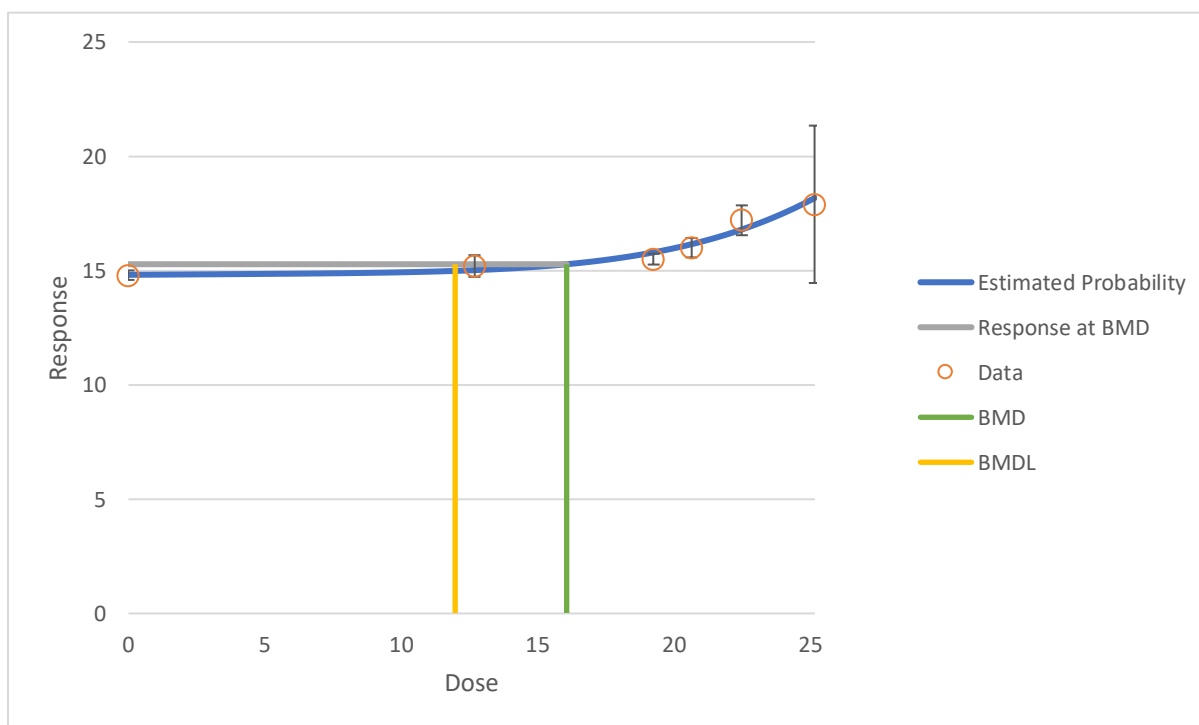


Figure B-10. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 5 Model for Time to Eye Opening using $AUC_{avg,pup,lact}$ in F1 Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $C_{max,pup,gest}$, the benchmark dose (BMD) modeling results for time to eye opening are summarized in Table B-30 and Figure B-11. 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 change in the mean equal to one standard deviation from the control mean ($BMDL_{1SD}$) from the selected Exponential 5 model is 24.9 mg/L.

Table B-30. Summary of Benchmark Dose Modeling Results for Time to Eye Opening using $C_{\max, \text{pup, gest}}$ in F₁ Male and Female CD-1 mice Following Exposure to PFOA (nonconstant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	BMD _{1SD} (mg/L)	BMDL _{1SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.5SD}	Dose Group near BMD _{1SD}	Control Dose Group					
Exponential 2	0.013	157.3	0.6	-0.9	0.62	5.5	4.4	11.0	8.7	EPA selected the Exponential 5 model. All models, except Exponential 2, Exponential 4, and Linear, 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	0.108	152.7	0.5	0.5	0.01	13.3	8.0	19.5	13.6	
Exponential 4	0.004	160.2	0.6	-1.0	0.56	5.2	4.2	10.4	8.3	
Exponential 5	0.175	152.1	1.4	-0.3	-0.67	21.4	20.6	25.9	24.9	
Hill	0.134	152.7	0.2	0.2	-0.96	24.4	10.8	27.9	16.6	
Polynomial Degree 5	0.118	152.5	0.5	0.5	-0.05	13.9	6.7	20.1	12.9	
Polynomial Degree 4	0.118	152.5	0.5	0.5	-0.05	13.9	6.6	20.1	13.0	
Polynomial Degree 3	0.118	152.5	0.6	0.6	-0.04	13.9	6.6	20.1	12.9	
Polynomial Degree 2	0.118	152.5	0.5	0.5	-0.06	13.8	7.3	20.0	13.2	
Power	0.120	152.5	0.5	0.5	-0.03	13.9	8.4	19.9	14.0	
Linear	0.009	158.2	0.6	-1.0	0.60	5.3	4.1	10.6	8.3	

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; 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.

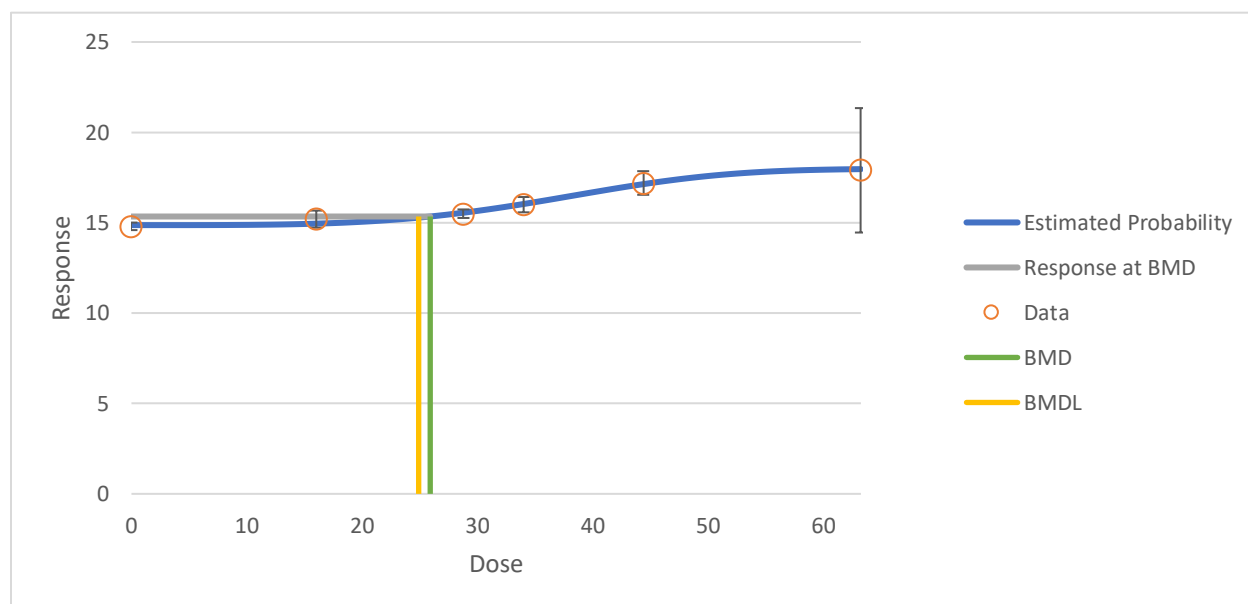


Figure B-11. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential Degree 5 Model for Time to Eye Opening using $C_{\max,pup,gst}$ in F₁ Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $C_{\max,pup,lact}$, the benchmark dose (BMD) modeling results for time to eye opening are summarized in Table B-31 and Figure B-12. The best fitting model was the Polynomial Degree 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 Polynomial Degree 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 change in the mean equal to one standard deviation from the control mean (BMDL_{1SD}) from the selected Polynomial Degree 5 model is 18.9 mg/L.

Table B-31. Summary of Benchmark Dose Modeling Results for Time to Eye Opening using $C_{\text{max,pup,lact}}$ in F1 Male and Female CD-1 mice Following Exposure to PFOA (nonconstant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	BMD _{1SD} (mg/L)	BMDL _{1SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.5SD}	Dose Group near BMD _{1SD}	Control Dose Group					
Exponential 2	<0.001	167.0	0.44	-1.37	0.4	6.4	5.0	12.7	9.9	EPA selected the Polynomial Degree 5 model. The Hill, Polynomial Degree 3, 4, and 5 had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 5 model had the lowest AIC.
Exponential 3	0.066	153.8	1.18	-0.97	-0.3	25.2	17.4	31.0	27.2	
Exponential 4	<0.0001	169.7	0.48	-1.43	0.5	6.2	4.8	12.4	9.6	
Exponential 5	0.029	155.7	1.25	-0.94	-0.4	25.8	18.4	31.4	24.9	
Hill	0.109	153.1	0.60	0.60	-1.1	35.2	33.7	37.7	30.7	
Polynomial Degree 5	0.169	151.1	0.65	0.65	-0.1	19.6	9.7	29.1	18.9	
Polynomial Degree 4	0.164	151.1	0.84	0.84	-0.1	22.0	11.1	29.5	20.4	
Polynomial Degree 3	0.111	152.1	1.00	1.00	-0.1	23.2	12.9	29.2	21.2	
Polynomial Degree 2	0.024	155.9	0.07	0.07	0.3	16.3	11.6	23.0	18.8	
Power	0.069	153.7	1.26	-0.91	-0.4	26.0	18.1	31.5	24.4	
Linear	<0.001	167.7	0.50	-1.44	0.5	6.2	4.8	12.4	9.6	

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 deviation(s) from the control mean; BMDL_{0.5SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation(s) 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 deviation from the control mean.

^aSelected model in bold.

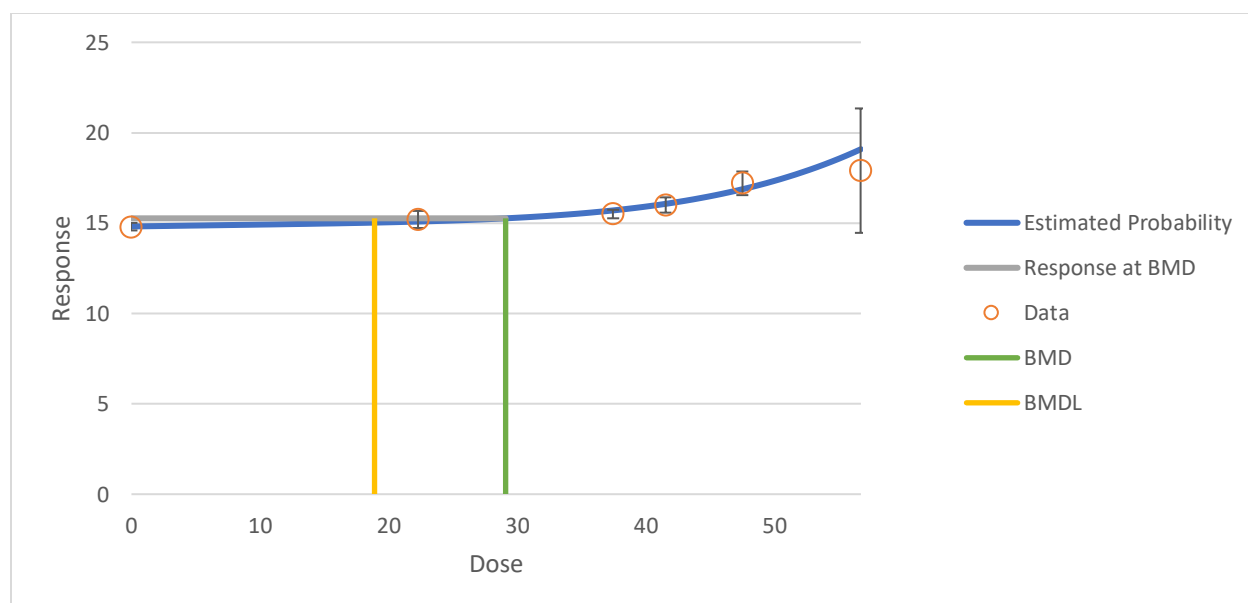


Figure B-12. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 5 Model for Time to Eye Opening using $C_{\max,pup,lact}$ in F₁ Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.7 Li, 2018, 5084746

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Li (2018, 5084746) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses fetal body weight in F₁ male and female Kunming mice and maternal body weight in P₀ female Kunming mice.

B.2.7.1 Fetal Body Weight

Decreased mean response of fetal body weight was observed in F₁ male and female Kunming 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-32. The AUC normalized per day during gestation ($AUC_{\text{avg,pup,gest}}$) 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 fetal body weight.

Table B-32. Dose-Response Modeling Data for Fetal Body Weight in F₁ Male and Female Kunming Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	10	1.5 ± 0.01
1	8.5	10	1.5 ± 0.01

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
5	22.9	10	1.3 ± 0.01
10	28.1	10	1.0 ± 0.10
20	34.9	10	0.9 ± 0.05

^aData are presented as mean ± standard deviation.

The benchmark dose (BMD) modeling results for fetal body weight 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 Fetal Body Weight in F₁ Male and Female Kunming Mice Following Exposure to PFOA (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	-62.6	-1.802	-1.8	2.7	2.2	No models had adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	-105.2	-1.496	0.7	11.7	9.7	
Exponential 4	<0.0001	-62.6	-1.802	-1.8	2.7	2.2	
Exponential 5	0.002	-142.0	0.004	2.1	18.5	17.3	
Hill	0.002	-142.0	-0.006	2.1	19.4	18.4	
Polynomial Degree 4	<0.0001	-102.3	-1.162	0.4	9.5	4.5	
Polynomial Degree 3	<0.0001	-102.3	-1.162	0.4	9.5	4.5	
Polynomial Degree 2	<0.0001	-103.1	-1.250	-0.1	8.5	6.4	
Power	<0.0001	-102.6	-1.240	0.5	10.1	7.8	
Linear	<0.0001	-72.2	-1.986	-2.0	2.9	2.4	

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.

B.2.7.2 Maternal Body Weight

Decreased mean response of maternal body weight was observed in P₀ female Kunming 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-34. The AUC normalized per day during gestation (AUC_{avg,dam,gest}) The AUC normalized per day during gestation (AUC_{avg,dam,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 body weight.

Table B-34. Dose-Response Modeling Data for Maternal Body Weight in P₀ Female Kunming Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	10	60.2 ± 1.3
1	33.9	10	63.1 ± 1.3
5	91.6	10	60.0 ± 0.8
10	112.6	10	44.8 ± 5.6
20	139.7	10	37.4 ± 6.6
40	185.0	10	29.0 ± 0.6

^aData are presented as mean ± standard deviation.

The benchmark dose (BMD) modeling results for maternal body weight are summarized in Table B-35. No models provided an adequate fit, therefore a NOAEL approach was taken for this endpoint.

Table B-35. Summary of Benchmark Dose Modeling Results for Maternal Body Weight in P₀ Female Kunming Mice Following Exposure to PFOA (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	412.9	1.7	-2.9	32.7	26.3	No models had adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	371.1	4.0	-1.6	70.4	59.8	
Exponential 4	<0.0001	412.9	1.7	-2.9	32.7	26.3	
Exponential 5	<0.0001	351.6	2.7	-1.6	85.5	78.2	
Hill	0.0003	345.4	1.9	-1.6	90.0	83.6	
Polynomial Degree 4	<0.0001	375.4	3.9	-1.3	70.9	54.1	
Polynomial Degree 3	<0.0001	375.4	3.9	-1.3	70.9	54.1	
Polynomial Degree 2	<0.0001	375.4	3.9	-1.3	70.9	54.1	
Power	<0.0001	375.4	3.9	-1.3	70.9	54.1	
Linear	<0.0001	376.9	4.1	-1.6	65.6	53.1	

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 one standard deviation from the control mean; BMDL_{1SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to one standard deviation from the control mean.

B.2.8 Loveless, 2008, 988599

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Loveless (2008, 988599) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses focal necrosis in male Crl:CD(SD)IGS BR rats and focal necrosis, individual cell necrosis, and IgM serum titer in male Crl:CD-1(ICR)BR mice.

B.2.8.1 Focal Necrosis in Male Crl:CD(SD)IGS BR Rats

Increased incidence of focal necrosis was observed in male 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* (USEPA, 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 effect resulting in focal necrosis.

Table B-36. Dose-Response Modeling Data for Focal Necrosis in Male Crl:CD(SD)IGS BR Rats Following Exposure to PFOA

Administered Dose (mg/kg/day)	Dose (mg/L)	Number per Group	Incidence
0	0	10	0
0.3	15.8	10	0
1	48.0	10	0
10	180.4	10	1
30	206.3	10	4

The benchmark dose (BMD) modeling results for focal necrosis are summarized in Table B-37 and Figure B-13. The best fitting model was the Multistage Degree 4 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Multistage Degree 4 model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level (BMDL_{10}) from the selected Multistage Degree 4 model is 74.1 mg/L.

Table B-37. Summary of Benchmark Dose Modeling Results for Focal Necrosis in Male Crl:CD(SD)IGS BR Rats Following Exposure to PFOA

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	1.000	26.0	-2.0×10^{-5}	-3.9×10^{-4}	180.4	95.2	EPA selected the Multistage Degree 4 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
Gamma	0.913	24.5	-0.6	-3.9×10^{-4}	166.4	82.1	
Log-Logistic	1.000	24.0	-2.2×10^{-7}	-3.9×10^{-4}	180.4	95.2	
Multistage Degree 4	0.915	23.0	-0.8	-3.9×10^{-4}	150.6	74.1	
Multistage Degree 3	0.863	23.4	-0.8	-3.9×10^{-4}	138.5	66.0	
Multistage Degree 2	0.613	26.0	-0.9	-3.9×10^{-4}	118.8	55.8	
Multistage Degree 1	0.533	25.8	-0.8	-3.9×10^{-4}	83.9	43.5	

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			
Weibull	1.000	26.0	-2.6×10^{-5}	-3.9×10^{-4}	180.4	96.2	4 model had the lowest AIC.
Logistic	1.000	24.0	8.8×10^{-4}	-2.0×10^{-3}	180.4	126.0	
Log-Probit	1.000	26.0	6.2×10^{-6}	-4.4×10^{-4}	180.4	90.7	
Probit	1.000	24.0	-4.8×10^{-7}	-1.2×10^{-8}	180.4	118.3	

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.

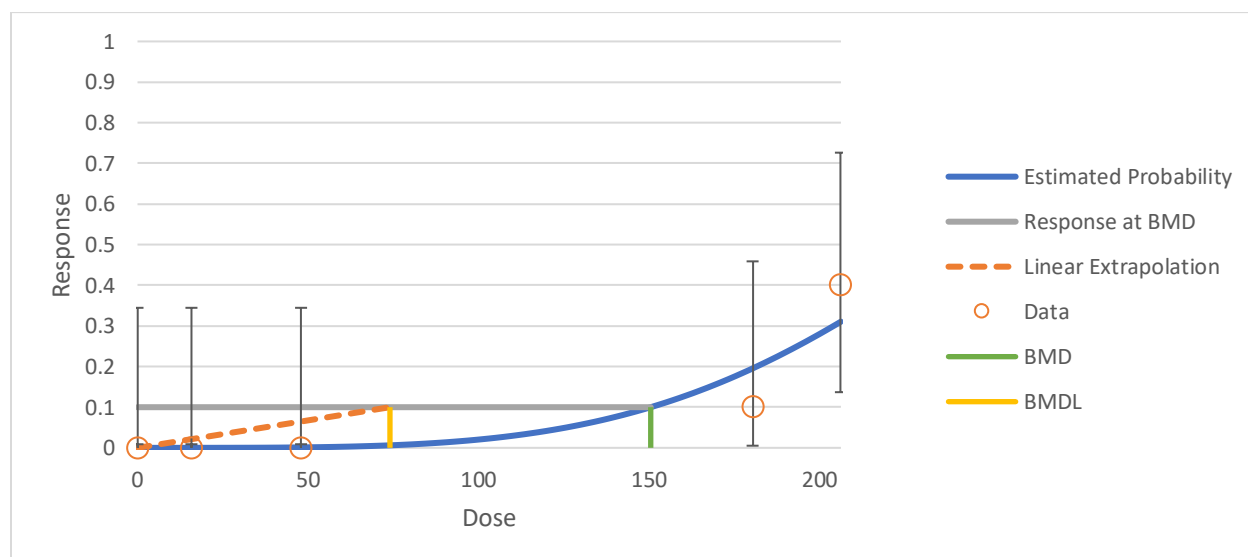


Figure B-13. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Focal Necrosis in Male Crl:CD(SD)IGS BR Rats Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.8.2 Focal Necrosis in Male Crl:CD-1(ICR)BR Mice

Increased incidence of focal necrosis was observed in male Crl:CD-1(ICR)BR mice.

Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (USEPA, 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 effect resulting in focal necrosis.

Table B-38. Dose-Response Modeling Data for Focal Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Dose (mg/L)	Number per Group	Incidence
0	0	19	0
0.3	36.3	20	1
1	111.5	20	3
10	221.4	20	4
30	305.0	19	7

The BMD modeling results for focal necrosis are summarized in Table B-39 and Figure B-14. The best fitting model was the Log-Probit 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 Log-Probit model had the lowest BMDL. The BMDL₁₀ from the selected Log-Probit model is 13.6 mg/L.

Table B-39. Summary of Benchmark Dose Modeling Results for Focal Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA

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	0.481	78.4	0.13	−0.0005	78.1	13.9	EPA selected the Log-Probit model as it had the lowest BMDL. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference).
Gamma	0.929	74.3	0.17	−0.0121	80.0	53.7	
Log-Logistic	0.920	74.4	0.13	−0.0005	78.1	45.8	
Multistage Degree 4	0.821	76.3	0.32	−0.0005	87.4	53.9	
Multistage Degree 3	0.808	76.3	0.28	−0.005	85.7	53.8	
Multistage Degree 2	0.930	74.3	0.22	−0.0005	82.7	53.7	
Multistage Degree 1	0.978	72.3	0.17	−0.0005	80.0	53.7	
Weibull	0.978	72.3	0.17	−0.0005	80.0	53.7	
Logistic	0.673	76.0	0.90	−0.8300	154.9	119.6	
Log-Probit	0.905	74.4	0.04	−0.0005	74.6	13.6	
Probit	0.707	75.7	0.84	−0.8300	144.9	111.2	

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.

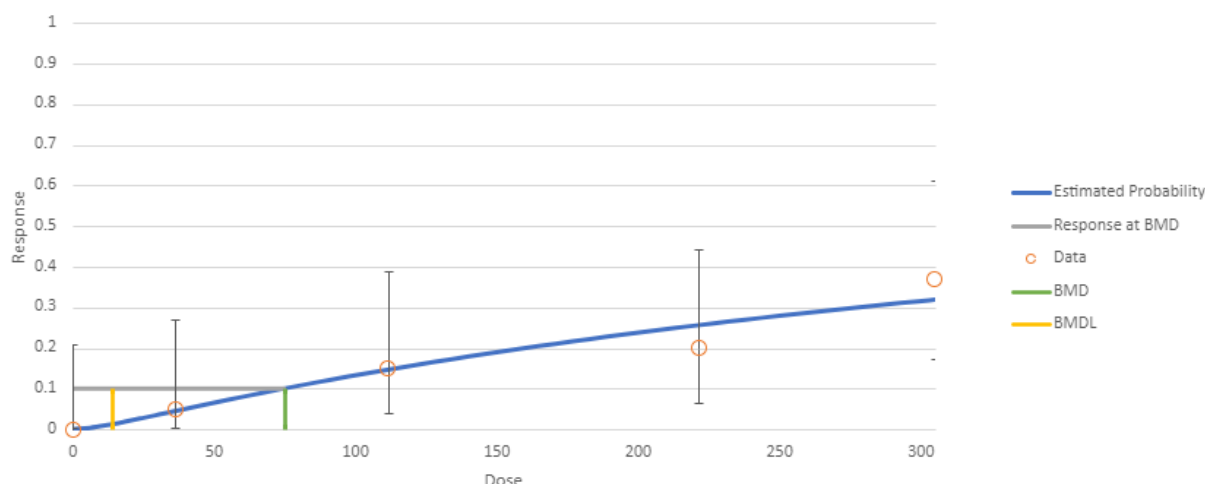


Figure B-14. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Probit Model for Focal Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.8.3 Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice

Increased incidence of individual cell necrosis was observed in male Crl:CD-1(ICR)BR mice. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA’s *Benchmark Dose Technical Guidance* (USEPA, 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 effect resulting in cell necrosis.

Table B-40. Dose-Response Modeling Data for Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Dose (mg/L)	Number per Group	Incidence
0	0	19	0
0.1	36.3	20	0
1	111.5	20	11
10	221.4	20	20
30	305.0	19	19

The BMD modeling results for individual cell necrosis are summarized in Table B-41 and Figure B-15. The best fitting model was the Log-Logistic 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 Log-Logistic model had the lowest Akaike information criterion (AIC). The BMDL₁₀ from the selected Log-Logistic model is 58.4 mg/L.

Table B-41. Summary of Benchmark Dose Modeling Results for Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA

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	1.000	29.5	-0.00003	-0.00054	97.4	58.4	EPA selected the Log-Logistic model. All models, except Multistage Degree 1, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Log-Logistic model had the lowest AIC.
Gamma	1.000	29.5	-0.00740	-0.00054	78.5	49.8	
Log-Logistic	1.000	29.5	-0.00001	-0.00054	97.4	58.4	
Multistage Degree 4	0.996	29.9	-0.41995	-0.00054	67.5	43.6	
Multistage Degree 3	0.964	30.7	-0.73665	-0.00054	57.2	39.8	
Multistage Degree 2	0.611	34.7	-1.37768	-0.00054	39.1	28.7	
Multistage Degree 1	0.005	53.5	-0.00054	-0.00054	11.5	8.7	
Weibull	0.995	31.7	-0.26573	-0.00117	73.4	72.9	
Logistic	1.000	29.5	0.00002	-0.00054	96.8	56.0	
Log-Probit	1.000	31.5	1.70×e ⁻⁸	-0.00054	95.3	53.3	
Probit	1.000	29.5	0.00020	-0.00003	87.7	50.7	

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.

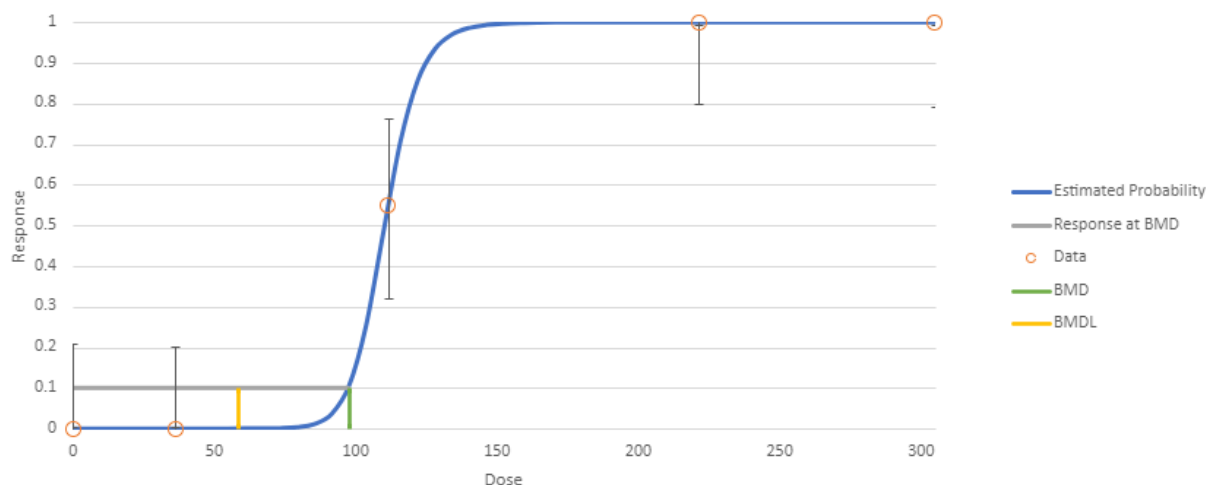


Figure B-15. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Logistic Model for Individual Cell Necrosis in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.8.4 IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice

Decreased mean response of IgM serum titer was observed in male Crl:CD-1(ICR)BR mice. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to one standard deviation from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (USEPA, 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 ($C_{7, \text{avg}}$) 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 effect resulting in decreased mean response of IgM serum titer.

Table B-42. Dose-Response Modeling Data for IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Dose (mg/L)	Number per Group	Mean Response (mg/dL) ^a
0	0	20	8.9 ± 0.6
0.1	36.3	20	8.9 ± 0.8
1	111.5	20	8.4 ± 0.7
10	221.4	20	7.2 ± 0.8
30	305.0	20	6.4 ± 0.8

^a Data are presented as mean ± standard deviation.

The BMD modeling results for IgM serum titer are summarized in Table B-43 and Figure B-16. The best fitting model was the Exponential 3 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 3 model had the lowest AIC. The BMDL_{1SD} from the selected Exponential 3 model is 90.7 mg/L.

Table B-43. Summary of Benchmark Dose Modeling Results for IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA (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	0.075	232.5	1.76	-1.5	78.3	65.6	EPA selected the Exponential 3 model. All models, except Exponential 2 and Exponential 4, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the
Exponential 3	0.728	228.2	0.30	-0.3	125.8	90.7	
Exponential 4	0.075	232.5	1.76	-1.5	78.3	65.6	
Exponential 5	0.463	230.1	0.28	-0.3	126.4	91.4	
Hill	0.848	229.6	0.04	-0.1	132.9	94.8	
Polynomial Degree 4	0.462	229.1	0.44	-0.5	120.8	84.0	
Polynomial Degree 3	0.462	229.1	0.44	-0.5	120.8	84.1	
Polynomial Degree 2	0.462	229.1	0.44	-0.5	120.8	84.1	

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			
Power	0.618	228.6	0.39	−0.4	123.4	87.8	Exponential 3 model had the lowest AIC.
Linear	0.205	230.2	1.44	−1.4	85.9	73.3	

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.

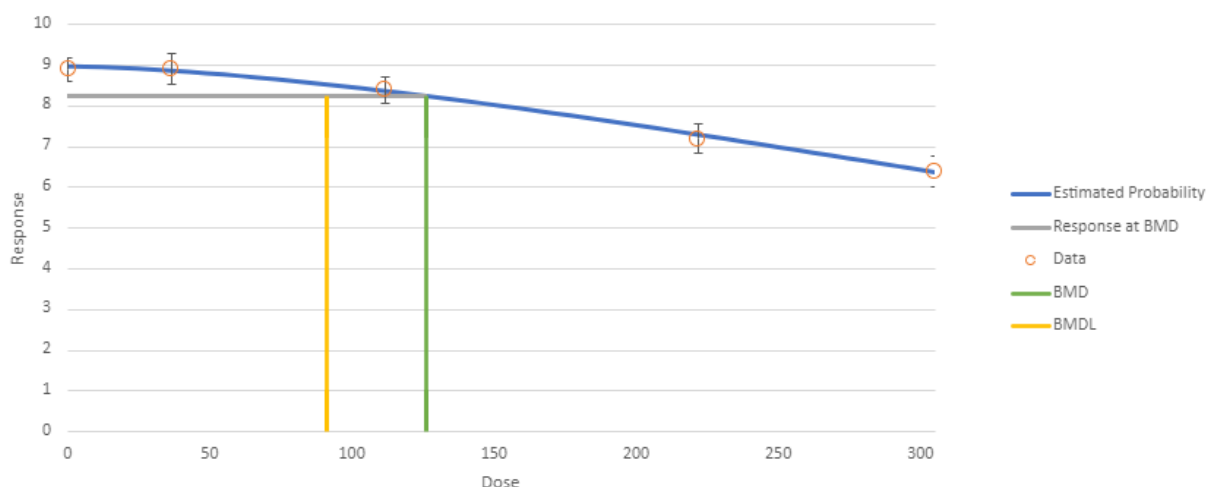


Figure B-16. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 3 Model for IgM Serum Titer in Male Crl:CD-1(ICR)BR Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.9 Macon, 2011, 1276151

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Macon (2011, 1276151) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses developmental scores for the mammary gland in F₁ female CD-1 mice.

B.2.9.1 Developmental Scores for the Mammary Gland

Decreased mean response of developmental scores for the mammary gland was observed in F₁ 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 and 1 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table B-44. The AUC normalized dose per day during gestation (AUC_{avg,pup,gest}) and the AUC normalized dose per day during gestation/lactation (AUC_{avg,pup,gest,lact}) were selected for this model rather than alternate metrics such as C_{max} because the AUC is expected to better correlate with an effect

leading to decreased mammary gland developmental scores across both the gestation and lactation lifestages.

Table B-44. Dose-Response Modeling Data for Developmental Scores for the Mammary Gland in F₁ Female CD-1 Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose		Number per Group	Mean Response (mg/dL) ^a
	AUC _{avg,pup,gest} (mg/L)	AUC _{pup,gest,lact} (mg/L)		
0	0	0	3	3.4 ± 0.5 ^b
0.3	2.7	3.6	5	1.9 ± 0.4
1	8.5	10.6	5	1.3 ± 0.2
3	18.7	19.0	3	1.6 ± 0.7

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

For AUC_{avg,pup,gest}, the benchmark dose (BMD) modeling results for developmental scores for the mammary gland are summarized in Table B-45 and Figure B-17. After visual inspection, the Exponential 4 model was selected. 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 0.2 mg/L.

Table B-45. Summary of Benchmark Dose Modeling Results for Developmental Scores for the Mammary Gland using AUC_{avg,pup,gest} in F1 Female CD-1 Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	BMD _{1SD} (mg/L)	BMDL _{1SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.5}	Dose Group near BMD _{1SD}	Control Dose Group					
Exponential 2	<0.001	37.8	-1.41	-1.41	1.80	2.1	1.0	4.6	2.1	EPA selected the Exponential 4 model. No other models had adequate fit (p-values greater than 0.1).
Exponential 3	<0.001	37.8	-1.41	-1.41	1.80	2.1	1.0	4.6	2.1	
Exponential 4	0.272	24.9	-0.02	-0.02	-0.02	0.2	0.1	0.4	0.2	
Exponential 5	— ^b	26.8	4.8×e ⁻⁵	4.8×e ⁻⁵	-1.3×e ⁻⁴	2.3	— ^c	2.4	0.2	
Hill	— ^b	26.8	-4.9×e ⁻⁸	-4.9×e ⁻⁸	2.6×e ⁻⁶	2.2	2.0	2.3	2.1	
Polynomial Degree 3	<0.0001	40.4	-1.07	-1.68	2.38	5.1	3.0	10.1	5.9	
Polynomial Degree 2	<0.0001	40.4	-1.07	-1.68	2.38	5.1	3.0	10.1	5.9	
Power	<0.0001	40.4	-1.07	-1.68	2.38	5.1	3.0	10.1	5.9	
Linear	<0.0001	40.4	-1.07	-1.68	2.38	5.1	3.0	10.1	5.9	

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; 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).

^cLower limit includes zero; BMDL not estimated.

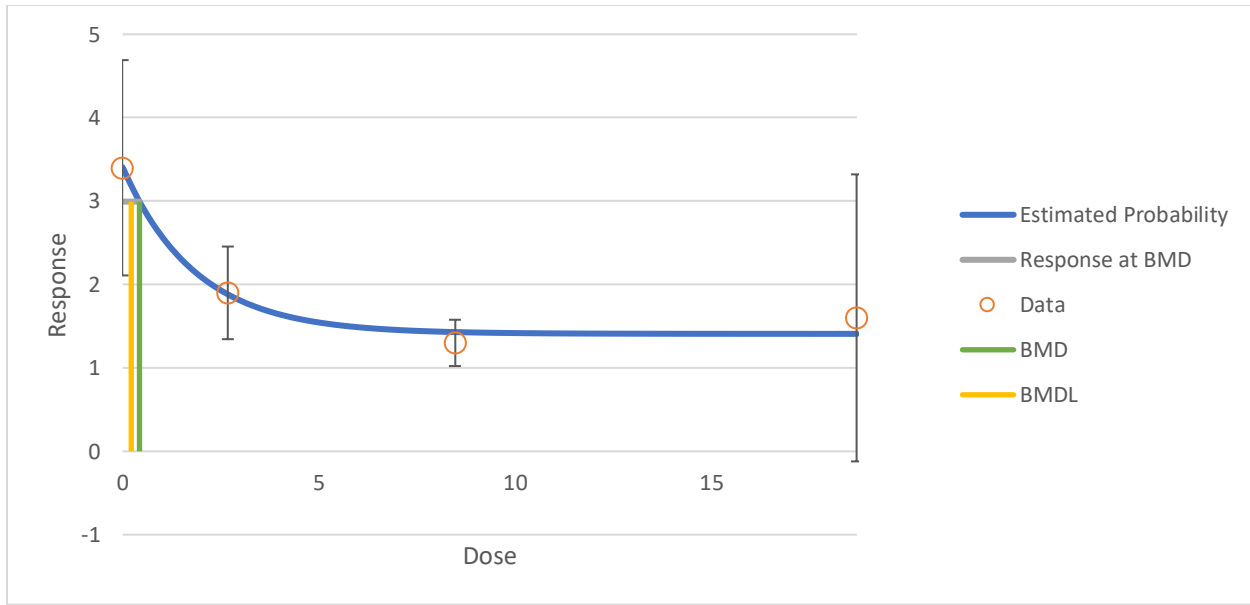


Figure B-17. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 4 Model for Developmental Scores for the Mammary Gland using $AUC_{avg,pup,gest}$ in F1 Female CD-1 Mice Following Exposure to PFOA

For $AUC_{avg,pup,gest,lact}$, the benchmark dose (BMD) modeling results for developmental scores for the mammary gland are summarized in Table B-46 and Figure B-18. After visual inspection and comparison of the viable models, the Exponential 4 model was selected as it 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 Exponential 4 model is 0.3 mg/L.

Table B-46. Summary of Benchmark Dose Modeling Results for Developmental Scores for the Mammary Gland using AUC_{avg,pup,gest,lact} in F1 Female CD-1 Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	BMD _{1SD} (mg/L)	BMDL _{1SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.5SD}	Dose Group near BMD _{1SD}	Control Dose Group					
Exponential 2	0.001	35.2	-1.53	-1.53	1.54	1.9	1.0	4.0	2.1	EPA selected the Exponential 4 model. The Exponential 4 and Hill model had adequate fit (p-values greater than 0.1), and the Exponential 4 model had the lower BMDL value.
Exponential 3	0.001	35.2	-1.53	-1.53	1.54	1.9	1.0	4.0	2.1	
Exponential 4	0.262	25.0	-0.02	-0.02	-0.02	0.3	0.1	0.6	0.3	
Exponential 5	– ^b	26.8	2.0×e ⁻⁷	2.0×e ⁻⁷	1.9×e ⁻⁷	1.2	0.1	1.6	0.3	
Hill	0.307	24.8	4.5×e ⁻⁶	4.5×e ⁻⁶	-3.1×e ⁻⁵	3.0	2.7	3.1	2.9	
Polynomial Degree 3	<0.001	38.4	-1.28	-1.46	2.17	4.2	2.6	8.5	5.3	
Polynomial Degree 2	<0.001	38.4	-1.28	-1.46	2.17	4.2	2.6	8.5	5.3	
Power	<0.001	38.4	-1.28	-1.46	2.17	4.2	2.6	8.5	5.3	
Linear	<0.001	38.4	-1.28	-1.46	2.17	4.2	2.6	8.5	5.3	

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; 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).

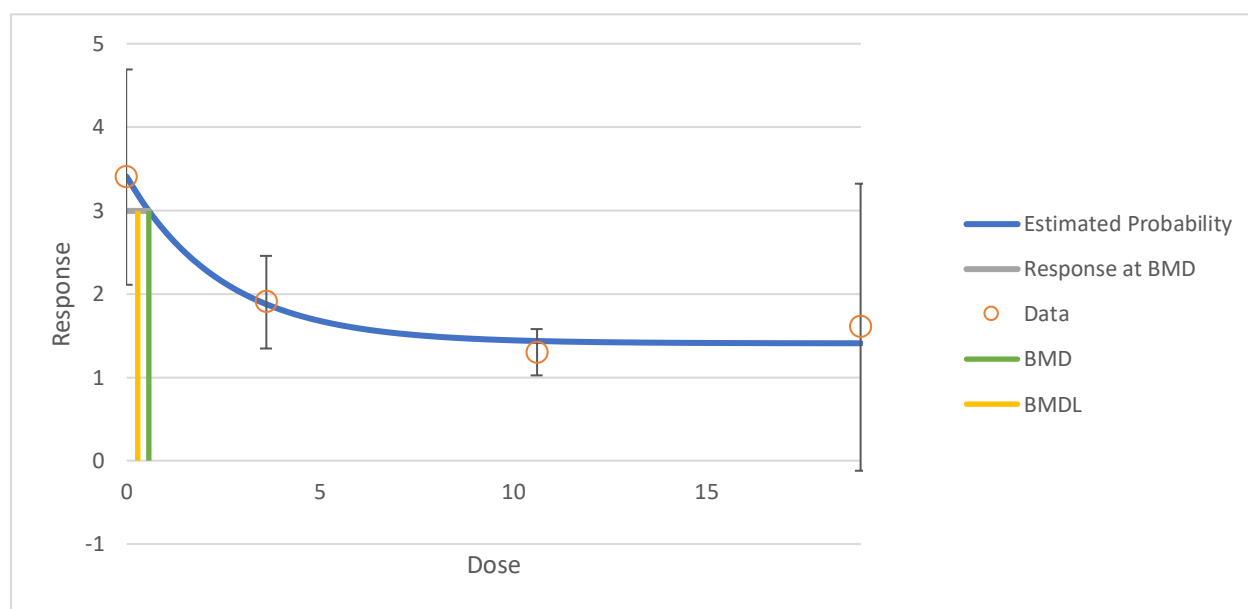


Figure B-18. Plot of Mean Response by Dose with Fitted Curve for the Selected Exponential 4 Model for Developmental Scores for the Mammary Gland using AUC_{avg,pup,gest,lact} in F1 Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.10 NTP, 2019, 5400977

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the NTP (2019, 5400977) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses serum free thyroxine (T4) levels in male Sprague-Dawley rats, serum thyroid stimulating hormone (TSH) levels in female Sprague-Dawley rats, and relative kidney weight in male Sprague-Dawley rats.

B.2.10.1 Serum Free Thyroxine (T4) Levels in Male Sprague-Dawley Rats

Decreased serum free T4 levels were 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-47. The C_{7,avg} was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of effects leading to a decreased serum free T4 levels.

Table B-47. Dose-Response Modeling Data for Serum Free Thyroxine (T4) Levels in Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (ng/dL) ^a
0	0	10	2.1 ± 0.41 ^b

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (ng/dL) ^a
0.625	31.3	10	0.4 ± 0.12
1.25	57.5	10	0.4 ± 0.06
2.5	97.0	10	0.3 ± 0.03
5	142.5	10	0.3 ± 0.06
10	180.3	10	0.3 ± 0.06

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for serum free T4 levels are summarized in Table B-48. No models had adequate fit (p-values greater than 0.1) therefore a LOAEL approach was taken for this endpoint.

Table B-48. Summary of Benchmark Dose Modeling Results for Serum Free Thyroxine (T4) Levels in Male Sprague-Dawley Rats Following Exposure to PFOA (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	29.9	0.3	0.3	3.9	2.9	No models had adequate fit (p-values greater than 0.1).
Exponential 3	<0.0001	29.9	0.3	0.3	3.9	2.9	
Exponential 4	0.982	-32.6	0.001	0.001	1.1	0.9	
Exponential 5	0.863	-30.4	-0.0002	-0.0002	10.4	0.9	
Hill	0.943	-30.6	0.00002	0.00002	4.0	0.0	
Polynomial Degree 4	<0.0001	102.2	-1.5	5.3	79.1	60.4	
Polynomial Degree 3	<0.0001	102.2	-1.5	5.3	79.1	60.4	
Polynomial Degree 2	<0.0001	102.2	-1.5	5.3	79.1	60.4	
Power	<0.0001	102.2	-1.5	5.3	79.1	60.4	
Linear	<0.0001	102.2	-1.5	5.3	79.1	60.4	

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.2.10.2 Serum Thyroid Stimulating Hormone (TSH) Levels in Female Sprague-Dawley Rats

Increased mean response of serum TSH 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are

listed in Table B-49. The $C_{7,avg}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of effects leading to an increased serum TSH.

Table B-49. Dose-Response Modeling Data for Serum Thyroid Stimulating Hormone (TSH) Levels in Female Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (ng/mL) ^a
0	0.0	10	10.1 ± 2.6 ^b
6.25	7.8	10	14.1 ± 3.7
12.5	13.9	10	13.0 ± 4.0
25	25.7	9	14.0 ± 4.5
50	48.5	10	17.8 ± 5.4
100	93.7	8	15.8 ± 6.2

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for serum TSH are summarized in Table B-50 and Figure B-19. 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 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 Hill model is 1.7 mg/L.

Table B-50. Summary of Benchmark Dose Modeling Results for Serum Thyroid Stimulating Hormone (TSH) Levels in Female Sprague-Dawley Rats Following Exposure to PFOA (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	0.026	338.3	1.5	-1.9	52.3	31.3	EPA selected the Hill 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 Hill model had the lowest AIC.
Exponential 3	0.026	338.3	1.5	-1.9	52.3	31.3	
Exponential 4	0.342	332.6	1.2	-0.3	7.9	2.9	
Exponential 5	0.342	332.6	1.2	-0.3	7.9	2.9	
Hill	0.405	332.1	1.0	-0.2	6.2	1.7	
Polynomial Degree 5	0.045	337.0	1.3	-1.7	41.3	22.5	
Polynomial Degree 4	0.045	337.0	1.3	-1.7	41.3	22.5	
Polynomial Degree 3	0.045	337.0	1.3	-1.7	41.3	22.5	
Polynomial Degree 2	0.045	337.0	1.3	-1.7	41.3	22.5	

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			
Power	0.045	337.0	1.3	-1.7	41.3	22.5	
Linear	0.045	337.0	1.3	-1.7	41.3	22.5	

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.

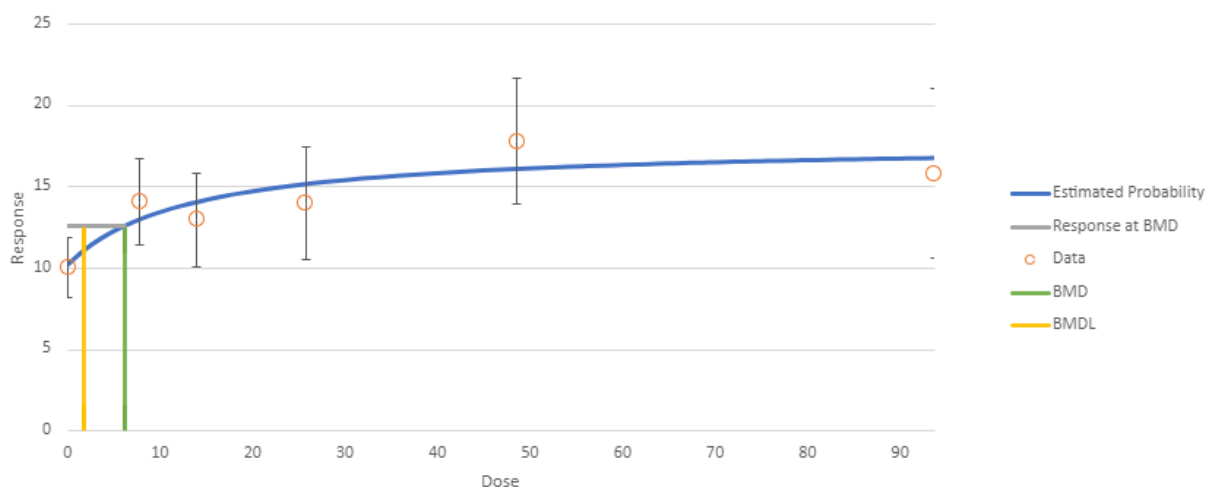


Figure B-19. Plot of Mean Response by Dose with Fitted Curve for the Selected Hill Model for Serum Thyroid Stimulating Hormone (TSH) Levels in Female Sprague-Dawley Rats Following Exposure to PFOS

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.10.3 Relative Kidney Weight in Male Sprague-Dawley Rats

Increased mean response of relative kidney weight 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-51. The $C_{7,avg}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of effects leading to increased relative kidney weight.

Table B-51. Dose-Response Modeling Data for Relative Kidney Weight in Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (mg organ weight/g body weight) ^a
0	0.0	10	2.8 ± 0.2 ^b
0.625	31.3	10	3.1 ± 0.1
1.25	57.5	10	3.4 ± 0.2
2.5	97.0	10	3.6 ± 0.2
5	142.5	10	3.6 ± 0.2
10	180.3	10	3.8 ± 0.0

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for relative kidney weight are summarized in Table B-52. No models had adequate fit (p-values greater than 0.1) therefore a LOAEL approach was taken for this endpoint.

Table B-52. Summary of Benchmark Dose Modeling Results for Relative Kidney Weight in Male Sprague-Dawley Rats Following Exposure to PFOA (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	-22.7	2.0	-2.7	71.5	48.7	No models had adequate fit (p-values were less than 0.1).
Exponential 3	<0.0001	-22.7	2.0	-2.7	70.7	48.7	
Exponential 4	0.034	-39.5	0.2	0.2	12.6	8.5	
Exponential 5	0.014	-37.6	0.1	0.1	13.9	8.6	
Hill	0.023	-38.6	-0.4	0.1	17.8	8.4	
Polynomial Degree 5	<0.0001	-24.6	2.2	-2.5	58.6	37.9	
Polynomial Degree 4	<0.0001	-24.6	2.2	-2.5	58.6	38.0	
Polynomial Degree 3	<0.0001	-24.6	2.2	-2.5	58.6	37.9	
Polynomial Degree 2	<0.0001	-24.6	2.2	-2.5	58.6	37.9	
Power	<0.0001	-24.6	2.2	-2.5	58.6	37.9	
Linear	<0.0001	-24.6	2.2	-2.5	58.6	37.9	

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.2.10.4 Absolute Body Weight in Male Sprague-Dawley Rats

Decreased mean response of Absolute Body Weight 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* (USEPA, 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 ($C_{7, \text{avg}}$) 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 decreased body weight.

Table B-53. Dose-Response Modeling Data for Absolute Body Weight in Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	10	347 ± 15.8 ^b
0.625	31.3	10	344 ± 22.1
1.25	57.5	10	331 ± 12.6
2.5	97.0	10	317 ± 22.1
5	142.5	10	304 ± 25.3
10	180.3	10	280 ± 31.6

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results Absolute Body Weight in Male Sprague-Dawley Rats are summarized in Table B-54 and Figure B-20. The best fitting model was the Linear 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 Linear model had the lowest AIC. The BMDL_{1SD} from the selected Linear model is 46.8 mg/L.

Table B-54. Summary of Benchmark Dose Modeling Results for Absolute Body Weight in Male Sprague-Dawley Rats Following Exposure to PFOA (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	0.651	545.9	0.2	-0.80	55.3	43.4	EPA selected the Linear model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and
Exponential 3	0.823	546.3	-0.3	-0.05	79.2	46.9	
Exponential 4	0.651	545.9	0.2	-0.80	55.3	43.4	
Exponential 5	0.823	546.3	-0.3	-0.05	79.2	46.9	
Hill	0.640	548.3	-0.3	-0.10	77.6	46.4	
Polynomial Degree 5	0.867	546.1	-0.2	-0.26	73.4	49.3	
Polynomial Degree 4	0.857	546.2	-0.2	-0.24	74.5	49.2	

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			
Polynomial Degree 3	0.846	546.2	−0.2	−0.22	75.8	49.1	the Linear model had the lowest AIC.
Polynomial Degree 2	0.838	546.3	−0.4	−0.14	77.9	49.0	
Power	0.835	546.3	−0.3	−0.07	78.9	49.0	
Linear	0.751	545.3	0.1	−0.71	58.6	46.8	

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.

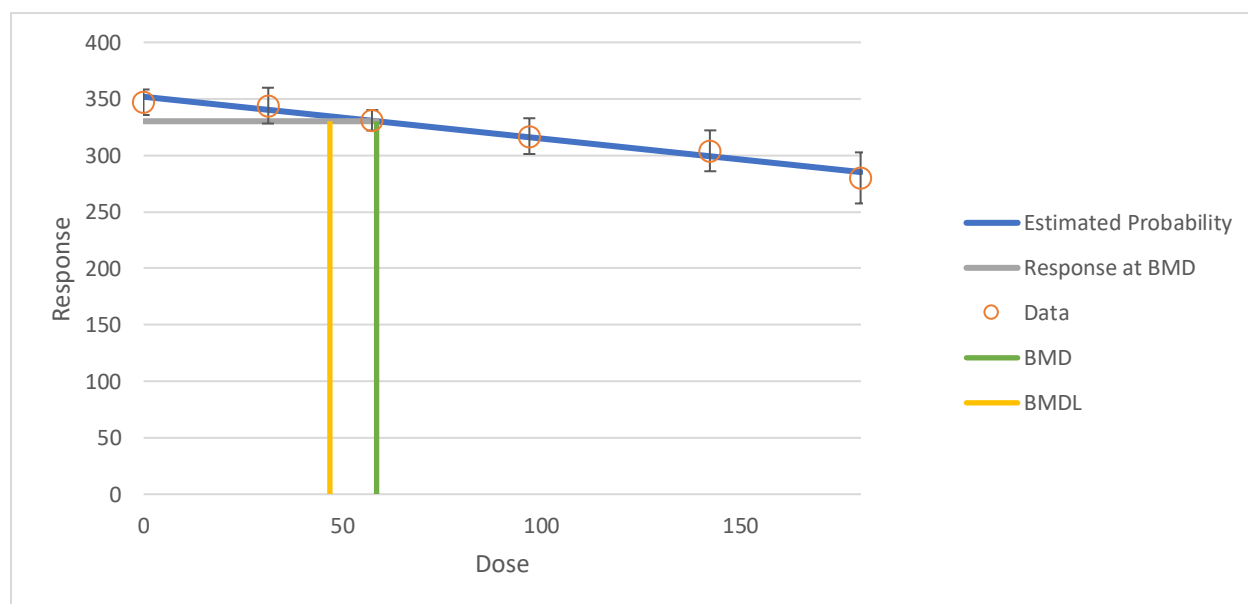


Figure B-20. Plot of Mean Response by Dose with Fitted Curve for the Selected Linear Model for Absolute Body Weight in Male Sprague-Dawley Rats Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.11 NTP, 2020, 7330145

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the NTP (2020, 7330145) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses hepatocyte single cell death, necrosis in the liver, relative kidney weight (right), hepatocellular adenomas, hepatocellular adenoma or carcinoma, and pancreatic acinar cell adenoma in F₁ male Sprague-Dawley rats and uterine adenocarcinoma in F₁ female Sprague-Dawley rats.

B.2.11.1 Hepatocyte Single Cell Death

Increased incidence of hepatocyte single cell death was observed in F₁ male Sprague-Dawley 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-55. The AUC normalized per day over entire study (AUC_{avg,pup,total}) 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 an increased incidence of hepatocyte single cell death.

Table B-55. Dose-Response Modeling Data for Hepatocyte Single Cell Death in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (ppm) ^a	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	1
300 / 0	0.3	50	1
0 / 20	72.6	50	1
300 / 20	73.5	50	3
0 / 40	113.5	50	11
300 / 40	115.1	50	5
0 / 80	161.7	50	24
300 / 80	161.7	50	29

^aDoses are presented as perinatal exposure/postnatal exposure.

Hepatocyte single cell death was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2).

The BMD modeling results for hepatocyte single cell death following postweaning exposure to PFOA are summarized in Table B-56 and Figure B-21. 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 77.1 mg/L.

Table B-56. Summary of Benchmark Dose Modeling Results for Hepatocyte Single Cell Death in F₁ Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA

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	— ^b	149.5	0.001	0.03	104.6	85.9	EPA selected the Multistage Degree 3 model. All models, except Dichotomous Hill, Multistage Degree 2, Multistage Degree 1, and Probit, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 3 model had the lowest AIC.
Gamma	0.308	148.6	0.643	0.29	98.8	82.2	
Log-Logistic	0.262	148.9	0.671	0.32	98.5	81.5	
Multistage Degree 3	0.354	148.2	-1.311	0.46	89.9	77.1	
Multistage Degree 2	0.064	152.8	-2.003	0.52	73.8	61.9	
Multistage Degree 1	0.001	162.8	-2.810	0.42	44.6	33.8	
Weibull	0.200	149.3	0.804	0.32	98.4	80.1	
Logistic	0.222	148.7	-1.236	1.02	92.3	77.8	
Log-Probit	0.389	148.3	0.522	0.27	98.5	82.8	
Probit	0.090	149.7	-1.372	1.67	86.8	72.1	

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.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

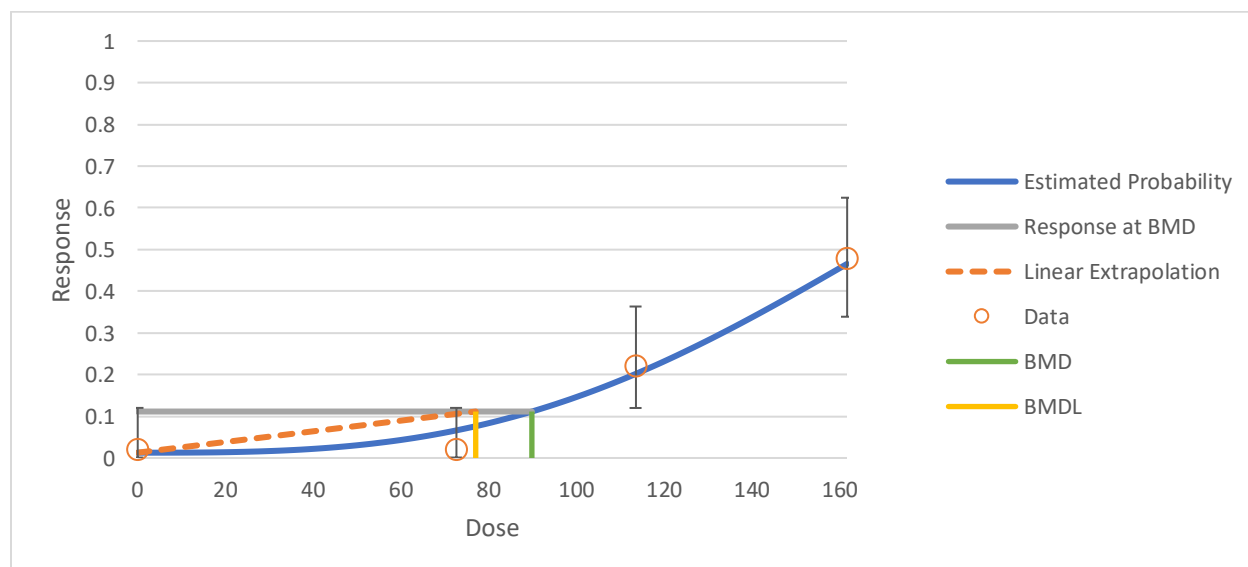


Figure B-21. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocyte Single Cell Death in F₁ Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The benchmark dose (BMD) modeling results for hepatocyte single cell death following perinatal and postweaning exposure to PFOA are summarized in Table B-57 and Figure B-22. The best fitting model was the Gamma 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 Gamma 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 Gamma model is 100.1 mg/L.

Table B-57. Summary of Benchmark Dose Modeling Results for Hepatocyte Single Cell Death in F₁ Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

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	— ^b	142.1	−0.07	−0.7	121.2	101.3	EPA selected the Gamma model. The Gamma, Log-Logistic, Weibull, and Log-Probit had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Gamma model had the lowest AIC.
Gamma	0.428	138.7	−0.69	−0.6	114.8	100.1	
Log-Logistic	0.320	140.1	−0.07	−0.7	121.1	101.3	
Multistage Degree 3	0.043	144.1	−0.30	0.4	88.6	77.4	
Multistage Degree 2	0.002	151.1	−1.19	0.5	72.4	61.5	
Multistage Degree 1	<0.0001	163.2	−2.14	0.4	43.0	32.7	
Weibull	0.330	140.0	−0.12	−0.6	121.1	98.2	
Logistic	0.044	141.9	−1.46	1.9	97.0	82.3	
Log-Probit	0.308	140.1	0.01	−0.7	121.0	105.1	
Probit	0.004	145.0	0.04	2.5	89.3	74.6	

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.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

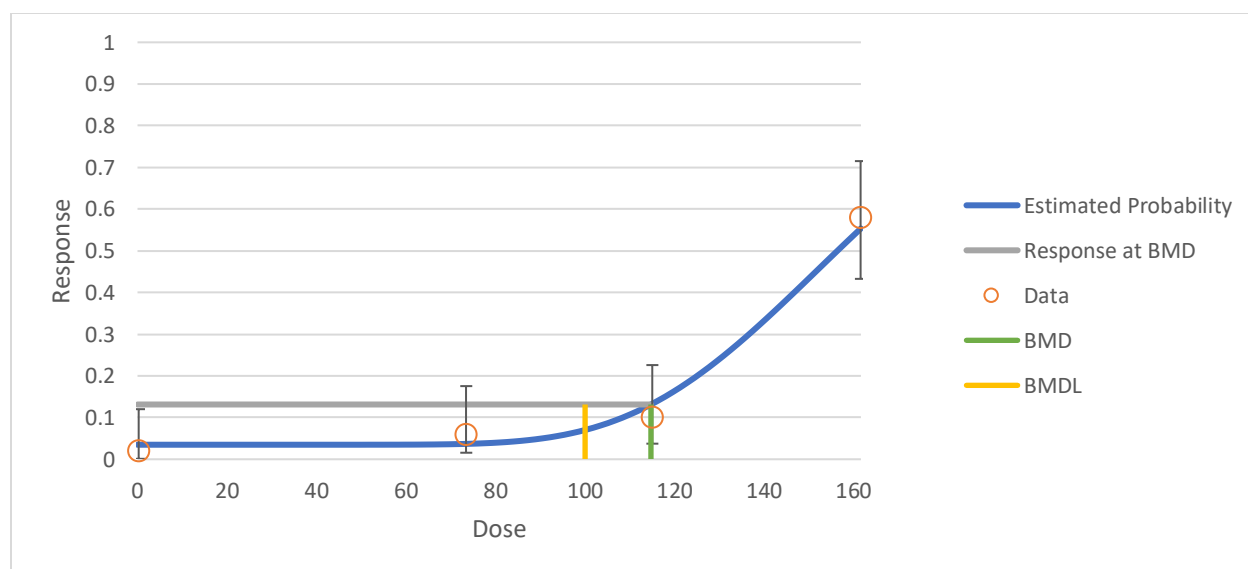


Figure B-22. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Gamma Model for Hepatocyte Single Cell Death in F1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The benchmark dose (BMD) modeling results for hepatocyte single cell death using a pooled method are summarized in Table B-58 and Figure B-23. The best fitting model was the Multistage Degree 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 Multistage Degree 4 model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level BMDL₁₀ from the selected Multistage Degree 4 model is 90.8 mg/L.

Table B-58. Summary of Benchmark Dose Modeling Results for Hepatocyte Single Cell Death in F1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled)

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	0.274	287.8	1.2	-0.07	105.3	92.4	EPA selected the Multistage Degree 4 model. All models, except Multistage Degree 7, Multistage Degree 2, Multistage Degree 1, and Probit, had adequate fit (p-values greater than
Gamma	0.380	285.9	1.1	-0.14	105.2	92.0	
Log-Logistic	0.400	285.8	1.2	-0.07	105.3	92.4	
Multistage Degree 7	0.081	291.7	1.2	0.01	104.9	91.0	
Multistage Degree 6	0.285	287.7	1.2	0.01	104.9	91.3	
Multistage Degree 5	0.413	285.7	1.2	0.01	104.9	91.5	

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 4	0.536	284.1	0.9	0.17	100.5	90.8	0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 4 model had the lowest AIC.
Multistage Degree 3	0.209	288.3	-0.3	0.43	89.3	82.6	
Multistage Degree 2	0.005	299.9	-1.2	0.52	73.1	66.0	
Multistage Degree 1	<0.0001	322.0	-2.8	0.46	43.8	35.9	
Weibull	0.413	285.7	1.2	0.00	104.8	91.7	
Logistic	0.160	287.0	0.8	1.39	94.6	84.2	
Log-Probit	0.351	286.1	1.1	-0.23	106.0	92.4	
Probit	0.015	290.9	-0.2	2.07	88.0	77.6	

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.

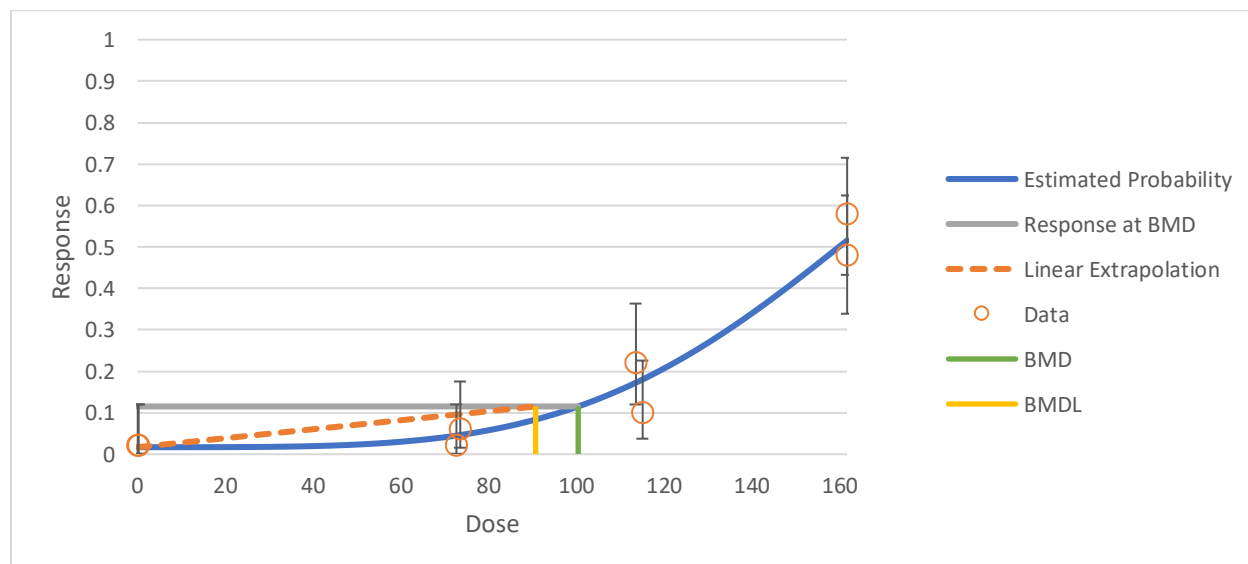


Figure B-23. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 4 Model for Hepatocyte Single Cell Death in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.11.2 Necrosis in the Liver

Increased incidence of necrosis was observed in F₁ 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-59. The AUC normalized per day over entire study

(AUC_{avg,pup,total}) 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 necrosis.

Table B-59. Dose-Response Modeling Data for Necrosis in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (ppm) ^a	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	2
300 / 0	0.3	50	1
0 / 20	72.6	50	17
300 / 20	73.5	50	11
0 / 40	113.5	50	23
300 / 40	115.1	50	14
0 / 80	161.7	50	20
300 / 80	161.7	50	21

^aDoses are presented as perinatal exposure/postnatal exposure.

Necrosis in the liver was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2).

The BMD modeling results for necrosis in the liver following postweaning exposure to PFOA are summarized in Table B-60 and Figure B-24. The best fitting model was the Log-Logistic model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Log-Logistic model had the lowest AIC. The BMDL₁₀ from the selected Log-Logistic model is 15.3 mg/L.

Table B-60. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver in F₁ Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA

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	— ^b	225.6	0.0	−0.001	−0.001	— ^c	EPA selected the Log-Logistic model. All models, except the Dichotomous Hill, Logistic and Probit, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently
Gamma	0.160	224.8	−0.2	−0.239	−0.239	20.7	
Log-Logistic	0.307	223.6	−0.1	−0.099	−0.099	15.3	
Multistage Degree 3	0.160	224.8	−0.2	−0.239	−0.239	20.7	
Multistage Degree 2	0.160	224.8	−0.2	−0.239	−0.239	20.7	
Multistage Degree 1	0.160	224.8	−0.2	−0.239	−0.239	20.7	

Model ^a	Goodness of Fit		Scaled Residual		BMD ₁₀ (mg/L)	BMDL ₁₀ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Weibull	0.160	224.8	−0.2	−0.239	−0.239	20.7	close (less than threefold difference), and the Log-Logistic model had the lowest AIC.
Logistic	0.008	231.4	1.4	1.440	−1.714	43.9	
Log-Probit	0.307	224.2	0.0	0.001	0.001	— ^c	
Probit	0.011	230.6	1.4	1.449	−1.537	41.6	

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.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^cLower limit includes zero; BMDL not estimated.

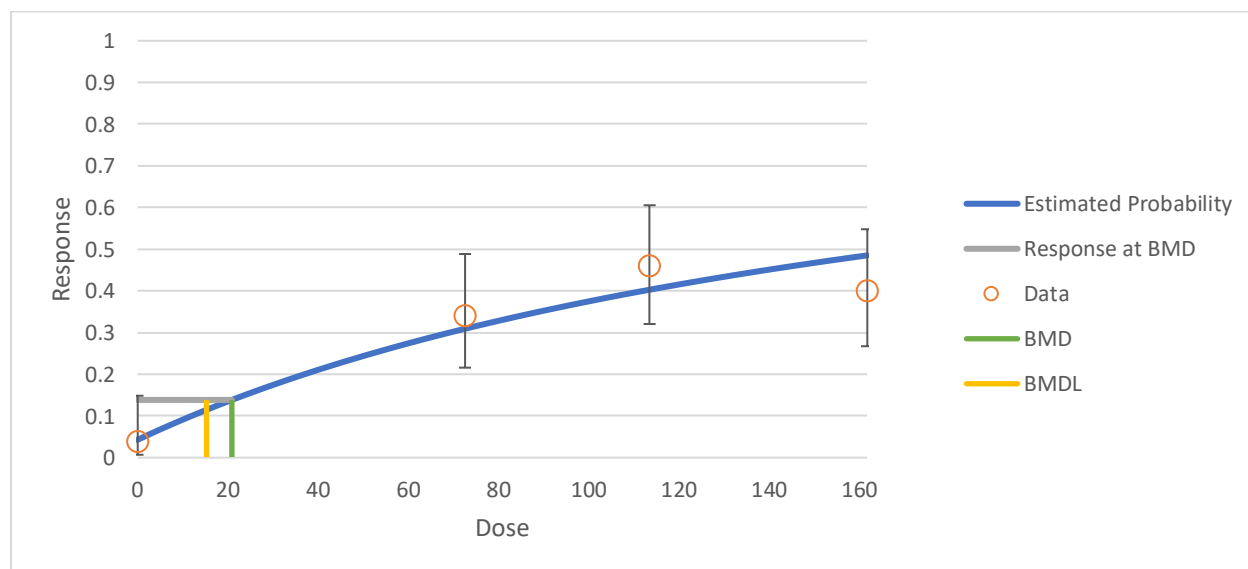


Figure B-24. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Log-Logistic Model for Necrosis in the Liver in F₁ Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for necrosis in the liver following perinatal and postweaning exposure to PFOA are summarized in Table B-61 and Figure B-25. The Dichotomous Hill model was saturated and while the Log-probit model had adequate fit, the BMD/BMDL ratio was larger than three. Of the remaining models, the selected model was the Multistage degree 1 model based on adequate p-values (greater than 0.1) and visual inspection. The BMDL₁₀ from the selected Multistage Degree 1 model is 26.9 mg/L.

Table B-61. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver in F1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

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	– ^b	198.1	0.225	–0.009	40.6	22.4	EPA selected the Multistage Degree 1 model. All models, except Dichotomous Hill, had adequate fit (p-values greater than 0.1). The log-probit model had a BMD/BMDL ratio greater than three. The Multistage Degree 1 model had the lowest AIC of the remaining models.
Gamma	0.611	196.1	0.212	–0.007	38.5	27.0	
Log-Logistic	0.585	196.1	0.224	–0.009	40.6	22.4	
Multistage Degree 3	0.645	196.0	0.266	–0.020	37.7	27.0	
Multistage Degree 2	0.627	196.1	0.246	–0.014	38.1	27.0	
Multistage Degree 1	0.869	194.1	0.013	0.013	34.8	26.9	
Weibull	0.614	196.1	0.220	–0.008	38.5	27.0	
Logistic	0.267	196.7	1.149	–1.063	67.9	57.2	
Log-Probit	0.567	196.2	0.224	–0.009	42.9	3.2	
Probit	0.348	196.0	1.095	–0.863	63.7	53.6	

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.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

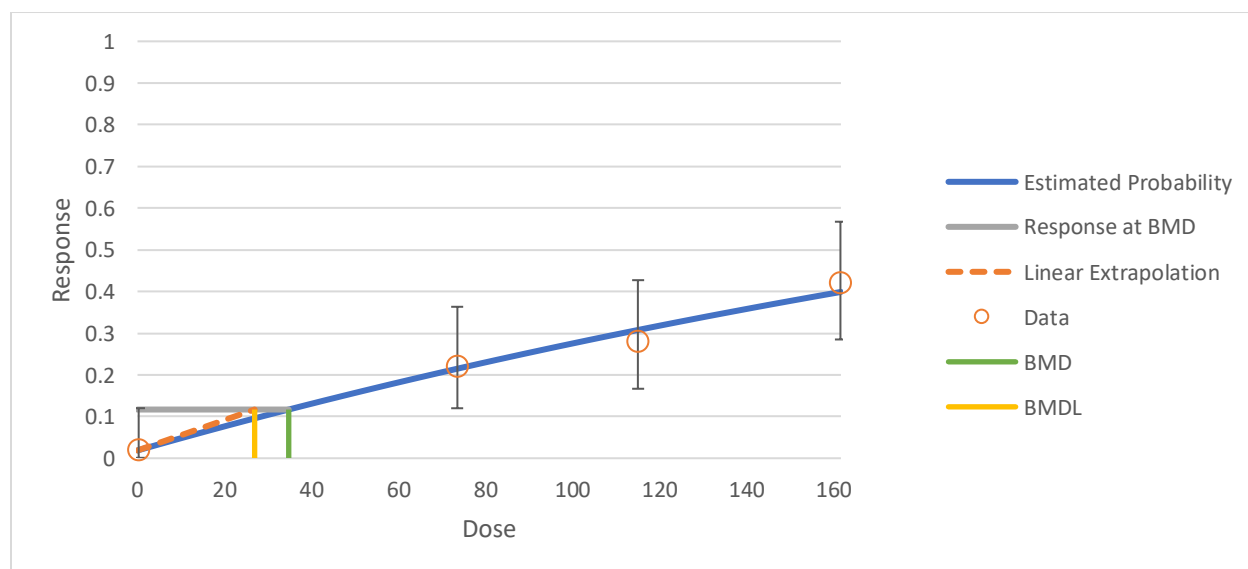


Figure B-25. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Necrosis in the Liver in F1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for necrosis using pooled methods are summarized in Table B-62. No models provided an adequate fit, therefore a lowest-observed-adverse-effect-level (LOAEL) approach was taken for this endpoint.

Table B-62. Summary of Benchmark Dose Modeling Results for Necrosis in the Liver in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled)

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	<0.0001	447.4	-0.7	0.7	19.4	7.8	No models had adequate fit (p-values were less than 0.1).
Gamma	<0.0001	443.6	-0.8	0.7	17.9	15.4	
Log-Logistic	<0.0001	445.4	-0.7	0.7	18.9	10.7	
Multistage Degree 7	<0.0001	443.6	-0.8	0.7	17.9	15.4	
Multistage Degree 6	<0.0001	443.6	-0.8	0.7	17.9	15.4	
Multistage Degree 5	<0.0001	443.6	-0.8	0.7	17.9	15.4	
Multistage Degree 4	<0.0001	443.6	-0.8	0.7	17.9	15.4	
Multistage Degree 3	<0.0001	443.6	-0.8	0.7	17.9	15.4	
Multistage Degree 2	<0.0001	443.6	-0.8	0.7	17.9	15.4	
Multistage Degree 1	<0.0001	443.6	-0.8	0.7	17.9	15.4	
Weibull	<0.0001	443.6	-0.8	0.7	17.9	15.4	
Logistic	<0.0001	454.9	4.1	-1.0	40.6	35.8	
Log-Probit	<0.0001	445.4	-0.7	0.7	20.7	4.6	
Probit	<0.0001	453.2	4.0	-0.7	38.3	34.0	

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.

B.2.11.3 Relative Kidney Weight (Right)

Increased mean response of relative kidney weight (right) was observed in F₁ 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-63. The AUC normalized per day over entire study (AUC_{avg,pup,total}) 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 increased kidney weight.

Table B-63. Dose-Response Modeling Data for Relative Kidney Weight (Right) in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (mg/kg/day) ^a	Internal Dose (mg/L)	Number per Group	Mean Response (mg/g) ^b
0 / 0	0	10	2.7 ± 0.2 ^c
300 / 0	0.3	10	2.7 ± 0.2
0 / 20	72.6	10	3.4 ± 0.2
300 / 20	73.4	10	3.3 ± 0.2
0 / 40	113.5	10	3.6 ± 0.1
300 / 40	115.1	10	3.4 ± 0.2
0 / 80	161.7	10	3.4 ± 0.2
300 / 80	161.7	10	3.5 ± 0.2

^aDoses are presented as perinatal exposure/postnatal exposure.

^bData are presented as mean ± standard deviation.

^cStandard deviations were calculated from standard errors.

Relative kidney weight (right) was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2).

The BMD modeling results for relative kidney weight (right) following postweaning exposure to PFOA are summarized in Table B-64. No models provided an adequate fit, therefore a LOAEL approach was taken for this endpoint.

Table B-64. Summary of Benchmark Dose Modeling Results for Relative Kidney Weight (Right) in F₁ Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA (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	13.4	2.19	-2.24	59.4	46.8	No models had adequate fit (p-values were less than 0.1)
Exponential 3	<0.0001	13.4	2.19	-2.24	59.4	46.8	
Exponential 4	0.012	-11.3	0.02	0.02	8.9	2.2	
Exponential 5	— ^a	-11.2	-0.02	2.4×e ⁻³	38.5	5.9	
Hill	0.035	-13.2	6.6×e ⁻⁴	-2.1×e ⁻⁵	62.0	4.3	
Polynomial Degree 3	<0.0001	10.9	2.16	-1.91	50.6	39.2	
Polynomial Degree 2	<0.0001	10.9	2.16	-1.91	50.6	39.2	
Power	<0.0001	10.9	2.16	-1.91	50.6	39.2	
Linear	<0.0001	10.9	2.16	-1.91	50.6	39.2	

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.

^aDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

The BMD modeling results for relative kidney weight (right) following perinatal and postweaning exposure to PFOA are summarized in Table B-65 and Figure B-26. 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 4.6 mg/L.

Table B-65. Summary of Benchmark Dose Modeling Results for Relative Kidney Weight (Right) in F₁ Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (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	0.001	-12.9	2.60	-1.82	43.0	34.9	EPA selected the Hill 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 Hill model had the lowest AIC.
Exponential 3	0.001	-12.9	2.60	-1.82	43.0	34.9	
Exponential 4	0.479	-25.6	-0.03	-0.03	13.2	8.2	
Exponential 5	0.479	-25.6	-0.03	-0.03	13.2	8.2	
Hill	0.576	-25.8	-0.01	-0.01	10.9	4.6	
Polynomial Degree 3	0.002	-15.4	2.49	-1.50	37.0	29.6	
Polynomial Degree 2	0.002	-15.4	2.49	-1.50	37.0	29.6	
Power	0.002	-15.4	2.49	-1.50	37.0	29.6	
Linear	0.002	-15.4	2.49	-1.50	37.0	29.6	

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.

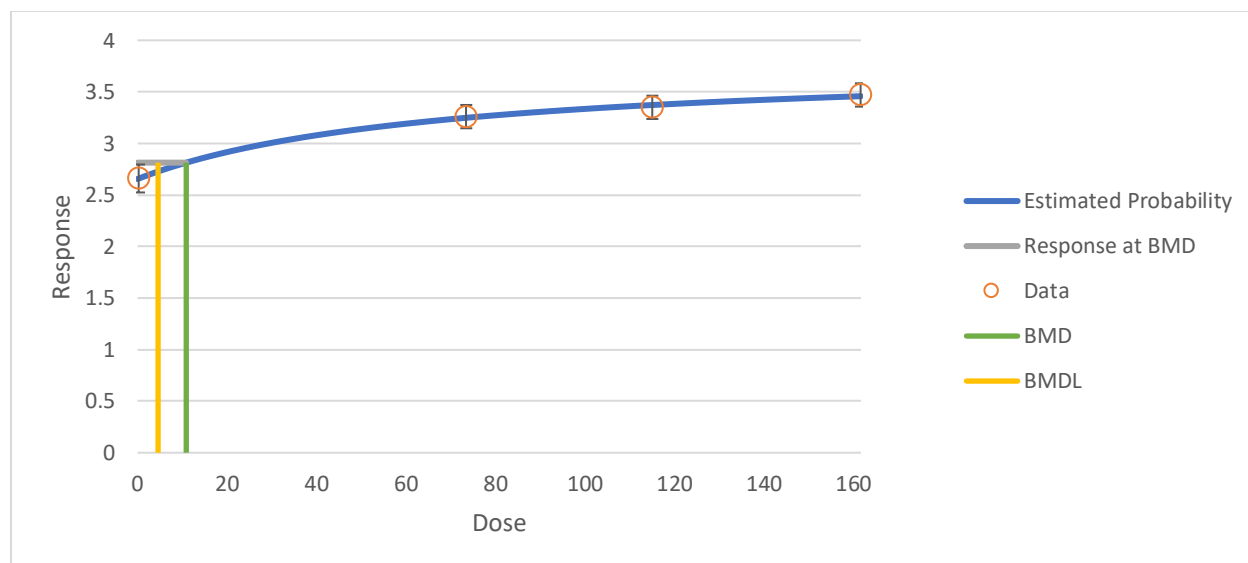


Figure B-26. Plot of Mean Response by Dose with Fitted Curve for the Selected Relative Kidney Weight (Right) in F1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA (constant variance)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for relative kidney weight (right) using pooled methods are summarized in Table B-66. No models provided an adequate fit, therefore a LOAEL approach was taken for this endpoint.

Table B-66. Summary of Benchmark Dose Modeling Results for Relative Kidney Weight (Right) in F1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled) (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	1.0	3.00	-2.006	52.6	44.6	No models had adequate fit (p-values were less than 0.1)
Exponential 3	<0.0001	1.0	3.00	-2.005	52.6	44.6	
Exponential 4	0.018	-37.7	-0.04	0.072	10.9	7.1	
Exponential 5	0.016	-37.1	0.92	-0.001	38.4	8.3	
Hill	0.015	-36.9	0.93	-0.001	52.3	4.9	
Polynomial Degree 7	<0.0001	-4.0	2.96	-1.685	45.1	39.8	
Polynomial Degree 6	<0.0001	-4.0	2.96	-1.685	45.1	40.1	
Polynomial Degree 5	<0.0001	-4.0	2.96	-1.685	45.1	42.6	
Polynomial Degree 4	<0.0001	-4.0	2.96	-1.685	45.1	37.8	

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			
Polynomial Degree 3	<0.0001	-4.0	2.96	-1.685	45.1	37.8	
Polynomial Degree 2	<0.0001	-4.0	2.96	-1.685	45.1	37.8	
Power	<0.0001	-4.0	2.96	-1.685	45.1	37.8	
Linear	<0.0001	-4.0	2.96	-1.685	45.1	37.8	

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.2.11.4 Hepatocellular Adenomas

Increased incidence of hepatocellular adenomas was observed in F₁ 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-67. The AUC normalized per day over entire study (AUC_{avg,pup,total}) was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of hepatocellular adenomas.

Table B-67. Dose-Response Modeling Data for Hepatocellular Adenomas in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (ppm) ^a	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	0
300 / 0	0.3	50	0
0 / 20	72.6	50	0
300 / 20	73.5	50	1
0 / 40	113.5	50	7
300 / 40	115.1	50	5
0 / 80	161.7	50	11
300 / 80	161.7	50	10

^aDoses are presented as perinatal exposure/postnatal exposure.

Hepatocellular adenomas were assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2).

The BMD modeling results for hepatocellular adenomas following postnatal exposure are summarized in Table B-68 and Figure B-27. The best fitting model was the Multistage Degree 3 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 3 model had the lowest AIC. The BMDL₁₀ from the selected Multistage Degree 3 model is 95.3 mg/L.

Table B-68. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenomas in F₁ Male Sprague-Dawley Rats Following Postnatal Exposure to PFOA

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	0.420	99.1	1.2	-0.001	117.1	95.3	EPA selected the Multistage Degree 3 model. All models, except Multistage Degree 1, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 3 model had the lowest AIC.
Multistage Degree 2	0.397	100.4	0.7	-0.001	108.8	88.7	
Multistage Degree 1	0.064	106.5	0.4	-0.001	94.1	65.3	

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.

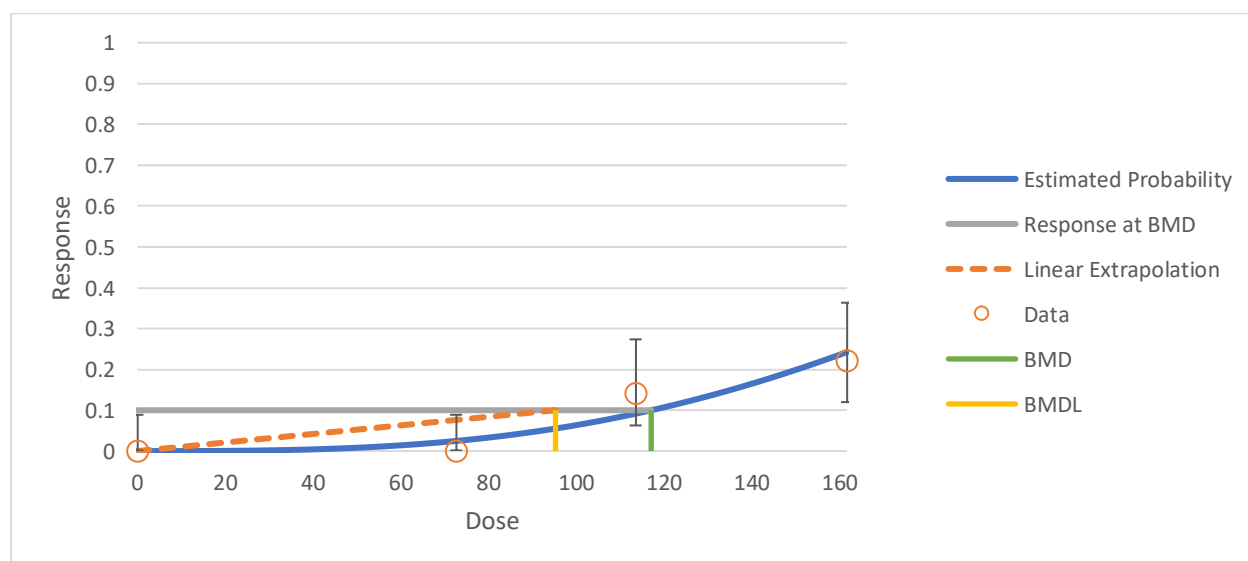


Figure B-27. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocellular Adenomas in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for hepatocellular adenomas following perinatal and postnatal exposure are summarized in Table B-69 and Figure B-28. 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 92.9 mg/L.

Table B-69. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenomas in F₁ Male Sprague-Dawley Rats Following Perinatal and Postnatal Exposure to PFOA

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	0.897	96.6	0.4	−0.003	122.0	96.6	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.
Multistage Degree 2	0.883	95.1	0.1	−0.007	116.7	92.9	
Multistage Degree 1	0.379	98.0	−0.1	−0.1	107.8	73.3	

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.

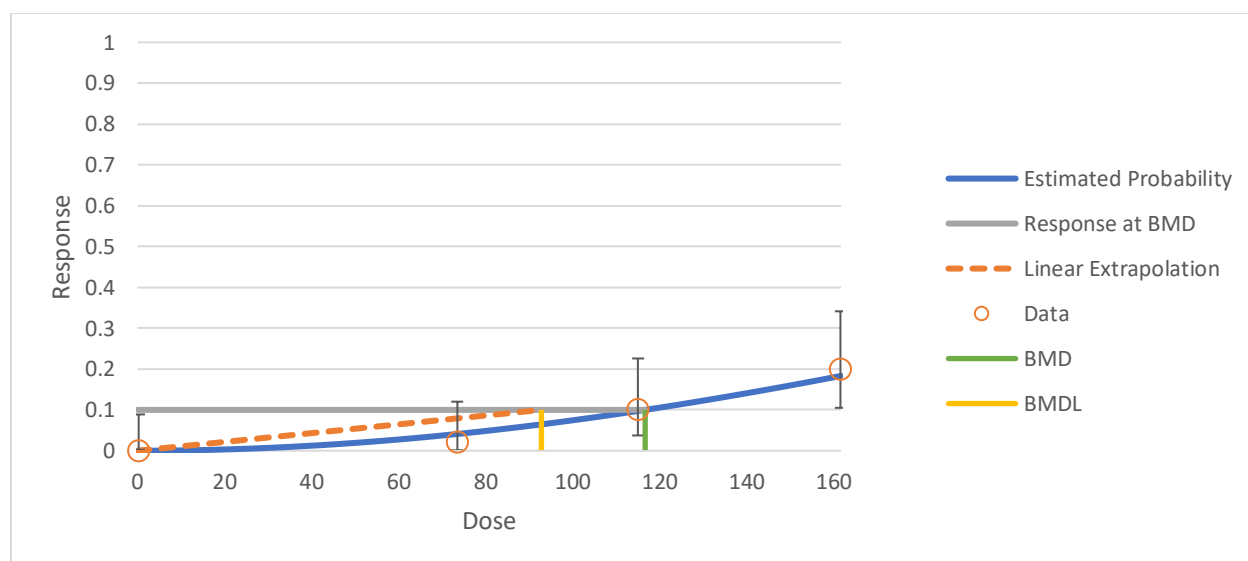


Figure B-28. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Hepatocellular Adenomas in F1 Male Sprague-Dawley Rats Following Perinatal and Postnatal Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for hepatocellular adenomas using pooled methods are summarized in Table B-70 and Figure B-29. The best fitting model was the Multistage Degree 3 model based on adequate p-values (greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models. Four models (Multistage Degree 3, 4, 5, and 6) had the same lowest AIC value. The BMDL₁₀ from the selected Multistage Degree 3 model is 104.1 mg/L.

Table B-70. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenomas in F1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled)

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 7	0.755	193.8	0.3	−0.001	119.9	104.1	EPA selected the Multistage Degree 3 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference).
Multistage Degree 6	0.844	191.8	0.3	−0.001	119.9	104.1	
Multistage Degree 5	0.844	191.8	0.3	−0.001	119.9	104.1	
Multistage Degree 4	0.844	191.8	0.3	−0.001	119.9	104.1	
Multistage Degree 3	0.844	191.8	0.3	−0.001	119.9	104.1	
Multistage Degree 2	0.787	193.7	0.9	−0.001	112.6	98.4	

Model ^a	Goodness of Fit		Scaled Residual		BMD ₁₀ (mg/L)	BMDL ₁₀ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Multistage Degree 1	0.180	202.7	0.6	–0.001	100.5	76.8	

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.

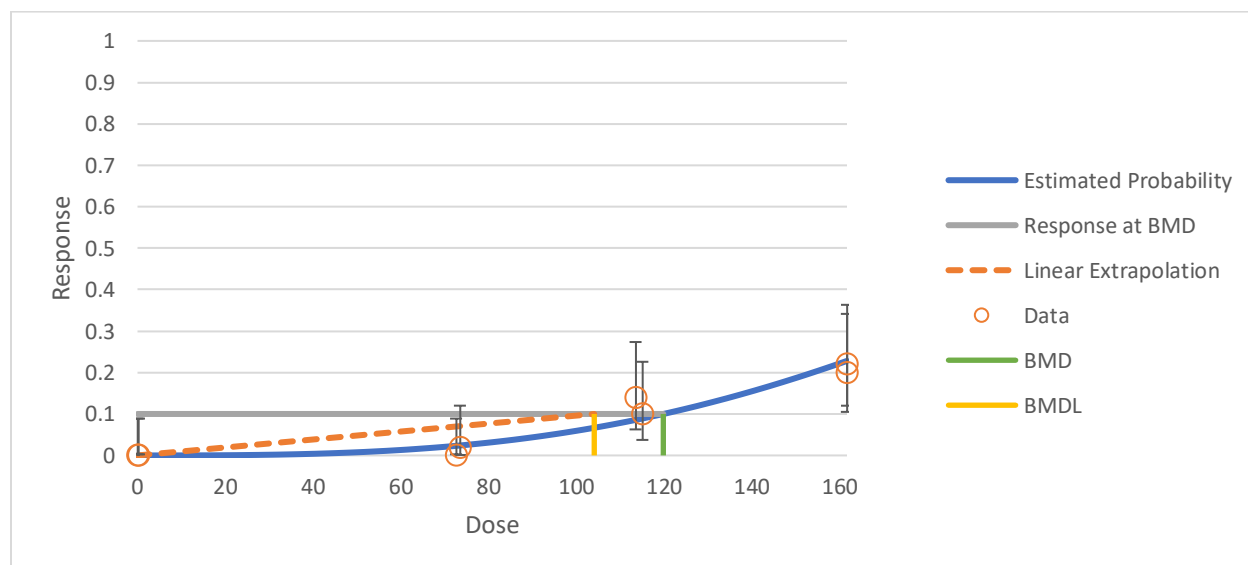


Figure B-29. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocellular Adenomas in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.11.5 Hepatocellular Adenoma or Carcinoma

Increased incidence of hepatocellular adenoma or carcinoma was observed in F₁ 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-71. The AUC normalized per day over entire study (AUC_{avg,pup,total}) was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of hepatocellular adenomas or carcinomas.

Table B-71. Dose-Response Modeling Data for Hepatocellular Adenoma or Carcinoma in F1 Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (ppm) ^a	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	0
300 / 0	0.3	50	0
0 / 20	72.6	50	0
300 / 20	73.5	50	1
0 / 40	113.5	50	7
300 / 40	115.1	50	5
0 / 80	161.7	50	11
300 / 80	161.7	50	12

^aDoses are presented as perinatal exposure/postnatal exposure.

Hepatocellular adenoma or carcinoma was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2). The dose response data (1) following postweaning exposure was the same between hepatocellular adenoma and hepatocellular adenoma or carcinoma therefore this modeling information can be found in Table B-68 and Figure B-27.

The BMD modeling results for hepatocellular adenoma or carcinoma following perinatal and postnatal exposure to PFOA are summarized in Table B-72 and Figure B-30. 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 88.6 mg/L.

Table B-72. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma in F1 Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

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	0.961	101.5	0.1	-0.001	117.5	95.7	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
Multistage Degree 2	0.753	100.8	-0.2	-0.007	109.3	88.6	

Model ^a	Goodness of Fit		Scaled Residual		BMD ₁₀ (mg/L)	BMDL ₁₀ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Multistage Degree 1	0.200	104.8	-0.4	-0.136	94.8	65.9	threefold difference), and the Multistage Degree 2 model had the lowest AIC.

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.

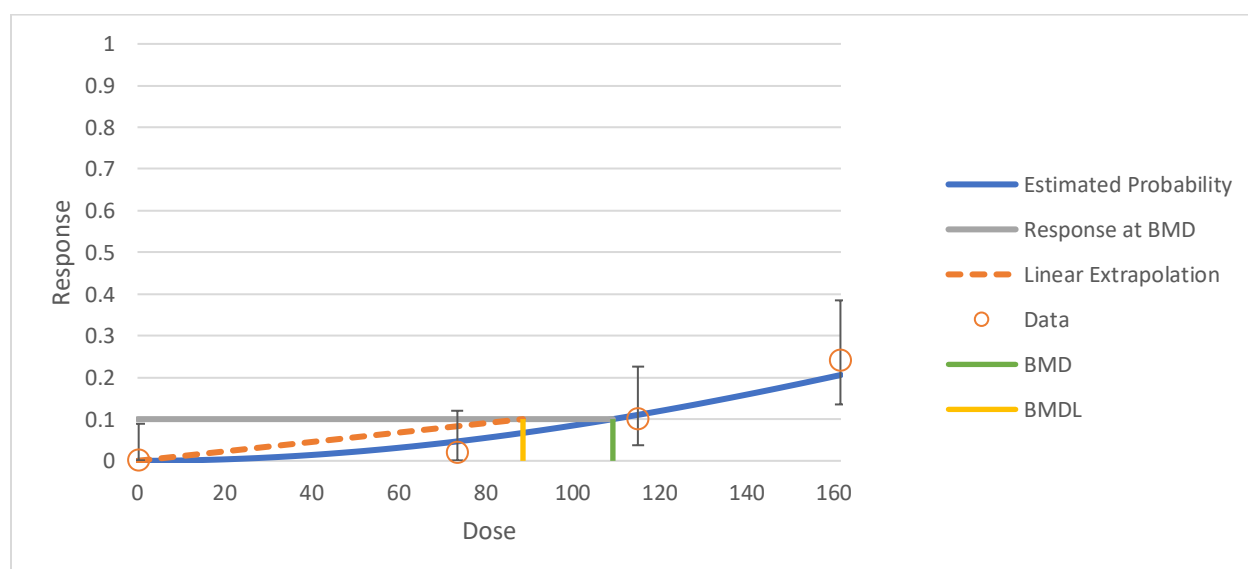


Figure B-30. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Hepatocellular Adenoma or Carcinoma in F₁ Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for hepatocellular adenoma or carcinoma using pooled methods are summarized in Table B-73 and Figure B-31. The best fitting model was the Multistage Degree 3 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models. Three models (Multistage Degree 3, 4, and 7) had the same lowest AIC value. The BMDL₁₀ from the selected Multistage Degree 3 model is 103.7 mg/L.

Table B-73. Summary of Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled)

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 7	0.820	198.6	0.1	−0.001	117.3	103.7	EPA selected the Multistage Degree 3 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 3 was one of the models with the lowest AIC.
Multistage Degree 6	0.714	200.6	0.1	−0.001	117.3	103.7	
Multistage Degree 5	0.714	200.6	0.1	−0.001	117.3	103.7	
Multistage Degree 4	0.819	198.6	0.1	−0.001	117.3	103.7	
Multistage Degree 3	0.820	198.6	0.1	−0.001	117.3	103.7	
Multistage Degree 2	0.760	199.2	0.7	−0.001	109.0	95.6	
Multistage Degree 1	0.180	207.3	0.5	−0.001	94.5	72.6	

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.

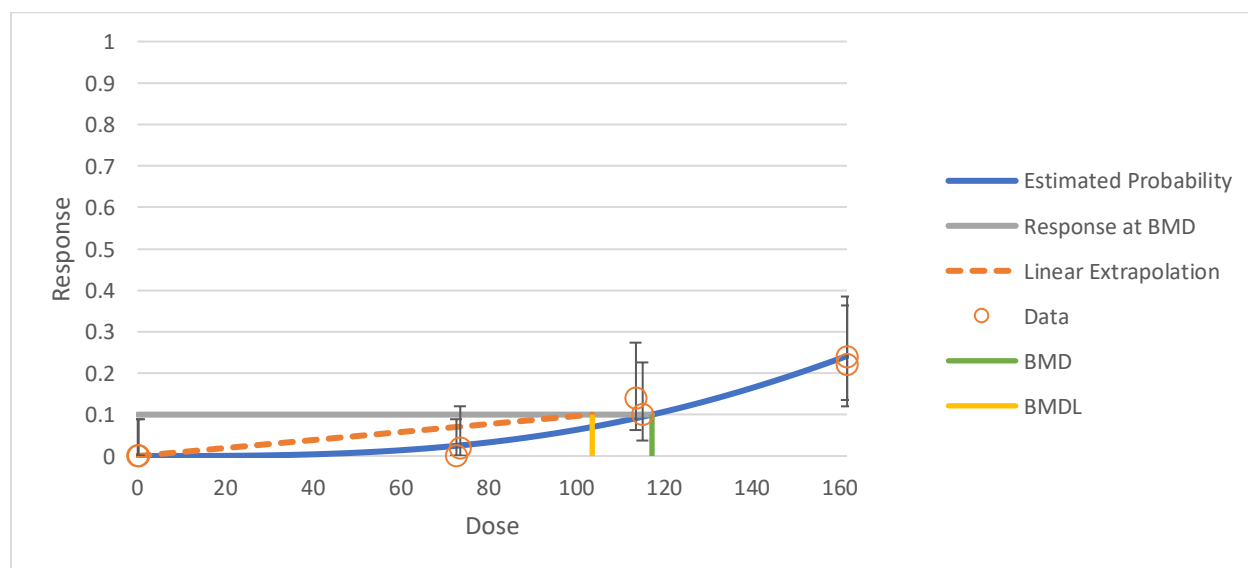


Figure B-31. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Hepatocellular Adenoma or Carcinoma in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.11.6 Pancreatic Acinar Cell Adenoma

Increased incidence of pancreatic acinar cell adenoma was observed in F₁ 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-74. The AUC normalized per day over entire study (AUC_{avg,pup,total}) was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of pancreatic acinar cell adenomas.

Table B-74. Dose-Response Modeling Data for Pancreatic Acinar Cell Adenoma in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA

Administered Dose (ppm) ^a	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	3
300 / 0	0.3	50	7
0 / 20	72.6	50	28
300 / 20	73.5	50	18
0 / 40	113.5	50	26
300 / 40	115.1	50	30
0 / 80	161.7	50	32
300 / 80	161.7	50	30

^aDoses are presented as perinatal exposure/postnatal exposure.

Pancreatic acinar cell adenoma was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2).

The BMD modeling results for pancreatic acinar cell adenoma following postweaning exposure to PFOA are summarized in Table B-75 and Figure B-32. All Multistage models had adequate p-values (greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference), and the same AIC values. The higher degree Multistage models identified model parameters of zero for higher power terms, so all the Multistage models give the same result. Degree 3 model was selected and shown below is the same as Degree 1 and 2 Multistage models. The BMDL₁₀ from the selected Multistage Degree 3 model is 12.6 mg/L.

Table B-75. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA

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	0.105	234.3	-0.3	-0.3	15.5	12.6	EPA selected the Multistage Degree 3 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the same AIC.
Multistage Degree 2	0.105	234.3	-0.3	-0.3	15.5	12.6	
Multistage Degree 1	0.105	234.3	-0.3	-0.3	15.5	12.6	

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.

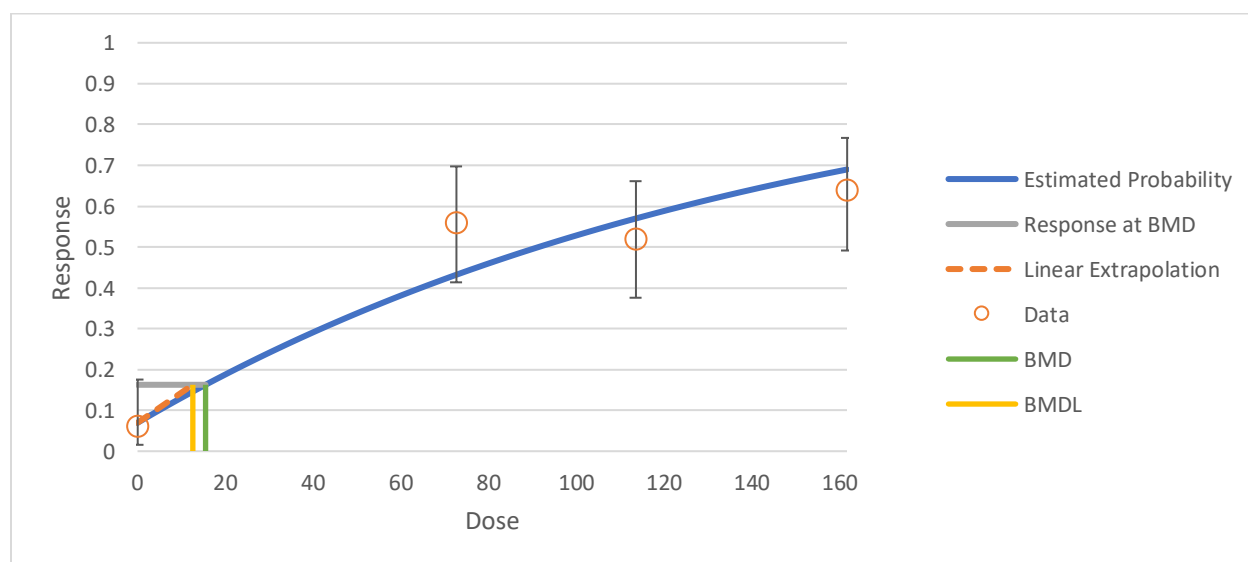


Figure B-32. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 3 Model for Pancreatic Acinar Cell Adenoma in F1 Male Sprague-Dawley Rats Following Postweaning Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for pancreatic acinar cell adenoma following perinatal and postweaning exposure are summarized in Table B-76 and Figure B-33. The best fitting model was the Multistage Degree 1 model based on adequate p-values (greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the

Multistage Degree 1 model had the lowest AIC. The BMDL₁₀ from the selected Multistage Degree 1 model is 15.7 mg/L.

Table B-76. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F₁ Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

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	0.178	248.3	0.1	0.1	20.6	15.7	EPA selected the Multistage Degree 1 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Multistage Degree 1 model had the lowest AIC.
Multistage Degree 2	0.178	248.3	0.1	0.1	20.6	15.7	
Multistage Degree 1	0.404	246.3	0.1	0.1	20.2	15.7	

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.

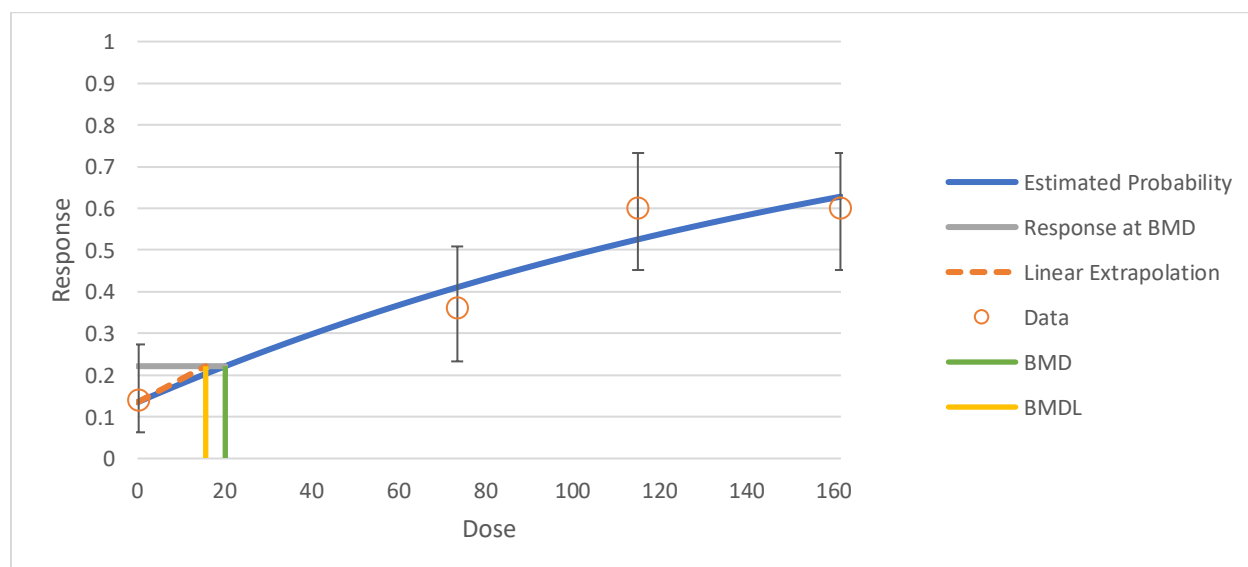


Figure B-33. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model Pancreatic Acinar Cell Adenoma in F₁ Male Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for pancreatic acinar cell adenoma using the pooled method are summarized in Table B-77 and Figure B-34. All Multistage models had adequate p-values (greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference), and the same AIC values. The higher degree Multistage models identified model parameters of zero for higher power terms, so all the Multistage models give the same result. Therefore, the Multistage Degree 1 model was selected. The BMDL₁₀ from the selected Multistage Degree 1 model is 15.0 mg/L.

Table B-77. Summary of Benchmark Dose Modeling Results for Pancreatic Acinar Cell Adenoma in F1 Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled)

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 7	0.238	478.4	0.8	-1.0	17.7	15.0	EPA selected the Multistage Degree 1 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the same AIC value.
Multistage Degree 6	0.238	478.4	0.8	-1.0	17.7	15.0	
Multistage Degree 5	0.238	478.4	0.8	-1.0	17.7	15.0	
Multistage Degree 4	0.238	478.4	0.8	-1.0	17.7	15.0	
Multistage Degree 3	0.238	478.4	0.8	-1.0	17.7	15.0	
Multistage Degree 2	0.238	478.4	0.8	-1.0	17.7	15.0	
Multistage Degree 1	0.238	478.4	0.8	-1.0	17.7	15.0	

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.

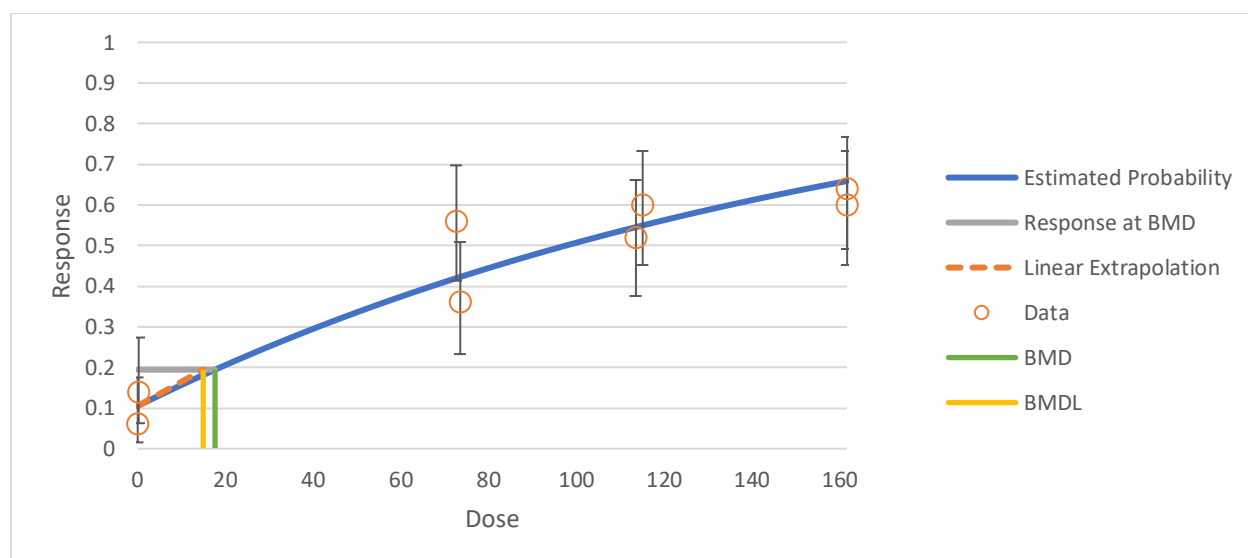


Figure B-34. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Pancreatic Acinar Cell Adenoma in F₁ Male Sprague-Dawley Rats Following Exposure to PFOA (Pooled)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.11.7 Uterine Adenocarcinoma

Increased incidence of uterine adenocarcinoma was observed in F₁ 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* (USEPA, 2012, 1239433). The doses and response data used for the modeling are listed in Table B-78. The AUC normalized per day over entire study (AUC_{avg,pup,total}) was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of uterine adenocarcinoma.

Table B-78. Dose-Response Modeling Data for Uterine Adenocarcinoma in F₁ Female Sprague-Dawley Rats Following Postweaning Exposure to PFOA

Administered Dose (ppm) ^a	Internal Dose (mg/L)	Number per Group	Incidence
0 / 0	0	50	1
0 / 300	18.1	50	5
150 / 300	18.1	50	3
0 / 1,000	48.6	50	8
300 / 1,000	48.4	50	5

^aDoses are presented as perinatal exposure/postnatal exposure.

Uterine adenocarcinoma was assessed (1) following postweaning exposure, (2) following perinatal and postweaning exposure, and (3) using a pooled method. The pooled method used the dose response data associated with both postweaning exposure (1) and perinatal and postweaning exposure (2).

The BMD modeling results for uterine adenocarcinoma following postweaning exposure to PFOA are summarized in Table B-79 and Figure B-35. All Multistage models had adequate p-values (greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference), and the same AIC value. The Multistage Degree 2 model identified a model parameter of zero for the higher power term, so the Multistage Degree 1 and 2 models give the same result. The Multistage Degree 2 model was selected and shown below is the same as Degree 1 Multistage model. The BMDL₁₀ from the selected Multistage Degree 2 model is 18.0 mg/L.

Table B-79. Summary of Benchmark Dose Modeling Results for Uterine Adenocarcinoma in F₁ Female Sprague-Dawley Rats Following Postweaning Exposure to PFOA

Model ^a	Goodness of Fit		Scaled Residual		BMD ₁₀ (mg/L)	BMDL ₁₀ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Multistage Degree 2	0.584	90.6	0.5	-0.1	30.5	18.0	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 same AIC value.
Multistage Degree 1	0.584	90.6	0.5	-0.1	30.5	18.0	

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.

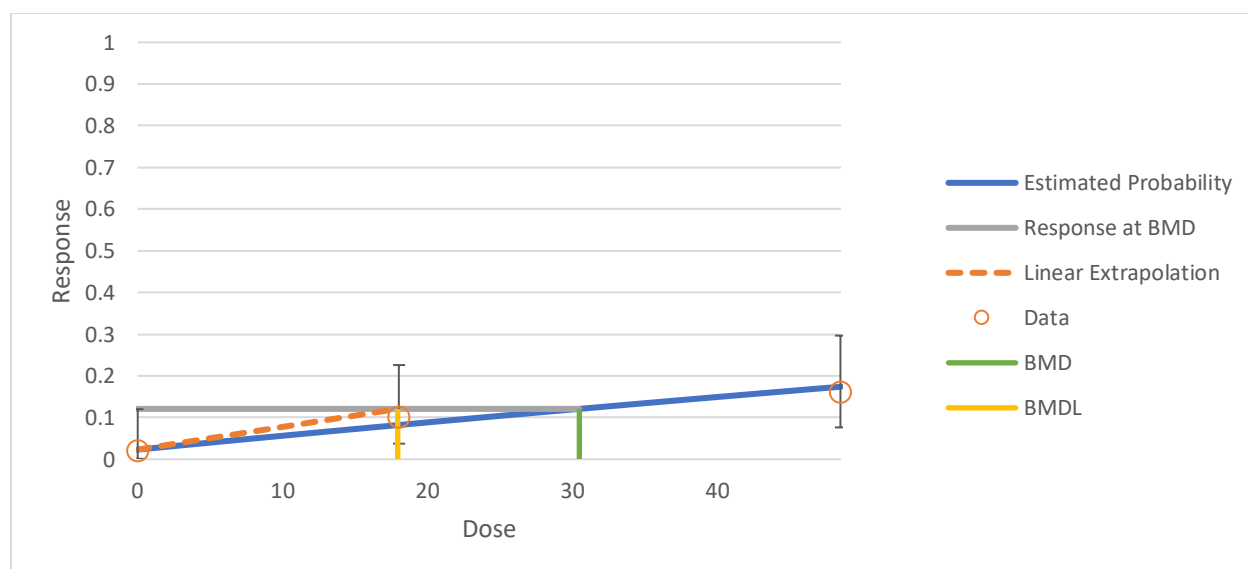


Figure B-35. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 2 Model for Uterine Adenocarcinoma in F1 Female Sprague-Dawley Rats Following Postweaning Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for uterine adenocarcinoma following perinatal and postnatal exposure are summarized in Table B-80 and Figure B-36. All Multistage models had adequate p-values (greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference), and the same AIC value. The Multistage Degree 2 model identified a model parameter of zero for the higher power term, so the Multistage Degree 1 and 2 models give the same result. The Multistage Degree 1 model was selected. The BMDL₁₀ from the selected Multistage Degree 1 model is 28.0 mg/L.

Table B-80. Summary of Benchmark Dose Modeling Results for Uterine Adenocarcinoma in F1 Female Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

Model ^a	Goodness of Fit		Scaled Residual		BMD ₁₀ (mg/L)	BMDL ₁₀ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Multistage Degree 2	0.808	69.1	−0.1	−0.1	57.5	28.0	EPA selected the Multistage Degree 1 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold
Multistage Degree 1	0.808	69.1	−0.1	−0.1	57.5	28.0	

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			
							difference), and the same AIC value.

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.

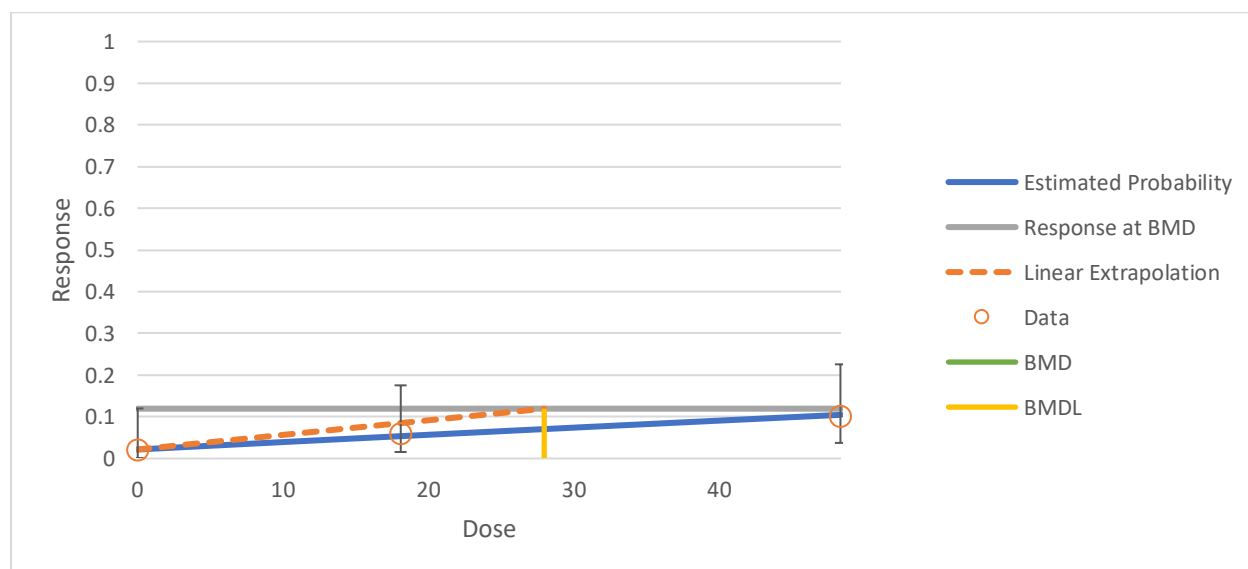


Figure B-36. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Uterine Adenocarcinoma in F1 Female Sprague-Dawley Rats Following Perinatal and Postweaning Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

The BMD modeling results for uterine adenocarcinoma using pooled methods are summarized in Table B-81 and Figure B-37. All Multistage models had adequate *p*-values (greater than 0.1), and the BMDLs were sufficiently close (less than threefold difference), and the same AIC value. The higher Degree Multistage models identified model parameters of zero for the higher power terms, so the Multistage Degree models all give the same result. The Multistage Degree 1 model was selected. The BMDL₁₀ from the selected Multistage Degree 1 model is 25.3 mg/L.

Table B-81. Summary of Benchmark Dose Modeling Results for Uterine Adenocarcinoma in F1 Female Sprague-Dawley Rats Following to PFOA (Pooled)

Model ^a	Goodness of Fit		Scaled Residual		BMD ₁₀ (mg/L)	BMDL ₁₀ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Multistage Degree 4	0.643	147.1	-0.8	-0.2	41.4	25.3	EPA selected the Multistage Degree 1 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the same AIC.
Multistage Degree 3	0.643	147.1	-0.8	-0.2	41.4	25.3	
Multistage Degree 2	0.643	147.1	-0.8	-0.2	41.4	25.3	
Multistage Degree 1	0.643	147.1	-0.8	-0.2	41.4	25.3	

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.

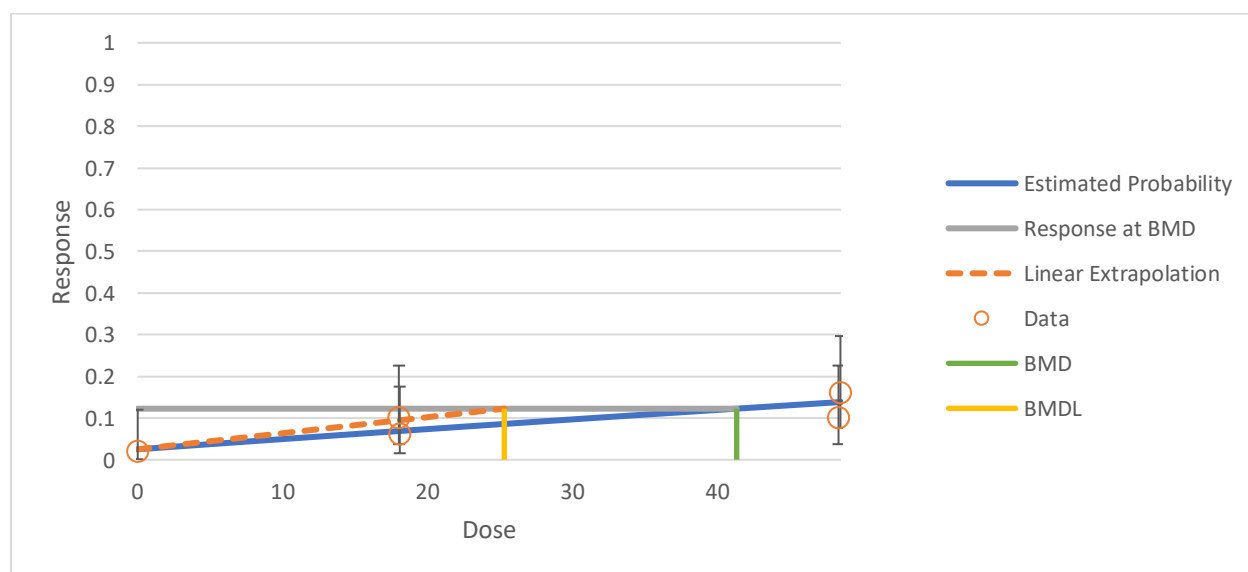


Figure B-37. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage Degree 1 Model for Uterine Adenocarcinoma in F1 Female Sprague-Dawley Rats Following to PFOA (Pooled)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.12 Song, 2018, 5079725

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Song (2018, 5079725) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses the number of Leydig cells in F₁ male Kunming mice and the offspring survival in F₁ male and female Kunming mice.

B.2.12.1 Number of Leydig Cells

Decreased mean response of number of Leydig cells was observed in F₁ male Kunming mice. Continuous models were used to fit dose-response data. Benchmark responses (BMR) of a change in the mean equal to 0.5 and 1 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table B-82. 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 AUC is expected to better correlate with an effect leading to decreased number of Leydig cells.

Table B-82. Dose-Response Modeling Data for Number of Leydig Cells in F₁ Male Kunming Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (incidence) ^a
0	0	5	67 ± 4.5 ^b
1	10.6	5	63 ± 2.2
2.5	17.9	5	50 ± 4.5
5	21.6	5	49 ± 6.7

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for number of Leydig cells are summarized in Table B-83 and Figure B-38. The best fitting model was the Polynomial Degree 2 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 Polynomial Degree 2 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 1 standard deviation from the control mean (BMDL_{1SD}) from the selected Polynomial Degree 2 model is 4.6 mg/L.

Table B-83. Summary of Benchmark Dose Modeling Results for Number of Leydig Cells in F₁ Male Kunming Mice Following Exposure to PFOA (nonconstant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	BMD _{1SD} (mg/L)	BMDL _{1SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.5SD}	Dose Group near BMD _{1SD}	Control Dose Group					
Exponential 2	0.018	128.9	−0.7	−0.7	−0.7	2.1	1.4	4.3	2.8	EPA selected the Polynomial Degree 2 model. Exponential 3, Polynomial 2 and 3 had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 2 model had the lowest AIC.
Exponential 3	0.103	125.4	0.5	0.5	−0.1	6.3	3.2	8.6	5.3	
Exponential 4	0.018	128.9	−0.7	−0.7	−0.7	2.1	1.4	4.3	2.8	
Exponential 5	— ^b	124.8	−0.1	−0.1	0.1	8.7	5.3	10.2	7.3	
Hill	— ^b	124.8	−0.1	−0.1	0.1	9.2	5.4	10.3	7.5	
Polynomial Degree 3	0.220	123.8	0.5	0.5	−0.1	6.1	2.2	8.6	4.4	
Polynomial Degree 2	0.220	123.8	0.5	0.5	−0.1	6.1	2.4	8.6	4.6	
Power	0.083	125.8	0.6	0.6	−0.1	5.9	2.7	8.4	4.9	
Linear	0.029	127.8	−0.7	−0.7	−0.7	2.3	1.5	4.6	3.1	

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; 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).

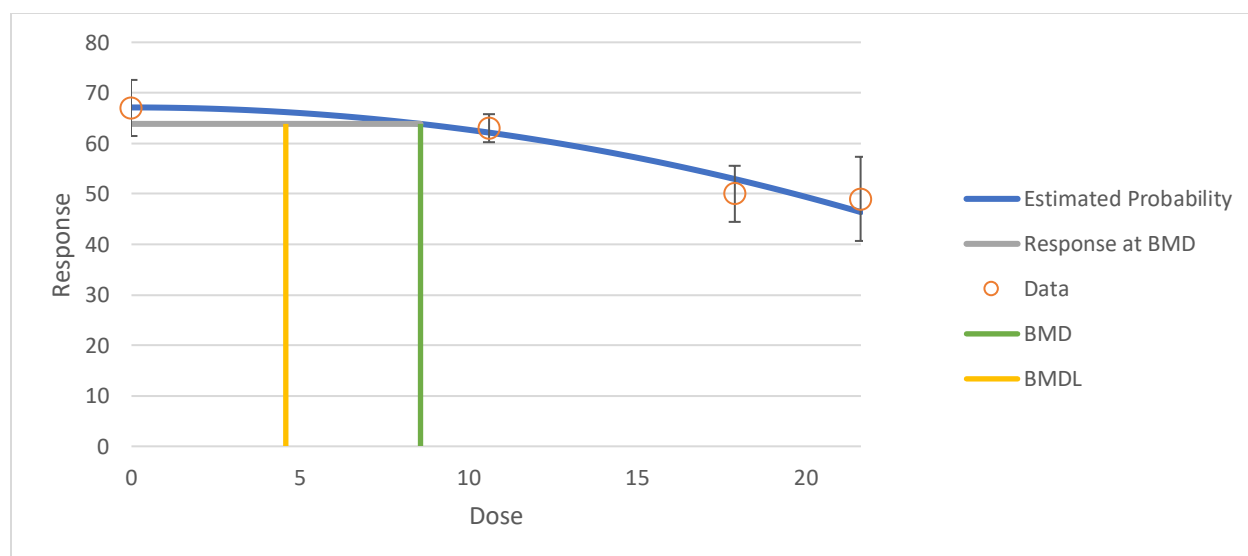


Figure B-38. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Polynomial Degree 2 Model for Number of Leydig Cells in F₁ Male Kunming Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.12.2 Offspring Survival

Decreased mean response of number of offspring survival was observed in F₁ male and female Kunming mice. Continuous models were used to fit dose-response data. Benchmark responses (BMR) of a change in the mean equal to 0.1 and 0.5 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table B-84. 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 considered and shown below because prenatal loss could be a result of exposure during a sensitive window of development where a Cmax 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-84. Dose-Response Modeling Data for Offspring Survival in F₁ Male and Female Kunming Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose					Number per group	Mean Response (incidences) ^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	10	15.1 ± 7.6 ^b
1	8.5	12.3	10.6	15.5	21.6	10	13.0 ± 14.5
2.5	17.0	18.6	17.9	27.0	35.7	10	12.0 ± 10.1
5	22.9	20.6	21.6	34.0	41.5	10	6.4 ± 17.1

^aData are presented as mean \pm standard deviation.

^bStandard deviations were calculated from standard errors.

For $AUC_{avg,pup,gest}$, the benchmark dose (BMD) modeling results for offspring survival are summarized in Table B-85 and Figure B-39. The best fitting model was the Polynomial Degree 2 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 Polynomial Degree 2 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 Polynomial Degree 2 model is 8.9 mg/L.

Table B-85. Summary of Benchmark Dose Modeling Results for Offspring Survival using $AUC_{avg,pup,gest}$ in F1 Male and Female Kunming Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.1SD} (mg/L)	BMDL _{0.1SD} (mg/L)	BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.1SD}	Dose Group near BMD _{0.5SD}	Control Dose Group					
Exponential 2	0.736	320.3	−0.15	0.53	−0.15	3.0	1.1	18.5	6.1	EPA selected the Polynomial Degree 2 model. All models, except the Hill model, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 2 model had the lowest AIC.
Exponential 3	0.709	321.9	0.04	−0.01	0.25	14.9	1.1	21.5	6.6	
Exponential 4	0.736	320.3	−0.15	0.53	−0.15	3.0	1.1	18.5	6.1	
Exponential 5	0.709	321.9	0.03	−0.01	0.25	15.0	1.1	21.5	6.6	
Hill	— ^b	323.9	4.4×e ^{−4}	−6.1×e ^{−4}	0.27	16.0	— ^c	20.7	— ^c	
Polynomial Degree 3	0.711	321.9	−0.25	−0.09	0.09	10.7	1.8	20.7	8.9	
Polynomial Degree 2	0.898	319.9	−0.23	−0.17	0.03	9.0	1.8	20.1	8.9	
Power	0.718	321.9	0.06	−0.01	0.22	14.2	1.8	21.5	8.9	
Linear	0.791	320.2	−0.16	0.53	−0.16	3.6	1.7	18.2	8.6	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{0.1SD} = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; BMDL_{0.1SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; BMD_{0.5SD} = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean; BMDL_{0.5SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.

^aSelected model in bold.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^cLower limit includes zero; BMDL not estimated.

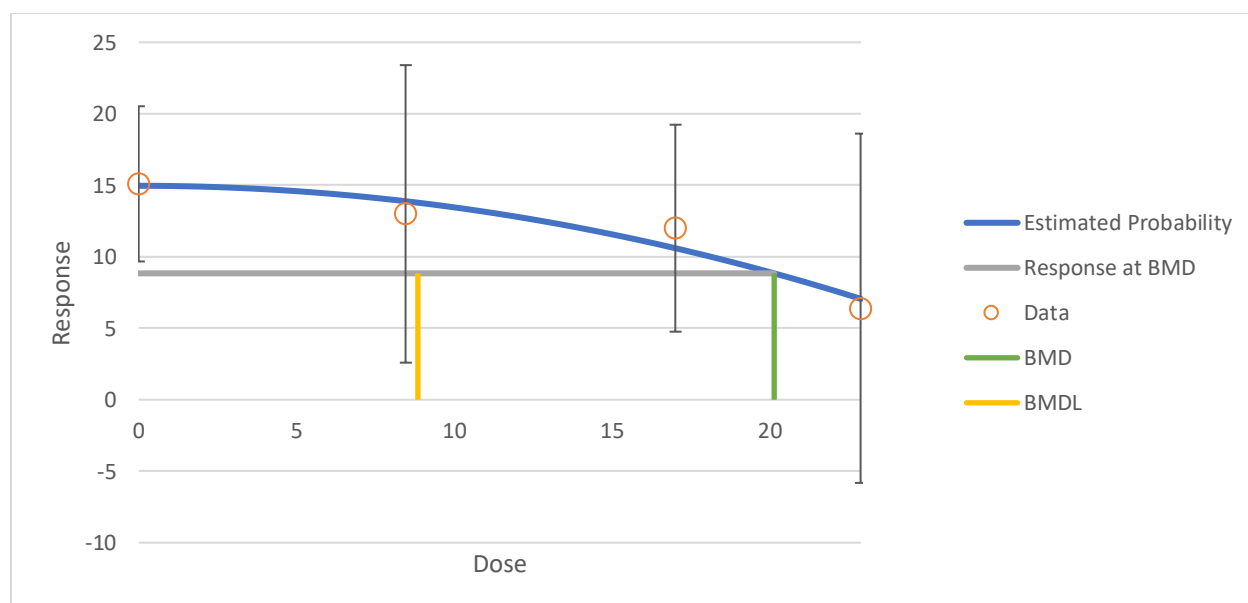


Figure B-39. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 2 Model for Offspring Survival using $AUC_{avg,pup,gest}$ in F₁ Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $AUC_{avg,pup,lact}$, the benchmark dose (BMD) modeling results for offspring survival are summarized in Table B-86 and Figure B-40. The best fitting model was the Polynomial 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 Polynomial 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 change in the mean equal to 0.5 standard deviations from the control mean ($BMDL_{0.5SD}$) from the selected Polynomial Degree 3 model is 8.9 mg/L.

Table B-86. Summary of Benchmark Dose Modeling Results for Offspring Survival using $AUC_{avg,pup,lact}$ in F1 Male and Female Kunming Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.1SD} (mg/L)	BMDL _{0.1SD} (mg/L)	BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.1SD}	Dose Group near BMD _{0.5SD}	Control Dose Group					
Exponential 2	0.590	320.8	-0.143	-0.775	-0.143	3.4	1.1	20.5	6.5	EPA selected the Polynomial Degree 3 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 3 model had the lowest AIC.
Exponential 3	0.701	321.9	0.003	-0.001	0.270	18.0	1.3	20.2	7.7	
Exponential 4	0.590	320.8	-0.142	-0.775	-0.142	3.4	1.1	20.5	6.5	
Exponential 5	0.701	321.9	0.003	-0.001	0.270	18.0	1.3	20.2	7.7	
Hill	— ^b	323.9	0.007	-0.002	0.267	17.8	— ^c	20.3	— ^c	
Polynomial Degree 3	0.784	320.2	-0.160	0.562	-0.023	11.4	1.8	19.5	8.9	
Polynomial Degree 2	0.733	320.4	0.009	0.600	-0.118	8.5	1.8	19.0	8.8	
Power	0.702	321.9	0.008	-0.002	0.267	17.8	1.9	20.3	9.3	
Linear	0.623	320.7	-0.179	0.569	-0.179	3.8	1.7	19.1	8.5	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{0.1SD} = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; BMDL_{0.1SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; BMD_{0.5SD} = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean; BMDL_{0.5SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.

^aSelected model in bold.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^cLower limit includes zero; BMDL not estimated.

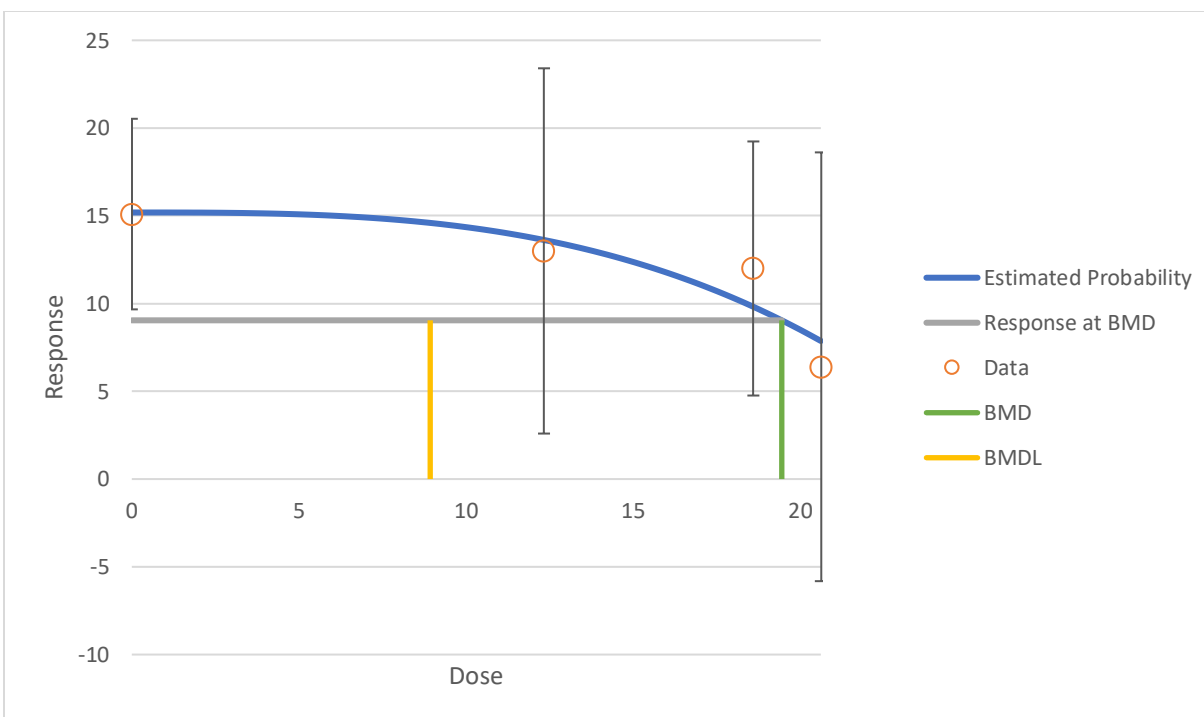


Figure B-40. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Offspring Survival using $AUC_{avg,pup,lact}$ in F1 Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $AUC_{avg,pup,gest,lact}$, the benchmark dose (BMD) modeling results for offspring survival are summarized in Table B-87 and Figure B-41. The best fitting model was the Polynomial 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 Polynomial 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 change in the mean equal to 0.5 standard deviations from the control mean ($BMDL_{0.5SD}$) from the selected Polynomial Degree 3 model is 8.9 mg/L.

Table B-87. Summary of Benchmark Dose Modeling Results for Offspring Survival using $AUC_{avg,pup,gest,lact}$ in F₁ Male and Female Kunming Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.1SD} (mg/L)	BMDL _{0.1SD} (mg/L)	BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.1SD}	Dose Group near BMD _{0.5SD}	Control Dose Group					
Exponential 2	0.659	320.6	-0.16	0.54	-0.16	3.2	1.1	19.3	6.3	EPA selected the Polynomial Degree 3 model. All models had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 3 model had the lowest AIC.
Exponential 3	0.705	321.9	0.02	-0.01	0.26	16.6	1.2	20.8	7.1	
Exponential 4	0.659	320.6	-0.16	0.54	-0.16	3.2	1.1	19.3	6.3	
Exponential 5	0.705	321.9	0.02	-4.8×e ⁻³	0.26	16.6	1.2	20.8	7.1	
Hill	0.701	321.9	-8.1×e ⁻⁵	-2.1×e ⁻⁴	0.27	17.3	— ^b	20.1	— ^b	
Polynomial Degree 3	0.882	320.0	-0.25	-0.19	0.05	11.7	1.8	20.0	8.9	
Polynomial Degree 2	0.831	320.1	-0.13	0.50	-0.06	8.6	1.7	19.3	8.7	
Power	0.710	321.9	0.04	-0.01	0.25	16.2	1.8	20.9	9.0	
Linear	0.706	320.4	-0.19	0.56	-0.19	3.7	1.7	18.3	8.4	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{0.1SD} = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; BMDL_{0.1SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; BMD_{0.5SD} = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean; BMDL_{0.5SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.

^aSelected model in bold.

^bLower limit includes zero; BMDL not estimated.

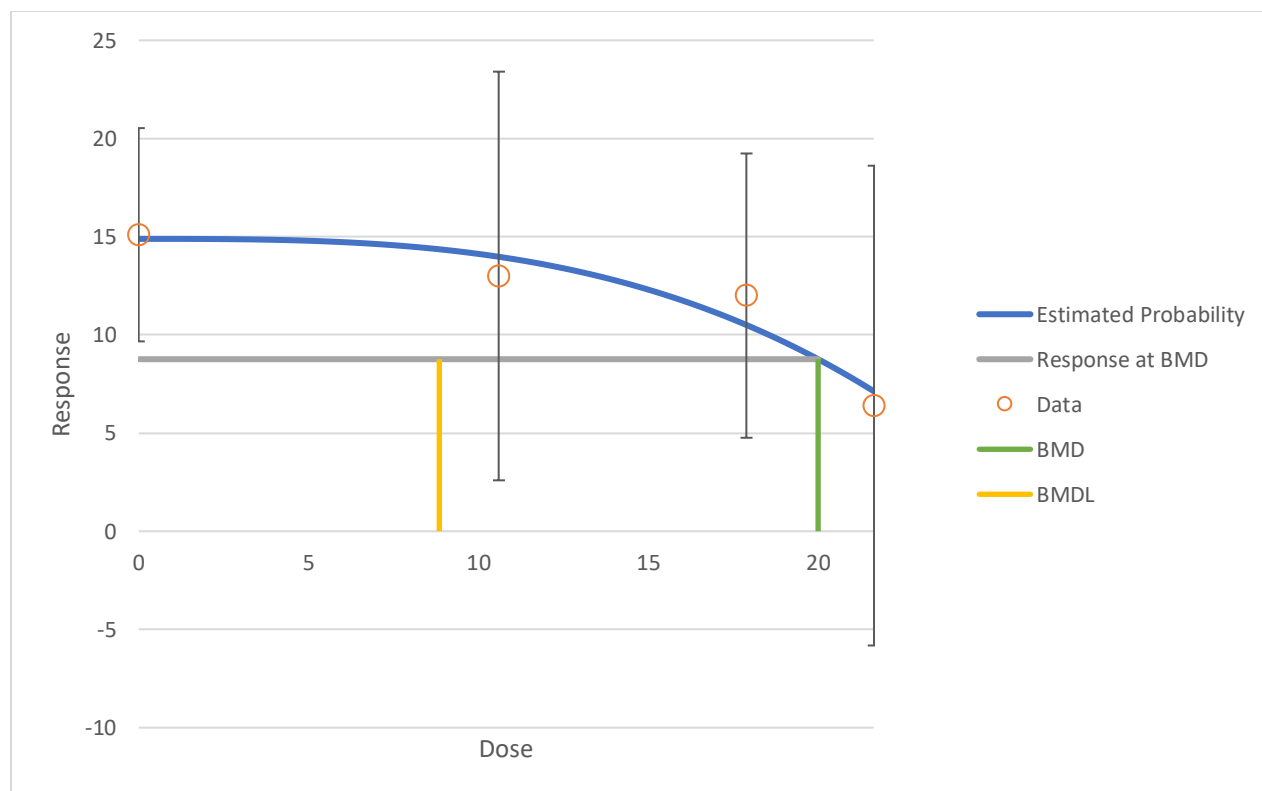


Figure B-41. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Offspring Survival using $AUC_{avg,pup,gest,lact}$ in F1 Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $C_{max,pup,gest}$, the benchmark dose (BMD) modeling results for offspring survival are summarized in Table B-88 and Figure B-42. The best fitting model was the Polynomial Degree 2 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 Polynomial Degree 2 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 Polynomial Degree 2 model is 13.5 mg/L.

Table B-88. Summary of Benchmark Dose Modeling Results for Offspring Survival using $C_{\text{max,pup,gest}}$ in F₁ Male and Female Kunming Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.1SD} (mg/L)	BMDL _{0.1SD} (mg/L)	BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.1SD}	Dose Group near BMD _{0.5SD}	Control Dose Group					
Exponential 2	0.686	320.5	-0.167	0.529	-0.167	4.8	1.7	29.1	9.5	EPA selected the Polynomial Degree 2 model. All models, except for the Exponential 5 model, had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 2 model had the lowest AIC.
Exponential 3	0.708	321.9	0.029	-0.008	0.252	24.5	1.8	32.3	10.6	
Exponential 4	0.686	320.5	-0.167	0.529	-0.167	4.8	1.7	29.1	9.5	
Exponential 5	— ^b	323.9	0.028	-0.008	0.253	24.5	— ^c	32.3	1.9	
Hill	0.701	321.9	4.2×e ⁻⁵	7.0×e ⁻⁵	0.271	26.0	— ^c	30.6	— ^c	
Polynomial Degree 3	0.667	321.9	-0.253	-0.132	0.073	17.8	2.7	31.1	13.6	
Polynomial Degree 2	0.867	320.0	-0.157	0.444	-0.040	13.4	2.7	29.9	13.4	
Power	0.717	321.9	0.056	-0.012	0.230	23.5	2.7	32.4	13.7	
Linear	0.738	320.3	-0.193	0.543	-0.193	5.6	2.6	27.8	13.0	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{0.1SD} = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; BMDL_{0.1SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; BMD_{0.5SD} = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean; BMDL_{0.5SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.

^aSelected model in bold.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^cLower limit includes zero; BMDL not estimated.

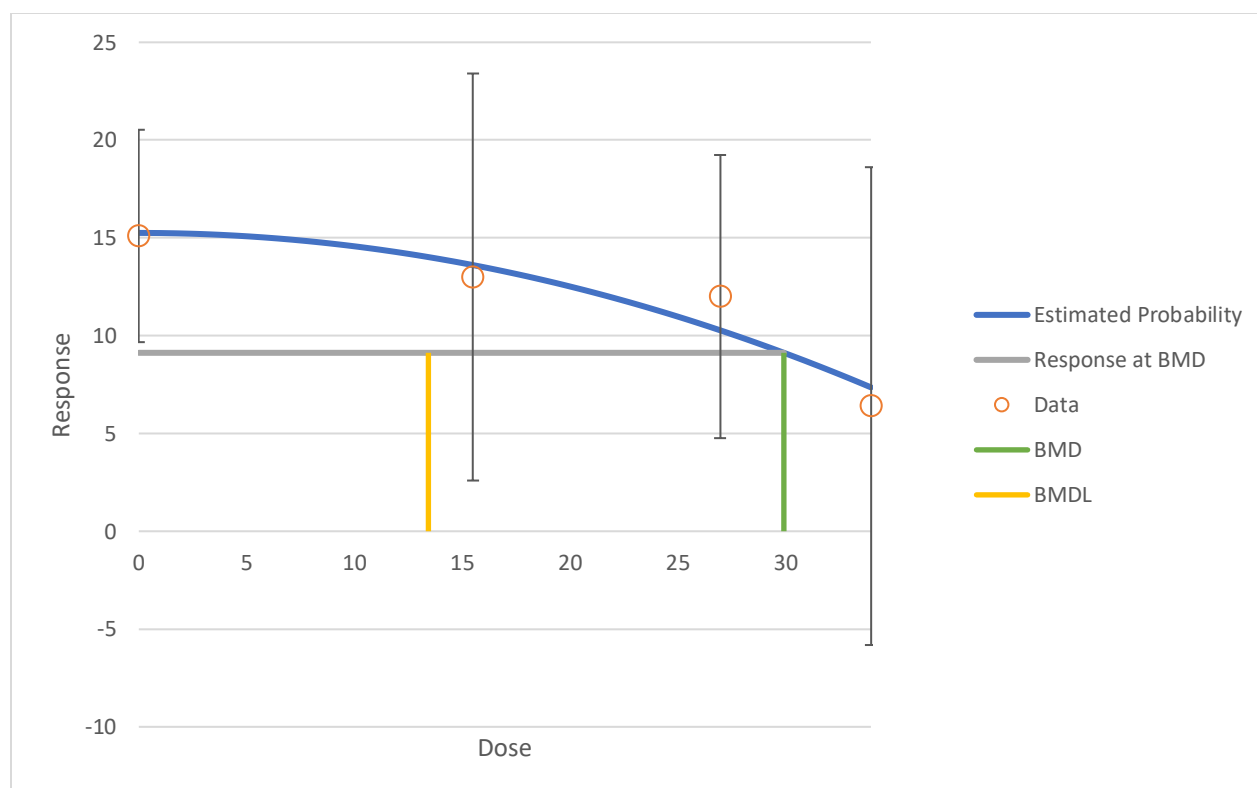


Figure B-42. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 2 Model for Offspring Survival using $C_{\max,pup,gest}$ in F1 Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

For $C_{\max,pup,lact}$, the benchmark dose (BMD) modeling results for offspring survival are summarized in Table B-89 and Figure B-43. The best fitting model was the Polynomial 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 Polynomial 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 change in the mean equal to 0.5 standard deviation from the control mean (BMDL_{0.5SD}) from the selected Polynomial Degree 3 model is 17.3 mg/L.

Table B-89. Summary of Benchmark Dose Modeling Results for Offspring Survival using $C_{\text{max,pup,lact}}$ in F₁ Male and Female Kunming Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.1SD} (mg/L)	BMDL _{0.1SD} (mg/L)	BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.1SD}	Dose Group near BMD _{0.5SD}	Control Dose Group					
Exponential 2	0.633	320.6	-0.154	0.545	-0.154	6.3	2.2	38.4	12.4	EPA selected the Polynomial Degree 3 model. All models, except the 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 Polynomial Degree 3 model had the lowest AIC.
Exponential 3	0.702	321.9	0.008	-0.002	0.267	33.8	2.4	40.2	14.2	
Exponential 4	0.633	320.6	-0.154	0.545	-0.154	6.3	2.2	38.4	12.4	
Exponential 5	— ^b	323.9	0.004	-0.001	0.269	34.0	— ^c	40.1	2.3	
Hill	0.701	321.9	6.0×e ⁻⁵	4.6×e ⁻⁶	0.271	34.5	— ^c	39.6	— ^c	
Polynomial Degree 3	0.839	320.1	-0.235	-0.267	0.030	22.6	3.5	38.7	17.3	
Polynomial Degree 2	0.789	320.2	-0.092	0.555	-0.077	16.8	3.4	37.5	17.1	
Power	0.705	321.9	0.018	-0.004	0.260	33.4	3.6	40.4	17.8	
Linear	0.673	320.5	-0.185	0.575	-0.185	7.3	3.3	36.3	16.5	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD_{0.1SD} = dose level corresponding to a change in the mean equal to 0.1 standard deviations from the control mean; BMDL_{0.1SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.1 standard deviations from the control mean; BMD_{0.5SD} = dose level corresponding to a change in the mean equal to 0.5 standard deviation from the control mean; BMDL_{0.5SD} = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean equal to 0.5 standard deviation from the control mean.

^aSelected model in bold.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^cLower limit includes zero; BMDL not estimated.

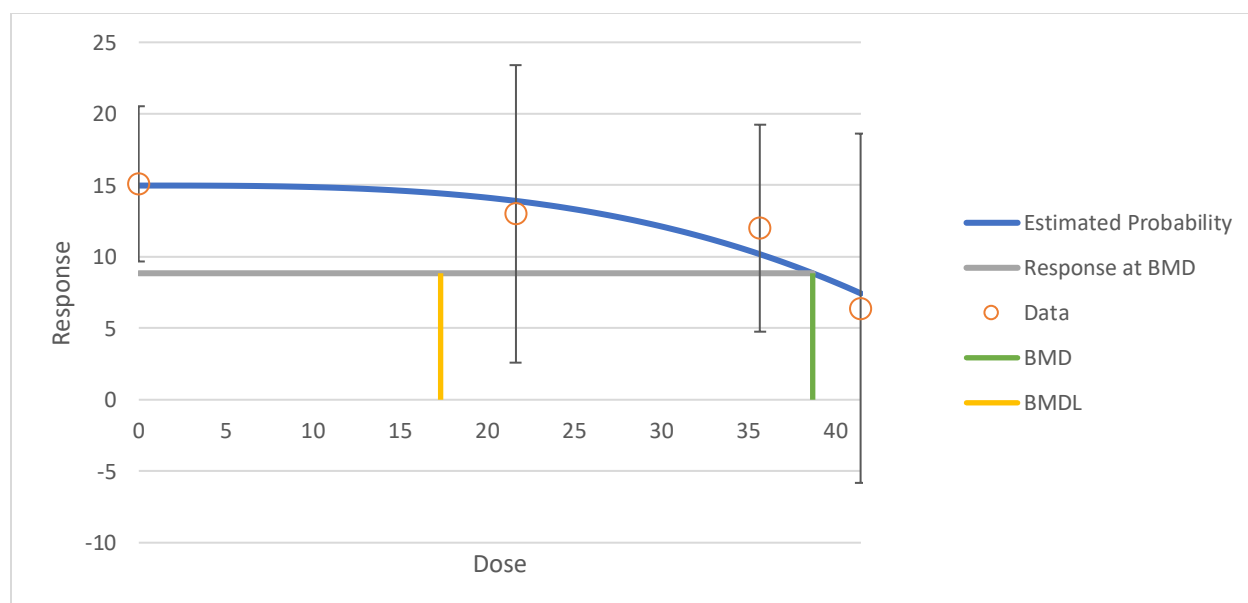


Figure B-43. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Offspring Survival using $C_{max,pup,lact}$ in F₁ Male and Female CD-1 Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.13 Wolf, 2007 1332672

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Wolf et al., 2007, 1332672 study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses pup body weight change in F₁ male and female CD-1 mice (in utero exposure), time to eye opening in F₁ male and female CD-1 mice (in utero and lactational exposure), and dams with whole litter loss (%) in P₀ female CD-1 mice.

B.2.13.1 Pup Body Weight Change

Decreased mean response of pup body weight change was observed in F₁ male and female CD-1 mice (in utero exposure). 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-90. The AUC normalized per day during gestation (AUC_{avg,pup,gest}) dose metric 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 change.

Table B-90. Dose-Response Modeling Data for Pup Body Weight Change in F₁ Male and Female CD-1 Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (g) ^a
0	0	14	12.4 ± 1.2 ^b
3	18.7	11	11.4 ± 1.3
5	22.9	13	9.6 ± 1.3

^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-91. No models provided an adequate fit, therefore a lowest-observed-adverse-effect-level (LOAEL) approach was taken for this endpoint.

Table B-91. Summary of Benchmark Dose Modeling Results for Pup Body Weight Change in F₁ Male and Female CD-1 Mice Following Exposure to PFOA (constant variance)

Model	Goodness of Fit		Scaled Residual		BMD _{0.5 SD} (mg/L)	BMDL _{0.5 SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD	Control Dose Group			
Exponential 2	0.006	135.1	-0.3	-0.3	6.1	4.3	No models had adequate fit (p-values greater than 0.1).
Exponential 3	— ^a	129.5	9.4×e ⁻⁷	-5.0	17.0	12.5	
Exponential 4	0.006	135.1	-0.3	-0.3	6.1	4.2	
Exponential 5	— ^a	129.5	-1.3	1.1	17.0	12.5	
Hill	— ^a	129.5	-2.3×e ⁻⁹	-3.7×e ⁻⁹	18.0	12.2	
Polynomial Degree 2	0.052	131.3	1.6	-0.5	11.2	6.9	
Power	— ^a	129.5	-1.0×e ⁻³	8.4×e ⁻⁴	16.9	12.2	
Linear	0.007	134.8	-0.3	-0.3	6.4	4.6	

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.2.13.2 Time to Eye Opening

Increased mean response of time to eye opening was observed in F₁ male and female CD-1 mice (in utero and lactational exposure). Continuous models were used to fit dose-response data. BMRs of a change in the mean equal to 0.5 and 1 standard deviations from the control mean were chosen. The doses and response data used for the modeling are listed in Table B-92. The dose metrics, AUC normalized per day during gestation (AUC_{avg,pup,gest}), lactation (AUC_{avg,pup,lact}), and 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 time of eye opening 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 time to eye opening could be due to exposure during the gestation or lactation lifestages.

Table B-92. Dose-Response Modeling Data for Time to Eye Opening F₁ Male and Female CD-1 Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose					Number per Group	Mean Response (days) ^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	14	14.8 ± 0.3 ^b
3	18.7	19.2	19.0	28.7	37.4	12	15.8 ± 0.7
5	22.9	20.6	21.6	34.0	41.5	12	15.9 ± 1.4

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for time to eye opening are summarized in Table B-93 for AUC_{avg,pup,gest}, Table B-94 for AUC_{avg,pup,lact}, Table B-95 for AUC_{avg,pup,gest,lact}, Table B-96 for C_{max,pup,gest}, and Table B-97 for C_{max,pup,lact}. No models provided an adequate fit. A lowest-observed-adverse-effect-level (LOAEL) approach was taken for this endpoint.

Table B-93. Summary of Benchmark Dose Modeling Results for Time to Eye Opening for AUC_{avg,pup,gest} in F₁ Male and Female CD-1 Mice Following Exposure to PFOA (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	0.736	101.4	0.3	-0.05	17.2	11.8	No models had adequate fit for constant and nonconstant variance (p-values were less than 0.05).
Exponential 3	0.736	101.4	0.3	-0.05	17.2	11.8	
Exponential 4	— ^a	103.3	3.9×e ⁻⁵	-1.9×e ⁻⁵	13.9	1.0	
Exponential 5	— ^a	103.3	2.0×e ⁻⁵	-7.2×e ⁻⁵	13.9	1.0	
Hill	— ^b	105.3	2.5×e ⁻⁴	1.5×e ⁻⁵	16.2	— ^c	
Polynomial Degree 2	0.751	101.4	0.2	-0.04	17.1	11.5	
Power	0.751	101.4	0.2	-0.04	17.1	11.5	
Linear	0.751	101.4	0.2	-0.04	17.1	11.5	

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.

^aDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^bDegrees of freedom are negative, (Goodness of fit test cannot be calculated).

^cLower limit includes zero; BMDL not estimated.

Table B-94. Summary of Benchmark Dose Modeling Results for Time to Eye Opening for AUC_{avg,pup,lact} in F₁ Male and Female CD-1 Mice Following Exposure to PFOA (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	0.945	101.3	-0.1	3.6×e ⁻³	16.2	11.1	No models had adequate fit for constant and nonconstant variance (p-values were less than 0.05).
Exponential 3	— ^a	103.3	-0.1	3.1×e ⁻³	16.2	11.1	
Exponential 4	— ^a	103.3	-0.1	3.5×e ⁻³	16.1	— ^b	
Exponential 5	— ^c	105.5	-0.1	3.2×e ⁻³	15.8	0.9	
Hill	<0.0001	105.3	-1.0×e ⁻⁷	2.1×e ⁻⁸	18.3	— ^b	
Polynomial Degree 2	— ^a	103.3	3.6×e ⁻³	1.1×e ⁻⁴	17.0	10.8	
Power	— ^a	103.3	-8.5×e ⁻³	1.3×e ⁻³	16.9	10.8	
Linear	0.939	101.3	-0.1	3.5×e ⁻³	16.1	10.8	

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).

^bLower limit includes zero; BMDL not estimated.

^cDegrees of freedom are negative, (Goodness of fit test cannot be calculated).

Table B-95. Summary of Benchmark Dose Modeling Results for Time to Eye Opening for AUC_{avg,pup,gest,lact} in F₁ Male and Female CD-1 Mice Following Exposure to PFOA (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	0.905	101.3	0.09	-0.01	16.6	11.3	No models had adequate fit for constant and nonconstant variance (p-values were less than 0.05).
Exponential 3	0.905	101.3	0.09	-0.01	16.6	11.3	
Exponential 4	— ^a	103.3	0.07	-0.01	16.4	1.0	
Exponential 5	— ^a	103.3	0.07	-0.01	16.3	— ^b	
Hill	— ^c	105.3	5.0×e ⁻⁴	-2.9×e ⁻⁴	17.8	— ^b	

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			
Polynomial Degree 2	0.916	101.3	0.08	−0.01	16.5	11.1	
Power	0.916	101.3	0.08	−0.01	16.5	11.1	
Linear	0.916	101.3	0.08	−0.01	16.5	11.1	

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.

^aDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^bLower limit includes zero; BMDL not estimated.

^cDegrees of freedom are negative, (Goodness of fit test cannot be calculated).

Table B-96. Summary of Benchmark Dose Modeling Results for Time to Eye Opening for C_{max,pup,gest} in F1 Male and Female CD-1 Mice Following Exposure to PFOA (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	0.816	101.3	0.18	−0.03	25.7	17.6	No models had adequate fit for constant and nonconstant variance (p-values were less than 0.05).
Exponential 3	0.816	101.3	0.18	−0.03	25.7	17.6	
Exponential 4	— ^a	103.3	0.15	−0.02	25.3	1.5	
Exponential 5	— ^a	103.3	0.15	−0.02	25.3	1.9	
Hill	— ^b	105.3	1.1×e ^{−3}	−2.2×e ^{−4}	25.9	— ^c	
Polynomial Degree 2	0.829	101.3	0.16	−0.02	25.5	17.2	
Power	0.829	101.3	0.16	−0.02	25.5	17.2	
Linear	0.829	101.3	0.16	−0.02	25.5	17.2	

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.

^aDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^bDegrees of freedom are negative, (Goodness of fit test cannot be calculated).

^cLower limit includes zero; BMDL not estimated.

Table B-97. Summary of Benchmark Dose Modeling Results for Time to Eye Opening ($C_{\max,pup,lact}$) in F1 Male and Female CD-1 Mice Following Exposure to PFOA (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	0.971	101.3	0.03	-0.002	32.1	21.9	No models had adequate fit for constant and nonconstant variance (p-values were less than 0.05).
Exponential 3	0.971	101.3	0.03	-0.003	32.1	21.9	
Exponential 4	— ^a	103.3	0.01	-8.8×e ⁻⁴	31.7	1.8	
Exponential 5	— ^a	103.3	0.01	-0.001	31.6	1.8	
Hill	<0.0001	105.3	-7.6×e ⁻⁸	-9.4×e ⁻⁸	35.4	— ^b	
Polynomial Degree 2	0.979	101.3	0.02	-0.002	31.8	21.5	
Power	0.979	101.3	0.02	-0.002	31.8	21.5	
Linear	0.979	101.3	0.02	-0.002	31.8	21.5	

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.

^aDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^bLower limit includes zero; BMDL not estimated.

B.2.13.3 Dams with Whole Litter Loss

Increased incidence of dams with whole litter loss was observed in P₀ female CD-1 Mice. Continuous models were used to fit dose-response data. Benchmark responses (BMR) of 5% and 10% extra risk were chosen. The doses and response data used for the modeling are listed in Table B-98. The AUC normalized per day during gestation (AUC_{avg,dam,gest}) dose metric was selected for this model to consider an accumulation of exposure where an AUC metric is expected to better correlate with the effect.

Table B-98. Dose-Response Modeling Data for Dams with Whole Litter Loss in P₀ Female CD-1 Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Incidence
0	0	39	1
3	74.9	25	1
5	91.6	30	5

The BMD modeling results for dams with whole litter loss are summarized in Table B-99 and Figure B-44. The best fitting model was the Gamma 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 Gamma 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% response level (BMDL₅) from the selected Gamma model is 29.2 mg/L.

Table B-99. Summary of Benchmark Dose Modeling Results for Dams with Whole Litter Loss in P₀ Female CD-1 Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD ₅ (mg/L)	BMDL ₀₅ (mg/L)	BMD ₁₀ (mg/L)	BMDL ₁₀ (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD ₅	Dose Group near BMD ₁₀	Control Dose Group					
Dichotomous Hill	65536	52.7	6.2×e ⁻⁵	5.9×e ⁻⁵	2.2×e ⁻⁵	81.1	— ^c	86.4	— ^c	EPA selected the Gamma model. All models, except the Dichotomous Hill, Log-Logistic, Weibull, and Log-Probit had adequate fit (p-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Gamma model had the lowest AIC.
Gamma	0.712	48.9	-0.3	0.1	0.1	79.1	29.2	87.2	59.9	
Log-Logistic	— ^b	50.7	5.8×e ⁻⁴	1.3×e ⁻⁴	2.9×e ⁻⁴	83.1	29.1	88.5	61.4	
Multistage Degree 2	0.256	50.2	-0.9	0.7	0.1	62.0	23.5	88.8	48.3	
Multistage Degree 1	0.189	50.1	-1.0	0.8	0.1	46.4	22.2	95.3	45.7	
Weibull	— ^b	50.7	-4.6×e ⁻⁵	-4.6×e ⁻⁵	1.5×e ⁻⁴	83.3	30.2	88.6	62.0	
Logistic	0.258	50.2	-0.9	0.6	0.3	59.4	41.5	86.3	63.9	
Log-Probit	— ^b	50.7	-9.2×e ⁻⁴	-6.4×e ⁻⁴	1.0×e ⁻³	82.4	— ^c	88.0	— ^c	
Probit	0.241	50.3	-0.9	0.7	0.2	57.2	38.7	87.3	61.3	

AIC = Akaike information criterion; BMD = benchmark dose; BMDL = benchmark dose lower limit; BMD₅ = dose level corresponding to a 5% response level; BMDL₅ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% response level; 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.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

^cLower limit includes zero; BMDL not estimated.

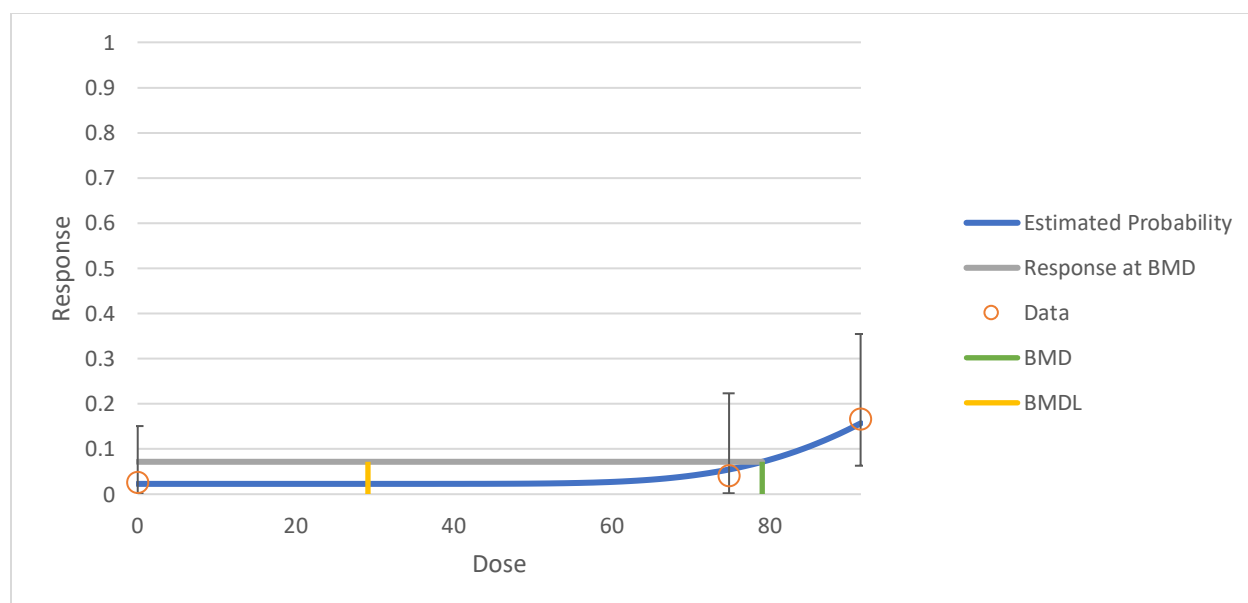


Figure B-44. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Gamma Model for Dams with Whole Litter Loss in P₀ Female CD-1 Mice Following Exposure to PFOA (constant variance)

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.14 Zhang, 2020, 650878

U.S. Environmental Protection Agency (EPA) conducted dose response modeling of the Zhang (2020, 650878) study using the Benchmark Dose Software (BMDS) 3.2 program. This study addresses length of diestrus and number of corpora lutea in female ICR mice.

B.2.14.1 Length of Diestrus

Increased mean response of length of diestrus was observed in female ICR mice. Continuous models were used to fit dose-response data. Benchmark responses (BMR) of a change in the mean equal to one standard deviation from the control mean was chosen. The doses and response data used for the modeling are listed in Table B-100. The $C_{7,avg}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of effects resulting in an increased length of diestrus.

Table B-100. Dose-Response Modeling Data for Length of Diestrus in Female ICR Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (days) ^a
0	0	8	2.9 ± 0.3 ^b
0.5	57.6	8	3.6 ± 0.6
2	166.1	8	5.9 ± 1.1
5	195.7	8	8.5 ± 2.8

^aData are presented as mean \pm standard deviation.
^bStandard deviations were calculated from standard errors.

The benchmark dose (BMD) modeling results for length of diestrus are summarized in Table B-101 and Figure B-45. The best fitting model was the Polynomial 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 Polynomial 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 change in the mean equal to 1 standard deviation from the control mean (BMDL_{1SD}) from the selected Polynomial Degree 3 model is 15.1 mg/L.

Table B-101. Summary of Benchmark Dose Modeling Results for Length of Diestrus in Female ICR Mice Following Exposure to PFOA (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	0.056	90.4	0.48	0.48	18.9	13.9	EPA selected the Polynomial Degree 3 model. Only the Polynomial Degree 3 model had adequate fit (p-value greater than 0.1), the BMDLs were sufficiently close (less than threefold difference), and the Polynomial Degree 3 model had the lowest AIC.
Exponential 3	0.050	90.4	0.17	-0.03	31.4	15.9	
Exponential 4	0.001	98.0	0.44	0.44	13.6	9.7	
Exponential 5	— ^b	93.4	0.09	-0.01	33.4	27.2	
Hill	— ^b	93.5	0.09	-0.01	33.4	18.9	
Polynomial Degree 3	0.192	87.9	-0.03	-0.03	27.1	15.1	
Polynomial Degree 2	0.036	91.0	0.05	0.01	30.0	16.0	
Power	0.028	91.4	0.10	-0.01	33.5	18.8	
Linear	0.003	96.0	0.44	0.44	13.6	9.7	

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.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

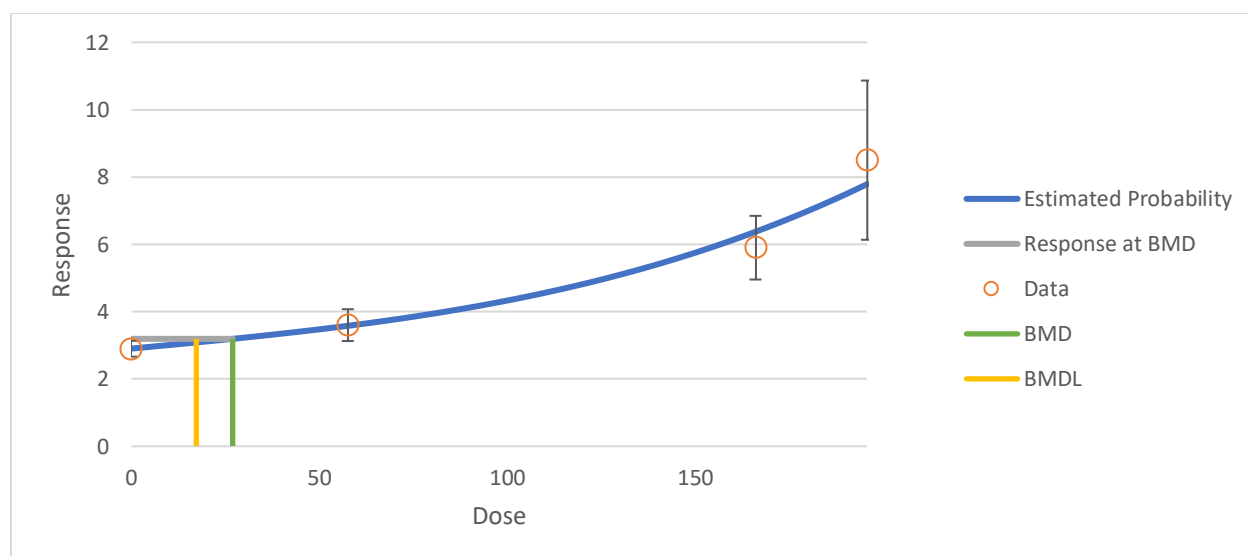


Figure B-45. Plot of Mean Response by Dose with Fitted Curve for the Selected Polynomial Degree 3 Model for Length of Diestrus in Female ICR Mice Following Exposure to PFOA

BMD = benchmark dose; BMDL = benchmark dose lower limit.

B.2.14.2 Number of Corpora Lutea

Decreased mean response of number of corpora lutea was observed in female ICR mice. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to one standard deviation from the control mean were chosen. The doses and response data used for the modeling are listed in Table B-102. The $C_{7,avg}$ was selected for this model rather than alternate metrics such as C_{max} because the AUC is expected to better correlate with an effect leading to decrease number of corpora lutea.

Table B-102. Dose-Response Modeling Data for Number of Corpora Lutea in Female ICR Mice Following Exposure to PFOA

Administered Dose (mg/kg/day)	Internal Dose (mg/L)	Number per Group	Mean Response (number per section) ^a
0	0	8	4.7 ± 1.1 ^b
0.5	57.6	8	4.0 ± 1.7
2	166.1	8	2.0 ± 0.6
5	195.7	8	1.7 ± 1.4

^aData are presented as mean ± standard deviation.

^bStandard deviations were calculated from standard errors.

The BMD modeling results for number of corpora lutea are summarized in Table B-103. No models provided an adequate fit for constant or non-constant variance models, therefore a no-observed-adverse-effect level (NOAEL) approach was taken for this endpoint.

Table B-103. Summary of Benchmark Dose Modeling Results for Number of Corpora Lutea in Female ICR Mice Following Exposure to PFOA (constant variance)

Model ^a	Goodness of Fit		Scaled Residual			BMD _{0.5SD} (mg/L)	BMDL _{0.5SD} (mg/L)	BMD _{1SD} (mg/L)	BMDL _{1SD} (mg/L)	Basis for Model Selection
	p-value	AIC	Dose Group near BMD _{0.5SD}	Dose Group near BMD _{1SD}	Control Dose Group					
Exponential 2	0.605	109.0	−0.4	0.8	−0.4	26.1	17.4	56.2	36.9	No models had adequate fit for constant or non-constant variance (p-values were less than 0.05).
Exponential 3	0.829	110.0	0.1	0.1	−2.3×e ^{−2}	49.6	18.7	82.9	39.8	
Exponential 4	0.605	109.0	−0.4	0.8	−0.4	26.1	17.4	56.2	36.9	
Exponential 5	— ^b	112.0	1.7×e ^{−6}	1.7×e ^{−6}	5.8×e ^{−7}	52.1	17.2	81.3	37.5	
Hill	— ^b	112.0	−3.3×e ^{−6}	−3.3×e ^{−6}	1.3×e ^{−6}	52.6	16.3	79.7	36.7	
Polynomial Degree 3	0.646	110.2	0.2	0.2	−0.1	42.1	27.8	81.5	55.5	
Polynomial Degree 2	0.646	110.2	0.2	0.2	−0.1	42.1	27.8	81.5	55.5	
Power	0.677	110.2	0.1	0.1	−0.1	45.4	27.8	83.8	55.6	
Linear	0.884	108.2	0.3	0.3	−0.2	37.2	27.7	74.5	55.4	

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.

^aSelected model in bold.

^bDegrees of freedom = 0, saturated model (Goodness of fit test cannot be calculated).

1 Appendix C. Detailed Information from Epidemiology Studies

2 C.1 Developmental

3 **Table C-1. Associations Between PFOA 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 1.7 (1.2–2.4)	BW (z-score): adequate, inadequate, and excess weight gain	Regression coefficient per log10 unit increase PFOA	BW: –0.1 (–0.34, 0.13) Females: –89.51 (–263.4, 84.38) Males: –35.51 (–198.99, 127.97) Adequate weight gain: –0.36 (– 0.85, 0.11) Excess weight gain: –0.08 (–0.44, 0.27) Inadequate weight gain: –0.08 (– 0.78, 0.63)
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	BL (cm), BW (g, z-score), gestational length (weeks), HC (cm), preterm birth	Regression coefficient per IQR increase and by quartiles OR per 0.1 ng/mL increase, per IQR increase, and by quartiles	BL: 0.1 (–0.1, 0.2) BW (g): 7 (–10, 23) Q2: 3 (–54, 59) Q3: 15 (–42, 72) Q4: 9 (–47, 64) BW (z-score): 0.02 (–0.02, 0.06) Q2: 0.009 (–0.13, 0.14) Q3: 0.04 (–0.09, 0.17) Q4: 0.02 (–0.1, 0.16) Gestational length: 0.1 (0, 0.2) Q2: 0 (–0.3, 0.2) Q3: 0.1 (–0.2, 0.3)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Q4: 0.1 (–0.2, 0.4)
							HC: 0.1 (0, 0.2)
							Q2: 0 (–0.2, 0.3)
							Q3: 0.1 (–0.2, 0.4)
							Q4: 0.1 (–0.1, 0.4)
							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: 1.10 (0.69–1.63) Twins: 1.01 (0.69–1.53)	BL (cm), BW (g), GA (weeks), HC (cm), ponderal index	Regression coefficient per log(PFOA+1) unit increase	BL S: 0.02 (–0.13, 0.17) T: 0.21 (–0.11, 0.52) BW S: –11.55 (–35.72, 12.62) T: 18.48 (–17.18, 54.13) GA S: 0.01 (–0.07, 0.08) T: –0.01 (–0.12, 0.11) HC S: 0.04 (–0.17, 0.26) T: 0.12 (–0.22, 0.46) Ponderal index S: –0.01 (–0.03, 0.01) T: –0.01 (–0.04, 0.02)
							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 = 0.92	BL (cm), BW (g), HC (cm)	Regression coefficient per IQR increase in serum PFOA	BL: 0.1 (–0.1, 0.2) Females: –0.2 (–0.5, 0) Males: 0.2 (0, 0.3), Interaction p-value = 0.008 BW: 18 (–9, 45) Females: –23 (–78, 31)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Males: 31 (6, 56)
							HC: 0.1 (0, 0.2) Females: -0.1 (-0.3, 0.1) Males: 0.2 (0.1, 0.3), Interaction p-value = 0.004
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	BL (cm), BW (g), GA at delivery (weeks), HC (cm), umbilical circumference (cm), upper arm length (cm), upper thigh length (cm)	Regression coefficient per SD increase in log-PFOA	BL: -0.23 (-0.35, -0.1) BW: -5.9 (-28.75, 16.94) GA: 0.01 (-0.08, 0.1) HC: -0.04 (-0.12, 0.03) Umbilical circumference: -0.06 (-0.19, 0.07) Upper arm length: -0.02 (-0.07, 0.03) Upper thigh length: -0.19 (-0.26, -0.12)
NICHD = National Institute of Child Health and Human Development							
Confounding: Maternal age, education, pre-pregnant body mass index, serum cotinine, infant sex, chemical-maternal race/ethnic interaction, mode of delivery							
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) Girls: 1.497 (0.920–2.642) Boys: 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 PFOA or by quartiles	BW -73.64 (-126.39, -20.88) Girls: -56.04 (-129.32, 17.24) Boys: -71.8 (-148.61, 5.00) p-value for interaction by sex = 0.958 GA -0.21 (-0.44, 0.02) Girls: -0.53 (-0.83, -0.23) Boys: 0.17 (-0.16, 0.51) p-value for interaction by sex = 0.002 Low BW 1.16 (0.52, 2.58)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Q2: 0.61 (0.14, 2.69) Q3: 0.27 (0.05, 1.42) Q4: 1.00 (0.23, 4.35) p-trend = 0.007 Preterm birth 1.49 (0.94, 2.36) Q2: 0.71 (0.23, 2.14) Q3: 1.60 (0.60, 4.23) Q4: 1.84 (0.72, 4.71) p-trend = 0.273
							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 2.35 (1.6–3.30)	AC, FL, BPD, estimated fetal weight at 12 weeks, 20 weeks, and 34 weeks	Percent change per twofold increase in PFOA	AC 12 wk: 0.8 (–2.4, 4.0) Girls: 2.9 (–1.7, 7.2) Boys: –1.5 (–6.0, 2.8) 20 wk: –0.5 (–3.7, 2.8) Girls: 2.7 (–1.9, 6.9) Boys: –3.1 (–7.5, 1.2) 34 wk: (1.1 (–2.1, 4.3) Girls: 1.2 (–3.2, 5.4) Boys: 1.1 (–3.3, 5.4) FL 12 wk: 1.9 (–1.4, 5.2) Girls: 4.2 (–0.5, 8.3) Boys: –0.6 (–5.0, 3.8) 20 wk: –1.4 (–4.6, 1.9) Girls: 0.2 (–4.3, 4.6) Boys: –3.0 (–7.5, 1.3) 34 wk: –0.2 (–3.5, 3.1) Girls: –1.8 (–6.3, 2.7) Boys: 1.2 (–3.4, 5.5)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							BPD 12 wk: -0.5 (-5.6, 4.5) Girls: 3.9 (-0.7, 8.2) Boys: -4.7 (-11.1, 1.8) 20 wk: 0.0 (-3.2, 3.3) Girls: 2.9 (-1.5, 7.3) Boys: -2.6 (-7.1, 1.8) 34 wk: 1.9 (-1.3, 5.1) Girls: 1.6 (-2.9, 6.0) Boys: 2.2 (-2.4, 6.6)
							Estimated Fetal Weight 12 wk: 1.2 (-2.1, 4.4) Girls: 3.3 (-1.4, 7.5) Boys: -1.2 (-5.7, 3.2) 20 wk: -0.8 (-4.0, 2.4) Girls: 2.0 (-2.5, 6.4) Boys: -3.5 (-8.0, 0.9) 34 wk: 1.3 (-1.9, 4.5) Girls: 0.7 (-3.8, 5.0) Boys: 2.1 (-2.4, 6.4)
INMA = Infancia y Medio Ambiente (Environment and Childhood) Project							
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 = 248	Cord blood 1.52 µL (1.10– 2.10 µL)	BW (g)	Regression coefficient per IQR change in PFOA	-34.5 (-129.02, 60.02)
FLEHS II = Flemish Environmental and Health Study II							
Confounding: GA, child's sex, smoking of the mother during pregnancy, parity, maternal pre-pregnancy BMI							
Huo et al., 2020, 6835452 High	China, 2013– 2016	Cohort	Mothers (aged ≥ 20 years) and their children from the	Maternal blood 11.85 (9.20– 15.26)	GA (weeks), preterm birth (indicated, non- spontaneous,	Regression coefficient (GA) per ln-unit increase in	GA: 0 (-0.14, 0.13) T1: 0.11 (-0.31, 0.54) T2: -0.69 (-1.75, 0.37) T3: 0.03 (-0.29, 0.35) OR T2: 0.11 (-0.03, 0.24)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			Shanghai Birth Cohort N = 2,849		spontaneous, and overall)	PFOA and per tertile OR (preterm birth) per ln-unit increase in PFOA and per tertile	OR T3: -0.01, -0.15, 0.12) Preterm birth, overall: 0.92 (0.61, 1.33) Females: 0.82 (0.44, 1.55) Males: 1.02 (0.59, 1.78) Preterm birth, indicated: 1.71 (0.8, 3.67) T2: 0.96 (0.44, 2.11) T3: 1.02 (0.47, 2.22) Preterm birth, non-spontaneous: Females: 2.64 (0.83, 8.39) Males: 1.23 (0.44, 3.39) Preterm birth, spontaneous: 0.73 (0.45, 1.19) T2: 0.71 (0.43, 1.17) T3: 0.76 (0.46, 1.22) Females: 0.54 (0.26, 1.13) Males: 0.95 (0.49, 1.81)
Results: Lowest tertile used as reference.							
Confounding: Maternal age, pre-pregnancy BMI, parity, parental education levels, pregnancy complicated with chronic disease, infant sex, GA at blood drawing							
Lauritzen et al., 2017, 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: 1.62 (Range = 0.31–7.97) Sweden: 2.33 (Range = 0.60–6.70)	BL (cm), BW (g), GA (weeks), HC (cm), SGA	Regression coefficient or OR (SGA) per ln-unit increase in PFOA	BL -0.49 (-0.99, 0.02); p-value = 0.06 NO: -0.1 (-0.7, 0.4); p-value = 0.656 SE: -1.3 (-2.3, -0.3); p-value = 0.01 SE-girls: -0.8 (-2.4, 0.8); p-value = 0.34 SE-boys: -1.6 (-2.9, -0.4) BW

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							<p>–81.7 (–202, 39.2); p-value = 0.185 NO: 37 (–99, 174); p-value = 0.59 SE: –359 (–596, –122), p-value = 0.003 SE-girls: –156 (541, 228); p-value = 0.419 SE-boys: –526 (–828, –222); p-value = 0.001</p> <p>GA –0.20 (–0.34, 0.14); p-value = 0.255 NO: –0.2 (–0.6, 0.2); p-value = 0.431 SE: –0.3 (–0.9, 0.3); p-value = 0.318 SE-girls: –0.1 (–1.1, 0.9); p-value = 0.802 SE-boys: –0.4 (–1.2, 0.5); p-value = 0.365</p> <p>HC –0.02 (–0.32, 0.27) NO: 0.2 (–0.2, 0.5); p-value = 0.354 SE: –0.4 (–1.0, 0.1); p-value = 0.115 SE-girls: –0.1 (–1.0, 0.7); p-value = 0.728 SE-boys: –0.6 (–1.3, 0.1); p-value = 0.103</p> <p>SGA 1.21 (0.69, 2.11) NO: 0.66 (0.33, 1.33); p-value = 0.246</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							SE: 5.25 (1.68, 16.4); p-value = 0.004 SE-girls: 4.73 (0.79, 28.3); p-value = 0.089 SE-boys: 6.55 (1.14, 37.45); p-value = 0.035
							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. 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 1.7 (1.1–2.3)	Anogenital distance (AGD) (mm); clitoral (AGDac), fourchette (AGDaf), penile (AGDap), scrotal (AGDas)	Regression coefficient per ln-unit increase in PFOA or by quartiles	AGDac –0.5 (–1.8, 0.8) p-trend by quartiles = 0.71 AGDaf 0.1 (–0.9, 1.1) p-trend by quartiles = 0.94 AGDap 0.1 (–1.1, 1.3) p-trend by quartiles = 0.71 AGDas –0.3 (–1.6, 1.0) p-trend by quartiles = 0.58 Quartile analysis did not show any statistically significant associations
							Results: Lowest quartile used as reference. Confounding: Age at examination, weight for age z-score, pre-pregnancy BMI, parity, smoking
Manzano-Salgado et al., 2017, 4238465 High	Spain, 2003–2008	Cohort	Mother (aged ≥16 years)-child pairs from INMA	Maternal plasma Mean = 2.35 (SD = 1.25)	BL (cm), BW (g), GA (weeks), HC (cm), low BW,	Regression coefficient per doubling of	BL: 0.01 (–0.28, 0.29) Q2: 0.01 (–0.28, 0.29) Q3: –0.06 (–0.36, 0.24) Q4: –0.03 (–0.34, 0.28)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			N = 1,202		low BW at term, preterm birth, SGA	PFOA and per quartiles	Females: 0.04 (–0.16, 0.24) Males: 0.01 (–0.18, 0.21)
						Low BW, low BW at term, preterm birth, SGA: OR per unit increase in maternal plasma log2 PFOA	BW: –9.33 (–38.81, 20.16) Q2: –29.6 (–92.82, 33.63) Q3: –32.99 (–97.08, 31.09) Q4: –32.77 (–97.65, 32.11) Females: 13.81 (–26.67, 54.3) Males: –24.75 (–66.71, 17.22)
							GA: –0.05 (–0.16, 0.07) Q2: –0.05 (–0.29, 0.2) Q3: 0.03 (–0.23, 0.28) Q4: –0.12 (–0.37, 0.17) Females: –0.04 (–0.2, 0.13) Males: –0.08 (–0.24, 0.08)
							HC: –0.07 (–0.17, 0.03) Q2: –0.01 (–0.22, 0.19) Q3: 0.04 (–0.17, 0.25) Q4: –0.16 (–0.38, 0.06) Females: 0.03 (–0.1, 0.17) Males: –0.13 (–0.27, 0)
							Low BW: 0.9 (0.63, 1.29) Females: 0.76 (0.48, 1.21) Males: 1.12 (0.64, 1.99)
							Low BW at term: 0.85 (0.53, 1.34) Females: 0.62 (0.36, 1.06) Males: 1.67 (0.72, 3.86), interaction p-value = 0.05
							Preterm birth: 0.92 (0.72, 1.19) Females: 1.19 (0.62, 2.31) Males: 0.74 (0.43, 1.25)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							SGA: 0.92 (0.72, 1.19) Females: 0.72 (0.5, 1.04) Males: 1.18 (0.82, 1.69), interaction p-value = 0.08
							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
Sagiv et al., 2017, 4238410 High	United States, 1999–2002	Cohort	Pregnant women and infants from Project Viva N = 1,644	Maternal blood 5.8 (IQR = 3.8)	Birth weight-for-GA (z-score), gestational length (weeks), preterm birth	Regression coefficient per IQR increase and by quartiles Preterm birth: OR per IQR increase and by quartiles	BW-for-GA: –0.02 (–0.08, 0.03) Q2: –0.04 (–0.17, 0.09) Q3: –0.12 (–0.25, 0.02) Q4: –0.07 (–0.21, 0.07) Gestational length: –0.05 (–0.16, 0.06) Q2: 0.05 (–0.22, 0.32) Q3: 0 (–0.28, 0.28) Q4: –0.04 (–0.33, 0.24) Preterm birth: 1 (0.9, 1.3) Q2: 1.1 (0.6, 2) Q3: 1.1 (0.6, 1.9) Q4: 1.2 (0.7, 2.2) BW-for-GA and gestational length: no statistically significant associations by sex
							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	Cohort	Pregnant women (aged ≥18 years) and their children at	Maternal blood 5.5 (3.8–7.7)	BW (z-score), length-for-age (z-score), rapid weight gain,	Regression coefficient by tertile (per	BW T2: 0.18 (–0.06, 0.42) T3: –0.15 (–0.4, 0.1)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
	years from recruitment		birth, 4 weeks and 2 years from the HOME study N = 345		weight-for-age (z-score), weight-for-length (z-score)	doubling in PFOA) Rapid weight gain: Relative risk by tertile	Length-for-age T2: 0.19 (–0.2, 0.5) T3: –0.32 (–0.72, 0.07) Weight gain T2: 1.08 (0.78, 1.5) T3: 0.8 (0.56, 1.15) Weight-for-age T2: –0.02 (–0.34, 0.29) T3: –0.46 (–0.78, –0.14), p-trend < 0.01 Weight-for-length T2: –0.31 (–0.56, –0.06) T3: –0.34 (–0.59, –0.08), p-trend = 0.02 BW, length-for-age, and weight gain: no statistically significant trends
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 1.1 (0.7–1.6)	Adiposity (% fat mass), BW (g)	Regression coefficient per 1-ln increase PFOA and by tertiles	Adiposity: –0.43 (–0.91, 0.04) T2: –0.34 (–1.06, 0.38) T3: –0.97 (–1.74, –0.2) BW: –51.4 (–97.2, –5.7) T2: –15.9 (–84.9, 53.2) T3: –92.4 (–166.2, –18.5)
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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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 1.0 (0.7–1.6)	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: 0.76 (–0.03, 1.55) T2: 1.4 (0.18, 2.62) T3: 1.16 (–0.18, 2.49) Females: 0.27 (–0.85, 1.4) T2: 1.71 (–0.06, 3.48) T3: 0.03 (–1.77, 1.83) Males: 1.53 (0.35, 2.71) T2: 1.2 (–0.56, 2.97) T3: 2.81 (0.79, 4.84) p-value for sex interaction = 0.07 WAZ: 0.01 (–0.14, 0.15) T2: 0.17 (–0.05, 0.39) T3: 0.08 (–0.16, 0.32) Females: –0.14 (–0.34, 0.06) T2: 0.01 (–0.3, 0.33) T3: –0.18 (–0.51, 0.14) Males: 0.17 (–0.05, 0.39) T2: 0.31 (–0.01, 0.63) T3: 0.38 (0.01, 0.75) No statistically significant interaction by sex WLZ: 0.01 (–0.16, 0.18) T2: 0.1 (–0.16, 0.35) T3: 0.07 (–0.21, 0.35) Females: –0.11 (–0.34, 0.12) T2: –0.01 (–0.38, 0.35) T3: –0.17 (–0.55, 0.2) Males: 0.14 (–0.11, 0.39) T2: 0.17 (–0.21, 0.55) T3: 0.33 (–0.1, 0.76) WAZ, growth from birth: 0.07 (– 0.08, 0.21)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							WAZ, rapid growth: 1.25 (0.77, 2.04)
							WLZ, growth from birth: 0.09 (–0.10, 0.27)
							WLZ, rapid growth: 1.43 (0.92, 2.22)
							Outcome: Rapid growth defined as change in WAZ or WLZ >0.67 between birth and 5 months
							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
Tanner et al., 2020, 6322293 High	Sweden, Recruitment: 2007–2010; followed up to 5.5 years	Cohort	Mother-infant pairs from SELMA study N = 1,334	Maternal serum Geometric mean = 1.6 (Range = 0.2–21.1)	Age of infant PGV (months), infant growth slope (log10), infant PGV (log10), infant spurt duration (log10), infant weight plateau (kg)	Regression coefficient per 1-log10 increase in PFOA	Age of infant PGV: 0.58 (0.17, 0.99), p-value = 0.01 Growth slope: –0.06 (–0.11, –0.01), p-value = 0.02 PGV: –0.02 (–0.05, 0.02) Spurt duration: 0.06 (0.01, 0.11), p-value = 0.02 Weight plateau: 0.81 (0.21, 1.41), p-value = 0.01
							SELMA = Swedish Environmental Longitudinal Mother and Child, Asthma and Allergy
							Outcome: PGV = peak growth velocity
							Confounding: Sex, preterm birth, mother's age, weight, parity, and smoking
Wang et al., 2016, 3858502 High	Taiwan Recruitment 2000–2001, assessment up to age 11	Cohort	Children from Taiwan Maternal and Infant Cohort Study, assessed at ages 2, 5, 8, and 11 years N = 106 girls, 117 boys	Maternal serum Girls: 2.34 (1.57–3.43) Boys: 2.37 (1.35–3.47)	Birth HC (cm), BL (cm), BW (kg), SGA, height z-score at each age, average childhood height z-score, weight z-score at each age, average childhood weight z-score	Regression coefficient per ln-unit increase in PFOA or by quartiles SGA: OR per ln-unit increase in PFOA Height and weight z-scores by age:	Birth HC Girls: 0.11 (–0.26, 0.47) Boys: 0.06 (–0.24, 0.36) BL Girls: –0.32 (–0.92, 0.28) Boys: 0.31 (–0.22, 0.84) BW Girls: –0.08 (–0.18, 0.01) Boys: 0.04 (–0.05, 0.12) SGA

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							regression coefficient for age and PFOA interaction Girls: 1.48 (0.63, 3.48) Boys: 0.63 (0.32, 1.13) Avg childhood height z-score Girls: -0.15 (-0.38, 0.08) Boys: 0.01 (-0.24, 0.25) Avg childhood weight z-score Girls: -0.14 (-0.39, 0.11) Boys: 0.03 (-0.11, 0.18) Girls' analysis by quartiles: no statistically significant associations Height and weight z-scores by age: NR, no significant interactions for either sex (p-value > 0.10)
Outcome: SGA defined as birth weight below the 10 th percentile for GA by sex using 1998–2002 Taiwan nationwide singleton birth weight charts. Results: Lowest quartile used as reference. Confounding: Family annual income, maternal age at delivery, maternal education, maternal previous live children, maternal pre-pregnancy BMI							
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 1.61 (1.11–2.30)	BW (g), SGA	Regression coefficient (BW) or OR (SGA) per ln-unit increase in PFOA or by quartiles	BW Per increase: -68 (-112, -24) Q2: 27 (-35, 89) Q3: -41 (-106, 23) Q4: -90 (-159, -91) Girls Per increase: -86 (-145, -26) Q2: 30 (-55, 115) Q3: -36 (-124, 52) Q4: -136 (-231, -40) Boys Per increase: -49 (-113, 15) Q2: 26 (-66, 116) Q3: -44 (-139, 50)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Q4: -47 (-147, 54)
							SGA Per increase: 1.43 (1.03, 1.99) Q2: 0.77 (0.45, 1.32) Q3: 0.96 (0.57, 1.61) Q4: 1.44 (0.86, 2.40) Girls Per increase: 1.96 (1.18, 3.28) Q2: 1.00 (0.40, 2.51) Q3: 1.64 (0.71, 3.83) Q4: 2.33 (1.00, 5.43) Boys Per increase: 1.16 (0.75, 1.78) Q2: 0.67 (0.34, 1.31) Q3: 0.66 (0.33, 1.29) Q4: 1.04 (0.54, 2.01)
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 = 2.37 µg/g (range: 0.8–6.9 µg/g)	Z-scores for BL, birth weight, and cranial circumference	Regression coefficient per log2-unit increase in PFOA	BL z-score -0.14 (-0.40, 0.13) Girls: -0.02 (-0.37, 0.32) Boys: -0.27 (-0.65, 0.10) Birth weight z-score -0.29 (-0.56, -0.01) Girls: -0.20 (-0.57, 0.16) Boys: -0.39 (-0.79, -0.01) Cranial circumference z-score -0.17 (-0.48, 0.15) Girls: -0.30 (-0.74, 0.13) Boys: -0.03 (-0.46, 0.15)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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 N = 1,954 singletons (S) (930 girls, 1,024 boys) and 902 twins (T)	Blood 1.1 (0.7–1.6)	BMI, BMI z- score, length (cm), length z- score, obesity, weight (g), weight z-score, rapid weight gain, weight- for-length (WFL) z-score	Regression coefficient or OR (rapid weight gain, obesity) per log- SD increase in PFOA or by quartiles	<p>BMI S: -0.11 (-0.17, -0.05); p-value < 0.05 S-girls: -0.18 (-0.27, -0.09); p-value < 0.05 S-boys: -0.05 (-0.12, 0.03) T: 0.04 (-0.06, 0.14)</p> <p>BMI z-score S: -0.08 (-0.12, -0.04); p-value < 0.05 Q2: -0.189 (-0.30, -0.07); p-value < 0.05 Q3: -0.22 (-0.33, -0.10); p-value < 0.05 Q4: -0.24 (-0.35, -0.12); p-value < 0.05 S-girls: -0.13 (-0.19, -0.07); p-value < 0.05 Q2: -0.16 (-0.32, 0.01) Q3: -0.23 (-0.39, -0.06); p-value < 0.05 Q4: -0.33 (-0.50, -0.16); p-value < 0.05 S-boys: -0.04 (-0.09, 0.02) Q2: -0.21 (-0.37, -0.05); p-value < 0.05 Q3: -0.20 (-0.37, -0.03); p-value < 0.05 Q4: -0.16 (-0.32, 0.01) T: 0.05 (-0.03, 0.12) Q2: 0.23 (0.03, 0.42); p-value < 0.05</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Q3: 0.21 (0.01, 0.40); p-value < 0.05 Q4: 0.19 (−0.02, 0.39)
							Length S: 0.13 (0.02, 0.25); p-value < 0.05 S-girls: 0.19 (0.01, 0.37) S-boys: 0.09 (−0.06, 0.25) T: 0.16 (−0.03, 0.34)
							Length z-score S: 0.05 (0.001, 0.11); p-value < 0.05 S-girls: 0.07 (−0.004, 0.15) S-boys: 0.04 (−0.03, 0.11) T: 0.07 (−0.01, 0.15)
							Weight S: −12.57 (−49.47, 24.33) S-girls: −30.22 (−84.05, 23.60) S-boys: 6.60 (−44.69, 57.89) T: 94.04 (33.82, 154.26); p-value < 0.05
							Weight z-score S: −0.03 (−0.07, 0.01) S-girls: −0.05 (−0.11, 0.01) S-boys: −0.01 (−0.06, 0.05) T: 0.09 (0.03, 0.16); p-value < 0.05
							WFL z-score S: −0.08 (−0.12, −0.04); p-value < 0.05 S-girls: −0.13 (−0.19, −0.06); p-value < 0.05 S-boys: −0.04 (−0.09, 0.02)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							T: 0.04 (–0.04, 0.12)
							Rapid weight gain, obesity: not statistically significant for all children
Outcome: Rapid weight gain defined as the child's weight gain SD above 0.5 for 4 or 9 months or about 0.67 for 12 months.							
Results: Lowest quartile used as reference.							
Confounding: Child's age at measurement, age squared, age cubed, sex-age interactions, maternal age, pre-pregnancy BMI category, maternal education, maternal race, private insurance, infertility treatment							
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 1.6 (Range = 0.3–5.2)	BW (g)	Regression coefficient per ln-PFOA for birth weight $\geq 2,500$ g vs. $< 2,500$ g)	0.599 (SE = 0.35)
Confounding: Gravida and mode of delivery							
Arbuckle et al., 2020, 6356900 Medium	Canada, 2008– 2011	Cohort	Pregnant women (age range = 17–42 years) and their infants from MIREC N = 205	Maternal blood 1.70 $\mu\text{g/L}$ (1.10–2.50 $\mu\text{g/L}$)	Anoclonitoris distance (ACD, mm), anofourchette distance (AFD, mm), anopenile distance (APD, mm), anoscrotal distance (ASD, mm)	Regression coefficient per ln-unit change in PFOA and by quartiles	ACD: 0.78 (–0.25, 1.82) Q2: 0.88 (–0.79, 2.54) Q3: 0.48 (–1.22, 2.17) Q4: 1.06 (–0.65, 2.76) AFD: 0.06 (–1.2, 1.32) Q2: –0.69 (–2.66, 1.28) Q3: 0.73 (–1.27, 2.74) Q4: –0.56 (–2.6, 1.48) APD: 0.1 (–0.94, 1.14) Q2: –0.76 (–2.65, 1.12) Q3: –0.02 (–1.91, 1.88) Q4: –0.51 (–2.5, 1.48) ASD: 1.36 (0.3, 2.41) Q2: 0.23 (–1.67, 2.13) Q3: –0.43 (–2.34, 1.47) Q4: 1.77 (–0.23, 3.77)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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	BMI (z-score, kg/m ²), height (z-score, cm), weight (z-score, kg)	Regression coefficient per ln increase in PFOA	At Birth BMI: –0.09 (–0.2, 0.02) Females: 0.02 (–0.13, 0.17) Males: –0.2 (–0.36, –0.04) Height: –0.04 (–0.16, 0.08) Females: –0.007 (–0.18, 0.17) Males: –0.05 (–0.22, 0.12) Weight: –0.07 (–0.18, 0.03) Females: 0.02 (–0.14, 0.17) Males: –0.15 (–0.3, –0.006)
Population: Infants were followed up at 4, 6, 13, 24, 60, 84, and 108 months Results: Regression coefficients reported at birth; BMI, height, and weight (overall and stratified by infant sex) at follow-up points were not statistically significant Confounding: Maternal age, pre-pregnancy BMI, education level, ln-cord blood cotinine, infant sex, preterm birth, postnatal ETS exposure, breastfeeding							
de Cock et al., 2014, 2713590 Medium	The Netherlands Recruitment: 2011–2013 Follow-up at 1, 2, 4, 6, 9, and 11 months after birth	Cohort	Mother-child pairs N = 89	Cord blood 870.0 ng/L (Range = 300–2,700 ng/L)	BMI (kg/m ²), HC (cm), height (cm), weight (kg)	Regression coefficient for quartiles of PFOA	BMI, HC, height, and weight: no statistically significant associations
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 870 ng/L (Range = 200–2,700 ng/L)	BW (g)	Regression coefficient by tertiles	T2: 24.6 (–270.12, 319.33) T3: 191.3 (–137.17, 519.73) Females T2: 238.1 (–183.42, 659.57) T3: –108 (–487.87, 466.34) Males

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							T2: –184.8 (–623.06, 253.41) T3: 168.4 (–239.18, 575.92) No statistically significant associations or trends by tertiles
							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 = 662	Cord blood 550 ng/L (299–1,200 ng/L)	SGA	OR per IQR increase of PFOA	1.637 (0.971, 2.761)
							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 Medium	Sweden, 1996–2011 and follow-up at 5 years of age	Cohort	Mother-infant pairs of singleton births from POPUP study N = 381	Maternal serum 2.3 (1.6–3.0)	BL (SD scores), BW (SD scores), gestational length (days), HC (SD scores), length (SD scores), weight (SD scores)	Regression coefficient per IQR increase in maternal PFOA	BL: 0.0014 (–0.1435, 0.1478) BW: –0.0579 (–0.1852, 0.0695) Gestational length: –0.2201 (–1.5028, 1.055) HC: –0.0219 (–0.1648, 0.121)
							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	Maternal serum 1.06 (Range = 0.10–7.26)	BW (g), GA at birth (weeks), HC (cm), preterm birth	Regression coefficient per 1 ln-ng/mL increase in PFOA	BW: –119 (–202, –36.6), p-value = 0.005 Females: –161 (–283, –40.1), p-value = 0.01 Males: –81.2 (–194, 31.2)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			children from ACCEPT N = 256			OR per 1 ln- ng/mL increase in PFOA	GA: 0.45 (0.17, 0.74), p-value = 0.002 Female: 0.48, p-value = 0.019 Male: 0.42, p-value = 0.043 HC Females: -0.51, p-value = 0.006 Preterm birth OR: -0.146, p-value = 0.011
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 1.62 (0.67–4.03)	Ponderal index standard deviation score (SDS)	Regression coefficient per 1-unit increase in PFOA	0.07 (0.01, 0.13), p-value = 0.02
Outcome: Ponderal index (kg/m ³) was calculated as weight (kg) divided by the length cubed (m ³)							
Results: PFOA pooled 3 and 18 months							
Confounding: Maternal age, parity, 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 and Children's Health N = 1,949	Plasma 2.0 (1.3–3.3)	Birth HC (cm), BL (cm), BW (g)	Regression coefficient per log10 change in PFOA	HC: 0.053 (-0.189, 0.295) Females: 0.039 (-0.32, 0.398) Males: 0.099 (-0.228, 0.425) Length: -0.032 (-0.309, 0.246) Females: -0.013 (-0.4, 0.373) Males: -0.041 (-0.442, 0.36) BW: -18.7 (-69.8, 32.4) Females: -1.8 (-75.1, 71.5) Males: -29.5 (-101.3, 42.3)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Confounding: GA, maternal age, pre-pregnancy BMI, parity, infant sex, maternal educational level, plasma cotinine concentration during pregnancy							HC, BL, and BW: no statistically significant associations overall or stratified by sex
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 1.4 (0.9–2.1)	BL (cm), BW (g)	Regression coefficient per 1-ln ng/mL change in PFOA	Length: 0.01 (–0.37, 0.4) BW: –494. (–130.4, 31.6) Length and BW: no statistically significant associations
Confounding: Maternal age, pre-pregnancy BMI, parity, maternal education, maternal smoking during pregnancy, GA, infant sex, maternal blood sampling period							
Kwon et al., 2016, Medium 3858531	Korea, 2006–2010	Cohort	Pregnant women and infants from EBGRC N = 268	Cord blood 0.91 (0.68–1.15)	BW (g)	Regression coefficient per 1 log-unit change in PFOA	–77.93 (–153.56, –2.3), p-value = 0.04
EBGRC = 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, 5617416 Medium	Greenland, Poland, and Ukraine 2002–2004	Cohort	Pregnant women and singleton infants from INUENDO N = 1,250	Maternal serum Geometric mean = 1.421 (2-SD ln-PFOA = 1.175)	BW at term (g)	Regression coefficient per 2-SD increase in ln-PFOA	–68.94 (–134.25, –3.63), p-value = 0.039
INUENDO = Biopersistent Organochlorines in Diet and Human Fertility Confounding: Study population, maternal age, pre-pregnancy BMI, parity							
Liu et al., 2020, 6833609 Medium	China, 2009–2013	Nested case-control	Pregnant women and infants N = 519	Maternal blood 0.79 (0.51–1.17)	Preterm birth (spontaneous)	OR per unit increase of log ₁₀ PFOA	1.08 (0.41, 1.6), p-value = 0.538 Q2: 1.22 (0.68, 2.16) Q3: 0.87 (0.48, 1.6) Q4: 1.02 (0.55, 1.88)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
						and by quartiles	No statistically significant association by quartiles
			Population: Cases, n = 144; controls, n = 375 Exposure Level: Cases: 0.74 (0.51–1.17); controls: 0.80 (0.51–1.18) Results: Lowest quartile used as reference. Confounding: Sampling time, maternal age, pre-pregnancy BMI, occupation, parity, gravidity, spontaneous abortion history, child gender, folic acid use, passive smoking, fasting status, medication use				
Manzano-Salgado et al., 2017, 4238509 Medium	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 = 2.32 (1.63–3.31)	Rapid growth, weight gain (z-score)	Relative risk per unit log2 PFOA Regression coefficient per unit increase in log2 PFOA	Rapid growth: 0.99 (0.86, 1.14) Weight gain z-score: 0.04 (–0.04, 0.12) Females: –0.03 (–0.14, 0.08) Males: 0.13 (0.01, 0.26) p-value for sex interaction = 0.28
			INMA = Infancia y Medio Ambiente [Environment and Childhood Project] Outcome: Rapid growth defined as a z-score >0.67 standard deviation for weight gain from birth until 6 months. Confounding: Maternal characteristics (i.e., region of residence, country of birth, previous breastfeeding, age, pre-pregnancy BMI), age and sex of child				
Meng et al., 2018, 4829851 Medium	Denmark, 1996–2002	Cohort	Pregnant women and their infants from DNBC N = 3,507	Maternal serum 4.6 (3.3–6.0)	BW (g), GA (days), low BW, preterm birth	BW and GA: Regression coefficient per doubling of PFOA and by quartiles Low BW and preterm birth: OR per doubling of PFOA and by quartiles	BW: –35.6 (–66.3, –5) Q2: –20.4 (–70, 29.2) Q3: –25.9 (–77.7, 25.9) Q4: –117 (–172.3, –61.6) Females: –25 (–71.4, 21.5) Males: –41.5 (–82.1, –0.9) GA: –0.4 (–1, 0.3) Q2: –1.4 (–2.4, –0.3) Q3: –1.2 (–2.2, –0.1) Q4: –1.7 (–2.9, –0.6) Females: –0.1 (–1.1, 0.9) Males: –0.6 (–1.4, 0.3) Low BW: 1 (0.7, 1.5) Q2: 1.5 (0.8, 3.1) Q3: 1.2 (0.5, 2.5)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Q4: 1.5 (0.7, 3.3)
							Preterm birth: 1.1 (0.8, 1.5) Q2: 3.2 (1.8, 5.6) Q3: 1.7 (0.9, 3.2) Q4: 1.9 (1, 3.6)
							Birth weight and GA: no statistically significant associations by sex
			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				
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 = 3.16 (95% CI = 2.92, 3.42) Boys: Geometric mean = 5.00 (95% CI = 4.70, 5.32)	BW (g), HC (cm), BL (cm), ponderal index (g/cm ³)	Regression coefficient for mean change per 1-SD increase in ln(maternal PFOA) and in ln(paternal PFOA)	Maternal PFOA Girls: BW: –61.64 (–159.15, 35.87) HC: –0.18 (–0.59, 0.23) BL: –0.17 (–0.74, 0.40) Ponderal Index: –0.02 (–0.09, 0.04) Boys: BW: 4.78 (–85.44, 95.01) HC: 0.18 (–0.25, 0.60) BL: –0.24 (–0.77, 0.29) Ponderal Index: 0.04 (–0.02, 0.10) Paternal PFOA Girls: BW: 19.82 (–69.37, 109.02) HC: –0.03 (–0.42, 0.36) BL: –0.27 (–0.79, 0.25) Ponderal Index: 0.06 (0.00, 0.12) Boys: BW: –11.04 (–112.32, 90.23) HC: –0.04 (–0.52, 0.43) BL: –0.26 (–0.86, 0.34)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Ponderal Index: 0.03 (–0.04, 0.10)
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 Geometric mean = 1.95 (SE = 0.08)	BMI z-score (BMIZ), height-for-age z-score (HAZ), weight-for-age z-score (WAZ)	Regression coefficient per 1-ln ng/mL increase in PFOA and by tertiles	BMIZ: –0.19 (–0.5, 0.12) T2: –0.3 (–0.6, 0.01) T3: –0.15 (–0.49, 0.2) Females: –0.45 (–1, 0.1) T2: –0.2 (–0.68, 0.29) T3: –0.31 (–0.9, 0.28) Males: –0.02 (–0.35, 0.3) T2: –0.38 (–0.7, –0.05) T3: –0.07 (–0.5, 0.37) HAZ: –0.31 (–0.67, 0.04) T2: –0.17 (–0.38, 0.03) T3: –0.28 (–0.65, 0.08) Females: –0.36 (–0.87, 0.14) T2: –0.25 (–0.45, –0.05) T3: –0.35 (–0.88, 0.17) Males: –0.28 (–0.7, 0.14) T2: –0.2 (–0.53, 0.13) T3: –0.23 (–0.64, 0.19) WAZ: –0.34 (–0.68, –0.01) T2: –0.33 (–0.63, –0.04) T3: –0.28 (–0.65, 0.08) Females: –0.53 (–1.18, 0.12) T2: –0.28 (–0.73, 0.16) T3: –0.43 (–1.08, 0.23) Males: –0.22 (–0.51, 0.08) T2: –0.42 (–0.77, –0.07) T3: –0.21 (–0.56, 0.15) No statistically significant associations trends by sex

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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							
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 3.31 (2.54–3.99)	HC (cm), body length (cm), BW (g)	Regression coefficient per doubling of PFOA	HC 0 (–0.22, 0.23) Girls: 0.10 (–0.23, 0.44) Boys: –0.05, (–0.36, 0.26) p-value for sex interaction = 0.90 Body length 0.03 (–0.29, 0.35) Girls: –0.01 (–0.48, 0.46) Boys: 0.02 (–0.42, 0.47) p-value for sex interaction = 0.64 BW –11 (–88, 67) Girls: 58 (–48, 164) Boys: –71 (–184, 42) p-value for sex interaction = 0.04
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 (107 cases) or without (108 controls) cryptorchidism N = 215	Cord blood 2.6 (5 th – 95 th percentile: 1.4–4.4)	Cryptorchidism	OR per ln-unit increase in PFOA or by tertiles	Continuous: 0.51 (0.21, 1.2) T2: 0.58 (0.28, 1.22) T3: 0.46 (0.20, 1.02) p-trend = 0.06
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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Wang et al., 2019, 5080598 Medium		Cross-sectional	Pregnant women and their children at birth N = 340 (171 girls, 169 boys)	Cord blood 1.99 (1.22–3.11)	BL (mm), BW (g), HC (mm), ponderal index (g/cm ³)	Regression coefficient per log10-unit increase in PFOA	<p>BL 0.94 (–3.88, 5.76); p-value = 0.702 Girls: –1.34 (–8.55, 5.86); p-value = 0.715 Boys: 0.61 (–5.93, 7.15); p-value = 0.855 p-value for interaction by sex = 0.913</p> <p>BW –33.42 (–149.6, 82.77); p-value = 0.573 Girls: –84.07 (–260.42, 92.28); p- value = 0.35 Boys: –21.24 (–171.66, 129.17); p- value = 0.782 p-value for interaction by sex = 0.959</p> <p>HC –3.7 (–7.0, –0.4); p-value = 0.028 Girls: –5.73 (–10.66, –0.81); p- value = 0.023 Boys: –3.47 (–7.89, –0.96); p- value = 0.124 p-value for interaction by sex = 0.992</p> <p>Ponderal index –0.05 (–0.10, 0.01); p-value = 0.103 Girls: –0.05 (–0.13, 0.03); p-value = 0.23 Boys: –0.03 (–0.10, 0.04); p-value = 0.401</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							p-value for interaction by sex = 0.980
Confounding: Pregnant age, family income, maternal education level, maternal career, husband's smoking, energy daily intake, daily physical activity, GA, parity, pre-pregnant maternal body mass index, gestational diabetes mellitus, infant sex, delivery mode, gestational weight gain							
Woods et al., 2017, 4183148 Medium	United States, Recruitment: 2003–2006; outcome assessed at birth	Cohort	Pregnant women and their children at birth from the HOME study N = 272	Maternal serum BW (g) 5.4 µg/L (3.8–8.1 µg/L)		Regression coefficient per log10- µg/L increase maternal PFOA	–13.1 (–53.2, 27.0)
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; CI = Confidence Interval; SD = Standard Deviation; SE = Standard Error; OR = Odds 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 PFOA 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	Maternal serum 1.64	Levels of FSH (IU/L), testosterone (nmol/L), LH (IU/L),	Regression coefficient (testosterone), or	FSH: 10% (–0.4, 21.4); p-value = 0.06

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			N = 208 boys		testosterone/LH ratio, DHEAS (nmol/L), DHEA (nmol/L), Androstenedione (nmol/L), 17-OHP (nmol/L)	percent change (%) per doubling of PFOA	Testosterone, LH, testosterone/LH, DHEAS, DHEA, androstenedione, 17-OHP: 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: 1.7	Penile width (mm), Anogenital distance (AGD)	Regression coefficient per ln-unit increase PFOA or by quartiles	Penile width: no statistically significant associations; p-trend by quartiles = 0.86 AGD: no statistically significant associations; p-trend by quartiles = 0.58
			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 1.60	In cord blood, log10-transformed levels of E2 (ng/mL), FSH (mIU/mL), Inhibin B (pg/mL), insulin-like 3 (ng/mL), LH (mIU/mL), progesterone (ng/mL), prolactin (ng/mL), SHBG (not log10-transformed, nmol/L), testosterone (pg/mL) Testosterone/E2 ratio, testosterone/SHBG ratio	Regression coefficient per log10-unit increase in PFOA, least squares mean (LSM) by quartiles	Inhibin B 0.197 (0.009, 0.384); p-value = 0.04 Q1: 36.9 (29.1, 46.7) Q2: 44.3 (36.0, 55.3) Q3: 48.5 (39.0, 60.7) Q4: 50.3 (39.2, 64.2) E2, FSH, insulin-like 3, LH, progesterone, prolactin, SHBG, testosterone, testosterone/E2, testosterone/SHBG: No statistically significant associations
			Confounding: Age, parity, BMI before pregnancy, annual income, smoking during pregnancy, caffeine consumption during pregnancy, gestational weeks of blood sampling for PFOS/PFOA measurement, gestational age at birth				

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Lopez-Espinosa et al., 2016, 3859832 Medium	United States 2005–2006	Cross-Sectional	Male children ages 6–9 years N = 1,169	Serum 34.8	Total testosterone (ln-ng/dL)	Percent difference between 75 th and 25 th percentile of ln-unit PFOA or by quartiles	Total testosterone: –4.9 (–8.7, –0.8) Q2: –3.2 (–10.6, 4.7) Q3: –10.4 (–17.6, –2.6) Q4: –10 (–17, –2.4) p-trend = 0.030
Results: Results by quartile used lowest quartile as reference. Confounding: Age, month, 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: 1.40	Levels (log10-ng/mL) of DHEA, androstenedione	Regression coefficient per log10-unit increase PFOA or by quartiles	DHEA: –0.312 (–0.642, –0.043); p-value = 0.025 Androstenedione: –0.23 (–0.49, 0.038); p-value = 0.093
Confounding: Gestational age, maternal age, parity, smoking and caffeine intake during pregnancy, maternal educational level, blood sampling period							
Liu et al., 2020, 6569227 Medium	China 2013–2014	Cross-sectional	Neonates N = 374 (183 males)	Serum Total cohort: 1.65	Cord blood levels (ng/mL) of 17-OHP, progesterone	Percent change per interquartile ratio increase PFOA	17-OHP: 7.82 (–0.22, 16.51); p-value = 0.57 Progesterone: 9.45 (3.23, 16.05); p-value <0.01
Confounding: Maternal age at delivery, pre-pregnancy BMI, maternal education status, passive smoking during pregnancy, parity, gestational weeks, sample collecting time.							
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: 5.1 Sample 2: 4.3	Age (months) at axillary hair attainment, voice break, first ejaculation, Tanner stages 2-5 for genital development or pubic hair growth; combined sex-specific puberty indicator	Regression coefficient per log2-unit increase in first trimester maternal serum PFOA Puberty indicator: mean difference in age at puberty by tertiles	No statistically significant associations
Confounding: Highest social class of parents, maternal age at menarche, maternal age at delivery, parity, prepregnancy body mass index, daily number of cigarettes smoked in first trimester							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Tian et al., 2019, 5390052 Medium	China 2012–2013	Cohort	Male infants at birth, 6 months, and 12 months N = 500	Maternal plasma 20.13	Anopenile distance (AGDap) (mm), anoscrotal distance (AGDas) (mm)	Regression coefficient per ln- unit increase in maternal PFOA or by quartiles	AGDap Birth: 0.28 (–0.62, 1.18); p-value = 0.533 6 mo.: –1.82 (–4.25, 0.62); p-value = 0.147 Q2: –3.57 (–6.73, –0.41); p-value <0.05 Q3: –1.44 (–4.70, 1.81) Q4: –3.05 (–6.19, 0.10) 12 mo.: –1.55 (–4.76, 1.66); p-value = 0.342 AGDas Birth: –0.16 (–0.92, 0.61); p-value = 0.686 6 mo.: –2.17 (–4.58, 0.24); p-value = 0.079 Q2: –3.36 (–6.51, –0.21); p-value <0.05 Q3: –2.39 (–5.62, 0.84) Q4: –2.58 (–5.71, 0.54) 12 mo.: 1.12 (–1.56, 3.79); p-value = 0.411
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)							
Arbuckle et al., 2020, 6356900 Medium	Canada 2008–2011	Cohort	Newborns from the MIREC cohort N = 205 boys	Maternal plasma 1.7	Anopenile distance (AGDap) (mm), anoscrotal distance (AGDas) (mm)	Regression coefficient per ln- unit increase in maternal PFOA, or by quartiles	AGDap Per ln increase: 0.1 (–0.94, 1.14) Q2: –0.76 (–2.65, 1.12) Q3: –0.02 (–1.91, 1.88) Q4: –0.51 (–2.50, 1.48) p-value for trend = 0.807 AGDas Per ln increase: 1.36 (0.30, 2.41); p-value <0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Q2: 0.23 (–1.67, 2.13) Q3: –0.43 (–2.34, 1.47) Q4: 1.77 (–0.23, 3.77) p-value for trend = 0.148
Results: Lowest quartile used as reference.							
Confounding: AGDap: recruitment site, education, active smoking status, gestational age; AGDas: household income, active smoking status, gestational age							
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: 4.70 Exposed: 7.35 Semen Unexposed controls: 0.1 Exposed: 0.24	AGD (cm), crown-to-pubis distance (cm), pubis-to-floor distance (cm), crown-to-pubis/pubis to floor ratio, penis circumference (cm), penis length (cm), testicular volume (mL), normal morphology (%), semen pH, immotile sperm (%), nonprogressive motility (%), progressive motility (%), total sperm count (10 ⁶), semen volume (mL), sperm concentration (10 ⁶ /mL), viability (%), FSH (U/L), testosterone (nmol/L)	Mann-Whitney test (Exposed vs. Unexposed 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	Chinese adult men N = 651	<p>Serum 8.57</p> <p>Semen 0.23</p>	<p>Serum levels (ln-transformed) of E2 (pmol/L), FSH (IU/L), LH (IU/L), SHBG (nmol/L), free</p>	Percent change per In-unit increase in serum or semen PFOA or by quartiles	<p>Free testosterone Serum PFOA: –2.7 (–4.83, –0.53); p-value = 0.015 p-trend by quartiles = 0.036</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
					testosterone, total testosterone (nmol/L); free androgen index, total testosterone/LH ratio		<p>Semen PFOA: -4.42 (-7.12, -1.55); p-value = 0.003 p-trend by quartiles = 0.001</p> <p>Total testosterone Serum PFOA: -3.1 (-5.32, -0.84); p-value = 0.008 p-trend by quartiles = 0.012 Semen PFOA: -5.56 (-8.4, -2.62); p-value <0.000 p-trend by quartiles <0.001</p> <p>E2, semen PFOA: -5.49 (-10.6, -0.17); p-value = 0.044 p-trend by quartiles = 0.031</p> <p>Total testosterone/LH, semen PFOA: -4.83 (-9.12, -0.35); p-value = 0.035 p-trend by quartiles = 0.018</p> <p>No other statistically significant associations or trends by quartile</p>
<p>Results: Lowest quartile used as reference. Confounding: Age, BMI, smoking status, blood sampling time, fasting status</p>							
Petersen, 2018, 5080277 Medium	Denmark 2007–2009	Cross-sectional	Faroese men born between 1981 and 1984 N = 263	Serum 2.8	Levels (log-transformed) of E2 (nmol/L), FSH (IU/L), free testosterone (pmol/L), inhibin B (pg/mL), LH (IU/L), SHBG (nmol/L), testosterone (nmol/L)	Regression coefficient per log-unit increase PFOA	<p>Free testosterone: -0.28 (-0.56, 0.002)</p> <p>Free testosterone/E2: -0.12 (-0.21, 0.02)^d; p-value = 0.02</p> <p>No other statistically significant associations</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
					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 $((10^6/\text{mL})^{1/3})$		
					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: 4.84 Poland: 5.19 Ukraine: 1.91	Y:X chromosome ratio of sperm	Linear regression adjusted r^2	0.013; p-value = 0.05
							Confounding: Age, abstinence time, alcohol intake, 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 = 4.0	Sperm DNA methylation level (% 5-mC) at LINE-1, Alu, or Sat-alpha; global DNA methylation level (FCM DGML channel no.)	Regression coefficient per ln-unit increase PFOA	LINE-1: 1.1 (–0.3, 2.5) Ukraine: 2.6 (0.3, 5.0); p-value <0.05 Greenland: –1.7 (–4.2, 0.7) Poland: 1.7 (–1.4, 4.8) Alu, Sat-alpha, or global methylation levels: No statistically significant associations
							Confounding: Site, age (ln-transformed), smoking status
Pan et al., 2019, 6315783	China 2015–2016	Cross-sectional	Adult men in Nanjing	Serum 8.567	Sperm normal morphology (%),	Regression coefficient per ln-	No statistically significant associations by serum PFOA

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Medium			N = 664	Semen 0.229	count $((10^6)^{1/3})$, concentration $((10^6/\text{mL})^{1/3})$, progressive motility (%), curvilinear velocity (VCL) ($\mu\text{m/s}$); straight-line velocity (VSL) ($\mu\text{m/s}$), DNA fragmentation index (DFI) (ln-%), high DNA stainability (HDS) (ln-%); semen volume (ln-mL)	unit increase PFOA in serum or in semen, or by quartiles	<p>levels; following results are by semen PFOA</p> <p>Sperm count 0.247 (0.061, 0.432) p-value = 0.05 Q2: 0.37 (0.02, 0.71) Q3: -0.08 (-0.43, 0.27) Q4: 0.42 (0.06, 0.78) p-trend = 0.2</p> <p>Sperm concentration 0.193 (0.075, 0.311) p-value = 0.02 Q2: 0.3 (0.08, 0.52) Q3: 0.06 (-0.16, 0.28) Q4: 0.36 (0.13, 0.59) p-trend = 0.2</p> <p>Progressive motility -2.377 (-3.94, -0.815) p-value = 0.03 Q2: 0.31 (-2.65, 3.27) Q3: -1.49 (-4.48, 1.50) Q4: -4.26 (7.30, 1.22) p-trend = 0.02</p> <p>Sperm VCL -1.155 (-2.064, -0.245) p-value = 0.06 Q2: -1.65 (-3.38, 0.07) Q3: -1.61, (-3.35, 0.12) Q4: -2.64 (-4.41, -0.87) p-trend = 0.08</p> <p>Sperm VSL</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							-0.92 (-1.676, -0.165) p-value = 0.08 Q2: -1.68 (-3.11, -0.24) Q3: -0.87 (-2.32, 0.57) Q4: -2.13 (-3.60, -0.66) p-trend = 0.1 Sperm DFI 0.136 (0.064, 0.209) p-value = 0.01 Q2: 0.05 (-0.09, 0.19) Q3: 0.14 (0, 0.28) Q4: 0.21 (0.07, 0.35) p-trend = 0.03 Sperm morphology, sperm HDS, semen volume: no statistically significant associations or trends

Results: Lowest quartile used as reference.

Confounding: Age, BMI, BMI², smoking, alcohol intake, abstinence time

- 1 17-OHP = 17-hydroxyprogesterone; AGD = anogenital distance; AGDap = anopenile distance; AGDas = anoscrotal distance; BMI = body mass index; DHEA =
- 2 dehydroepiandrosterone; DFI = DNA fragmentation index; DNA = deoxyribonucleic acid; E2 = estradiol; FSH = follicle stimulating hormone; HDS = high DNA stainability; LH
- 3 = luteinizing hormone; LSM = least squares mean; MIREC = Maternal-Infant Research on Environmental Chemicals; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane
- 4 sulfonic acid; SHBG = sex hormone-binding globulin; VCL = curvilinear velocity; VSL = straight-line velocity.
- 5 ^aExposure levels reported as median in ng/mL unless otherwise specified.
- 6 ^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.
- 7 ^cConfounding indicates factors the models presented adjusted for.
- 8 ^dValues are reproduced as reported in publication.

1 **C.2.2 Female**2 **Table C-3. Associations between PFOA Exposure and Female Reproductive Health Effects in Female Children and**
3 **Adolescents**

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
Jensen et al., 2020, 6311643 High	Denmark, 2010–2012	Cohort	Female infants from the Odense Child Cohort, Age 4 months, N = 165	Maternal serum, 1.70 (5 th –95 th percentile = 0.67, 3.70)	Levels of 17- OHP (nM), DHEA (nM), FSH (IU/L), LH (IU/L)	Percent change per doubling in PFOA	17-OHP 3 (–7.9, 15.2) DHEA –4.7 (–15.5, 7.4) FSH 3.8 (–6.4, 15) LH 13.3 (–4.8, 34.9)
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, 34.67 (20.48, 57.84)	Levels of estradiol (log10- pg/mL), testosterone (log10-ng/mL), testosterone to estradiol ratio	Regression coefficient per log10-unit increase in PFOA	Estradiol 0.03 (–0.01, 0.07) Testosterone 0.07 (–0.03, 0.17) Testosterone to estradiol ratio 0.04 (–0.05, 0.13)
Confounding: Maternal age, pre-pregnancy BMI, parity, mode of delivery, passive smoking during pregnancy, gestational age, household income level among female infants separately							
Ernst et al., 2019, 5080529 Medium	Denmark, 1999–2017	Cohort	Female adolescents from the Danish National Birth Cohort, N = 555	Maternal blood, Sample 1: 4.8 (10 th –90 th percentile = 2.7, 8.2)	Breast development, pubic hair development, age at attainment of axillary hair (months), age at menarche	Regression coefficient per log10-unit increase in PFOA	Breast development –1.37 (–6.14, 3.4) Pubic hair development 3.05 (–0.94, 7.04) Axillary hair –1.49 (–4.56, 1.58) Menarche –1.09 (–3.25, 1.07)
Exposure Levels: For Sample 2, median = 4.1 (10 th –90 th percentile = 2.3, 6.4). Samples 1 and 2 combined for analysis.							
Outcome: Age in months at Tanner stage 5 used to measure breast development and pubic hair development.							

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
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							
Donley et al., 2019, 5381537 Medium	United Kingdom, Recruitment 1991–1992, outcome assessed at adolescence	Case- control	Mothers and their daughters from ALSPAC, N = 446	Maternal serum, 3.7 (2.8, 4.8)	AMH (log10- ng/mL)	Regression coefficient per unit increase in PFOA	Complete AMH data 0.05 (0.01, 0.09) Multiple imputation model 0.04 (–0.01, 0.09)
Results: N for complete data = 173; N for imputation model = 446 Confounding: Maternal age at delivery, pre-pregnancy BMI, maternal education							
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, 1.40 (<LOD-5.30)	Levels of androstenedione (log10-ng/mL), DHEA (log10- ng/mL)	Regression coefficient per log10-unit increase in PFOA	Androstenedione –0.17 (–0.46, 0.07) DHEA –0.10 (–0.27, 0.11)
Confounding: Gestational age, maternal age, parity, smoking and caffeine intake during pregnancy, maternal educational level, blood sampling period							
Itoh et al., 2016, 3981465 Medium	Japan, 2002–2005	Cohort	Female infants from the Sapporo Cohort of the Hokkaido Study, N = 106	Maternal serum, 1.35 (0.80, 2.00)	Levels of estradiol (log ₁₀ - ng/mL), progesterone (log10-ng/mL), prolactin (log10- ng/mL), SHBG (nmol/L), testosterone (log10-pg/mL)	Regression coefficient per log10 increase in PFOA	Estradiol –0.04 (–0.19, 0.11) Progesterone 0.04 (–0.22, 0.29) Prolactin –0.16 (–0.36, 0.05) SHBG –0.12 (–0.29, 0.05) Testosterone –0.03 (–0.27, 0.20)
Confounding: Age, parity, BMI before pregnancy, annual income, smoking during pregnancy, caffeine consumption during pregnancy, gestational weeks of blood sampling for PFOS/PFOA measurement							
Liu et al., 2020, 6569227 Medium	China, 2013–2014	Cross- sectional	Female neonates, N = 191	Cord blood, 1.65 (1.31, 2.11)	Levels of progesterone	Percent change per IQR-unit increase in PFOA	Progesterone –0.03 (–5.64, 5.9) 17-OHP

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
					(ng/mL), 17-OHP (ng/mL)		0.69 (–5.98, 7.84)
							Confounding: Maternal age at delivery, pre-pregnancy BMI, maternal education status, passive smoking during pregnancy, parity, gestational weeks, sample collecting time
Lopez-Espinosa et al., 2016, 3859832 Medium	United States, 2005–2006	Cross- sectional	Females from the C8 Health Project, Ages 6–9, N = 1,123	Serum, 30.1 (13.5, 74.0)	Levels of estradiol (ln- pg/mL)	Percent difference by quartiles of PFOA	Q2: 12.6 (3, 23.1) Q3: 6.2 (–3, 16.4) Q4: 8.1 (–1.2, 18.4)
							Results: Lowest quartile used as the reference group. Confounding: Age, month of sampling
Maisonnet et al., 2015, 3859841 Medium	United Kingdom, 1991–1992	Cohort	Female adolescents from ALSPAC, Age 15, N = 72	Maternal serum, 3.6 (2.7, 4.7)	Levels of serum total testosterone (nmol/L), SHBG (nmol/L)	Regression coefficient by tertiles of PFOA	Testosterone T2: 0.15 (–0.02, 0.32) T3: 0.24 (0.05, 0.43) SHBG T2: 0.32 (–15.97, 16.61) T3: 5.02 (–13.07, 23.11)
							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, GM = 2.74 (GSD = 2.95)	Levels of serum FSH (ln- mIU/mL), serum SHBG (ln- nmol/L)	Means by quartile of PFOA	FSH Q1: 1.47 (SE = 0.2) Q2: 1.38 (SE = 0.21) Q3: 1.23 (SE = 0.25) Q4: 1.35 (SE = 0.29) SHBG Q1: 3.5 (SE = 0.24), p-value <0.05 Q2: 3.5 (SE = 0.25), p-value <0.05 Q3: 3.45 (SE = 0.29), p-value <0.05 Q4: 2.96 (SE = 0.34), p-value <0.05

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
Confounding: Age, gender, BMI, high fat diet							
Wang et al., 2019, 5080598 Medium	China, 2013	Cross-sectional	Pregnant women and their children, N = 171	Cord blood, 1.99 (1.22–3.11)	Levels of estrone (log10-ng/mL), b-estradiol (log10-ng/mL), estriol (log10-ng/mL)	Regression coefficient per ln-unit increase in PFOA	Estrone 0.07 (–0.07, 0.21) b-estradiol 0.14 (–0.04, 0.32) Estriol 0.29 (0.02, 0.56), p-value = 0.034
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							

17-OHP = 17-hydroxyprogesterone; ALSPAC = Avon Longitudinal Study of Parents and Children; AMH = anti-Mullerian hormone; BMI = body mass index; DHEA = dehydroepiandrosterone; DNBC = Danish National Birth Cohort; FSH = follicle stimulating hormone; LH = luteinizing hormone; GM = geometric mean; GSD = geometric standard deviation; Q1 = quartile one; Q2 = quartile two; Q3 = quartile three; Q4 = quartile four; SD = standard deviation; SE = standard error; SHBG = sex hormone binding globulin; T1 = tertile one; T2 = tertile two; 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.

8 Table C-4. Associations between PFOA 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, 11.85 (9.18, 15.29)	Gestational hypertension, hypertensive disorders of pregnancy, preeclampsia	OR per ln-unit increase in PFOA	Gestational hypertension 1.37 (0.76, 2.48) Hypertensive disorders 1.09 (0.72, 1.66) Preeclampsia 0.89 (0.5, 1.57)
Confounding: Maternal age, pre-pregnancy BMI, parity, parental educational levels, gestational age of blood drawn, fetal sex ^c							

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
Mitro et al., 2020, 6833625 High	United States, 1999–2005	Cohort	Females from Project Viva, N = 812	Plasma, 5.6 (4.0, 7.6)	Levels of SHBG (nmol/L)	Percent difference per log2-unit increase in PFOA	–1.5 (–9.3, 7) Women under 35 years during pregnancy –0.9 (–11.4, 10.8) Women over 35 years during pregnancy –1.8 (–13.7, 11.6)
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 = 1.65 (95% CI: 1.61, 1.70)	Gestational hypertension, preeclampsia, DBP (mmHg), SBP (mmHg)	OR (GH, PE) or regression coefficient (DBP, SBP) per log2-unit increase in PFOA	Gestational hypertension 1.06 (0.84, 1.35) Preeclampsia 1.36 (0.9, 2.08) DBP 0.64 (0.24, 1.05), p-value = 0.002 SBP 0.82 (0.23, 1.42), p-value = 0.006
Confounding: Maternal age, education, smoking status, pre-pregnancy BMI, parity							
Huang et al., 2019, 5083564 Medium	China, 2011–2012	Cross-sectional	Females from mother-infant pairs, N = 687	Cord blood plasma, 6.98 (4.95, 9.54)	Gestational hypertension, hypertensive disorders of pregnancy, preeclampsia	OR per increase in standardized PFOA	Gestational hypertension 0.95 (0.61, 1.48) Hypertensive disorders of pregnancy 1.02 (0.73, 1.44) Preeclampsia 1.12 (0.68, 1.84)
Results: Standardized PFOA calculated by subtracting PFOA concentration from mean PFOA concentration and dividing by the SD.							
Confounding: Age, pre-pregnancy BMI, parity, education level							
Liew et al., 2016, 6387285 Medium	Denmark, 1996–2002	Case-control	Females from the Danish National Birth Cohort, N = 438	Plasma, Cases: 3.96 (3.02, 5.22) Controls: 3.56 (2.76, 4.66)	Miscarriage	OR per doubling of PFOA and by quartiles	1.4 (1, 1.9) Q2: 1 (0.5, 1.8) Q3: 1.4 (0.8, 2.6) Q4: 2.2 (1.2, 3.9) p-value for trend <0.01
Results: Lowest quartile used as the reference group.							

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
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 ≤24, 24– 29, 30–34, ≥35, N = 344	Serum, Pregnant women: 3.3 (2.2, 4.9) Infertile females: 3.2 (2.5, 4.3)	Pregnancy loss	HR per log-unit increase in PFOA	0.93 (0.75, 1.16)
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, 1.5 (10 th –90 th percentile = 0.7, 3.1)	Menstrual cycle length (long), irregularity	OR per log-unit increase in PFOA and by tertile	Length 1.5 (1.0, 2.1) T2: 1.4 (0.8, 2.3) T3: 1.8 (1.0, 3.3) Irregularity 1.3 (0.8, 1.9) T2: 1.3 (0.8, 2.3) T3: 1.3 (0.7, 2.3)
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, Ages >18, N = 336	Serum, 5.5 (3.8, 7.7)	Breastfeeding termination at 3 months and at 6 months	RR by quartiles of PFOA	Breastfeeding termination At 3 months Q2: 1.32 (0.92, 1.88) Q3: 1.63 (1.16, 2.28) Q4: 1.77 (1.23, 2.54) p-value = 0.003 At 6 months Q2: 1.25 (0.96, 1.62) Q3: 1.38 (1.06, 1.79) Q4: 1.41 (1.06, 1.87) p-value for trend = 0.038
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							

Reference, Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
Rylander et al., 2020, 6833607 Medium	Sweden, 1989	Case-control	Females with or without pre-eclampsia, Ages 15–44, N = 876	Serum, Primiparous cases: 2.82 (Minimum, Maximum = 0.55, 10.9)	Preeclampsia	OR by quartiles of PFOA	Q2: 0.94 (0.56, 1.57) Q3: 1.42 (0.87, 2.31) Q4: 1.13 (0.68, 1.87)
Exposure Levels: [Multiparous cases] Median = 1.96 ng/mL (Minimum, Maximum = 0.42, 6.93 ng/mL); [Primiparous controls] Median = 2.83 ng/mL (Minimum, Maximum = 0.39, 9.38 ng/mL); [Multiparous controls] Median = 1.81 ng/mL (Minimum, Maximum = 0.40, 9.34 ng/mL).							
Confounding: Maternal age, BMI in early pregnancy, maternal smoking in early pregnancy, parity							
Starling et al., 2014, 2446669 Medium	Norway, 2003–2007	Nested case-control	Females from MoBa, Ages 16–44, N = 976	Plasma, 2.78 (2.14, 3.57)	Preeclampsia onset	HR per ln-unit increase in PFOA	0.89 (0.65, 1.22)
Confounding: Maternal age, pre-pregnancy BMI, education completed, smoking during pregnancy							
Timmermann et al., 2017, 3981439 Medium	Denmark, 1997–2000, 2007–2009	Cohort	Pregnant and postpartum females, N = 987	Serum, 2.40 (1.45, 3.59)	Total breastfeeding duration (months), exclusive breastfeeding duration (months)	Regression coefficient per doubling of PFOA	Total breastfeeding duration –1.3 (–1.9, –0.7) Exclusive breastfeeding duration –0.5 (–0.7, –0.3)
Confounding: Cohort, maternal age, pre-pregnancy BMI, pregnancy alcohol intake, pregnancy smoking, education, employment, parity							
Wikstrom et al., 2019, 5387145 Medium	Sweden, 2007– 2010	Cohort	Females from the SELMA study, Ages 28–35, N = 1,773	Serum, 1.61 (1.12, 2.31)	Preeclampsia	OR per log2-unit increase in PFOA	PE All women: 1.31 (0.93, 1.87) Nulliparous women: 1.38 (0.90, 2.21)
Population: N for nulliparous women = 812							
Confounding: Parity, women's age, body weight, smoke exposure							

- 1 BMI = body mass index; CI = confidence interval; DBP = diastolic blood pressure; GM = geometric mean; GSD = geometric standard deviation; HR = hazard ratio; OR = odds
- 2 ratio; Q1 = quartile one; Q2 = quartile two; Q3 = quartile three; Q4 = quartile four; RR = relative risk ratio; SBP = systolic blood pressure; SD = standard deviation; SE = standard
- 3 error; SHBG = sex hormone binding globulin; LIFE = Longitudinal Investigation of Fertility and the Environment Study; MIREC = Maternal Infant Research on Environmental
- 4 Chemicals; MoBa = Norwegian Mother and Child Cohort Study; SELMA = Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study; HOME = Health
- 5 Outcomes and Measures of the Environment; INUENDO = Biopersistent Organochlorines in Diet and Human Fertility.

- 1 ^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.
 2 ^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.
 3 ^cConfounding indicates factors the models presented adjusted for.

4 **Table C-5. Associations between PFOA 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, 4.0 (2.8, 5.7)	Natural menopause	HR per doubling of PFOA and by tertiles	1.11 (0.99, 1.24) T2: 1.12 (0.9, 1.4) T3: 1.31 (1.04, 1.65) p-value for trend = 0.01
Results: Lowest tertile used as the reference group. Confounding: 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, 2.79 (2.48, 3.16)	Cycle-specific time to pregnancy, day-specific time to pregnancy; levels of AMH (ln-ng/mL)	Times to pregnancy: FR per ln-unit increase in PFOA AMH: Regression coefficient per ln-unit increase in PFOA	Cycle-specific time to pregnancy 1.15 (0.66, 2.01) Day-specific time to pregnancy 0.96 (0.31, 1.94) AMH –0.56 (p-value = 0.75)
Confounding: Age, mean cycle length (for cycle-specific outcome)							
Dhingra et al., 2016, 3981432 Medium	United States, 2005–2006, 2008–2011	Cohort	Females from the C8 Science Panel, Age >40, N = 9,192	Serum, measured and modeled Measured: Mean = 69.2 µg/mL (SD = 195.6) Modeled:	Natural menopause	OR per ln-unit increase in PFOA, or by quintiles, or by deciles	Measured 1.09 (1.002, 1.18), p-value = 0.04 Quintile 2: 1.68 (1.21, 2.35), p-value = 0.002 Quintile 3: 1.45 (1.04, 2.02), p-value = 0.03

Reference Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
				Mean = 81.8 µg/mL (SD = 175.0)			<p>Quintile 4: 1.39 (1, 1.93), p-value = 0.05 Quintile 5: 1.58 (1.14, 2.19), p-value = 0.006</p> <p>Modeled 0.98 (0.7, 1.37) Quintile 2: 0.98 (0.7, 1.37) Quintile 3: 1.05 (0.75, 1.45) Quintile 4: 0.78 (0.56, 1.08) Quintile 5: 0.92 (0.65, 1.3)</p> <p>Dose-response by deciles: increased up to the 4th decile and then, except for a drop at the 5th decile, remained approximately level thereafter</p>
<p>Results: Lowest quintile used as the reference group. Confounding: Age, parous/nulliparous status, smoking status, education, BMI, birth year</p>							
Kim et al., 2020, 6833596 Medium	Australia, 2006–2011	Cross- sectional	Females undergoing fertility treatment, Ages 23–42, N = 97	Follicular fluid Mean = 2.4 (Minimum- Maximum = 0.3, 14.5)	Fertilization rate	Regression coefficient per unit increase in PFOA	0.71 (–2.22, 3.63)
Confounding: Age							
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: 3.1 (2.5, 4.0) Women with 25 to 31-day cycle: 3.5 (2.3, 5.0)	Day-specific probability of pregnancy, menstrual cycle length	Regression coefficient by tertiles of PFOA	<p>All women: Day-specific probability of pregnancy T2: 1 (0.7, 1.5) T3: 0.7 (0.5, 1.5) Menstrual cycle length T2: 0.98 (0.95, 1.01) T3: 0.98 (0.96, 1)</p>

Reference Confidence	Location, Year(s)	Study Design	Population, Ages, N	Exposure Matrix, Levels ^a (ng/mL)	Outcome	Comparison	Results ^b
				Women with ≥ 32 - day cycle: 3.1 (2.0, 4.7)			
							Results: Lowest tertile used as the reference group. Exposure Levels: Presented for females with 25–31-day cycles. The study also present exposure levels for females with 24-day cycles or shorter and females with cycles longer than 31 days. Results: Lowest tertile used as the reference group. Confounding: For menstrual cycle length: female age, BMI, active smoking at enrollment; For day-specific probability of pregnancy: 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, GM = 2.74 (GSD = 2.95)	Levels of FSH in serum (ln- mIU/mL), SHBG in serum (ln-nmol/L)	Means by quartile of PFOA	FSH Q1: 1.69(SE = 0.24) Q2: 1.65 (SE = 0.24) Q3: 1.64 (SE = 0.25) Q4: 1.79 (SE = 0.26) SHBG Q1: 3.83 (SE = 0.21) Q2: 3.86 (SE = 0.2) Q3: 3.81 (SE = 0.22) Q4: 3.78 (SE = 0.23)
							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: 14.67 (7.32, 23.73)	Endometriosis- related infertility	OR by tertiles of PFOA	T2: 0.89 (0.5, 1.59) T3: 1.05 (0.58, 1.91)
							Population: [Cases] Females with endometriosis; [Controls] Females from couples seeking treatment for male infertility Exposure Levels: [Control] Median = 12.09 (25 th –75 th percentile = 7.33, 22.59) Results: Lowest tertile used as the reference group. Confounding: Age, BMI, household income, and education

1 17-OHP = 17-hydroxyprogesterone; ALSPAC = Avon Longitudinal Study of Parents and Children; BMI = body mass index; DHEA = dehydroepiandrosterone; DNBC = Danish
2 National Birth Cohort; FR = fecundability ratio; FSH = follicle stimulating hormone; HR = hazard ratio; LH = luteinizing hormone; GM = geometric mean; GSD = geometric
3 standard deviation; OR = odds ratio; Q1 = quartile one; Q2 = quartile two; Q3 = quartile three; Q4 = quartile four; SD = standard deviation; SE = standard error; SHBG = sex
4 hormone binding globulin; T1 = tertile one; T2 = tertile two; T3 = tertile 3.

5 ^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.

6 ^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

7 ^cConfounding indicates factors the models presented adjusted for.

1 C.3 Hepatic

2 **Table C-6. Associations Between PFOA Exposure and Hepatic Effects in Epidemiology 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 Age >20, N = 108–3,562	Serum	Levels of ALT (log ₁₀ -IU/L), AST (log ₁₀ -IU/L)	Regression coefficient per log-unit increase	ALT Non-obese, GF-1: 0.009 GF-2: 0.047, p-value = 0.02 GF-3A: 0.001 GF-3B/4: -0.001 Obese, GF-1: 0.077, p-value <0.01 GF-2: 0.035 GF-3A: 0.057 GF-3B/4: 0.164, p-value <0.01 AST Non-obese, GF-1: 0.014 GF-2: 0.028 GF-3A: 0.002 GF-3B/4: 0.055, p-value = 0.03 Obese, GF-1: 0.039, p-value <0.01 GF-2: 0.029 GF-3A : 0.036, p-value = 0.03 GF-3B/4: 0.050, p-value <0.01
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, Age 70 N = 1,002 Age 75 N = 817	Plasma Age 70 3.31 (2.52, 4.39) Age 75 3.81 (2.71, 5.41) Age 80	Levels of ALT (μkat/l)	Regression coefficient per ln-unit increase	0.04 (0.03, 0.06), p-value <0.0016

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
			Age 80 N = 603	2.53 (1.82, 3.61)			
			Confounding: Sex, LDL and HDL cholesterol, serum triglycerides, BMI, fasting glucose levels, statins use, and 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 6.19 (4.08–9.31)	Levels of ALT (In-U/L), AST (In-U/L)	Percent change per In-unit increase	ALT 7.4 (3.9, 11.0) AST 2.9 (0.7, 5.2)
			Confounding: Age, sex, career, income, education, drink, smoke, giblet, seafood consumption, exercise, BMI				
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: -218.3; Contractors: 32.1	Levels of ALT (IU/L), AST (IU/L)	Regression coefficient per unit increase	ALT -0.0097 (SD = 0.005), p-value = 0.00495 AST -0.0032 (SD = 0.003)
			Confounding: Sex, age at baseline, BMI at baseline, alcohol consumption at baseline				
Wang et al., 2012, 2919184 Low	China 2010–2011	Cross-sectional	Male fluorochemical plant workers and near-by residents, N = 55–132	Serum Residents: 284.34 (Range = 10.20–2436.91); Workers: 1635.96 (Range = 84.98–7737.13)	Levels of ALT (In-IU/L), AST (In-IU/L)	Regression coefficient per In-unit increase	ALT Residents: -0.1 (-0.19, 0.00), p-value = 0.05 Workers: 0.04 (-0.06, 0.15) AST Residents: -0.04 (-0.10, 0.02) Workers: -0.12 (-0.22, -0.02), p-value = 0.02
			Confounding: None				
Darrow et al., 2016, 3749173 Medium	United States 2005–2006	Cohort and Cross-sectional	Adults from the C8 Health Project, N = 30,723	Modeled cumulative PFOA, 20 th –80 th percentile: 191–3998 y-ng/mL; Estimated in serum 16.5 (range = 2.6–3,559)	Levels of ALT (IU/L), Liver (enlarged, fatty, or cirrhosis), Liver disease (any)	Regression coefficient per In-unit increase or by quintiles Liver (enlarged, fatty, or	ALT Modeled, 0.012 (0.008, 0.016) Quintile 2: 0.023 (0.006, 0.040) Quintile 3: 0.035 (0.018, 0.052) Quintile 4: 0.039 (0.022, 0.056) Quintile 5: 0.058 (0.040, 0.076) p-trend<0.0001 Estimated, 0.012 (0.009, 0.016) Quintile 2: 0.001 (-0.016, 0.018)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
						cirrhosis) and disease (any): HR per 1-ln y-ng/mL increase or by quintiles	<p>Quintile 3: 0.023 (0.007, 0.040) Quintile 4: 0.036 (0.019, 0.053) Quintile 5: 0.048 (0.031, 0.066) p-trend<0.001</p> <p>Liver (enlarged, fatty, or cirrhosis) No lag, 0.97 (0.91, 1.04) Quintile 2: 0.90 (0.65, 1.25) Quintile 3: 0.83 (0.60, 1.15) Quintile 4: 0.75 (0.54, 1.03) Quintile 5: 0.83 (0.60, 1.16)</p> <p>10-year lag, 1.00 (0.94, 1.07) Quintile 2: 1.04 (0.72, 1.50) Quintile 3: 0.91 (0.64, 1.31) Quintile 4: 0.84 (0.59, 1.21) Quintile 5: 0.87 (0.61, 1.25)</p> <p>Liver disease (any) No lag, 0.97 (0.92, 1.03) Quintile 2: 1.19 (0.88, 1.59) Quintile 3: 1.08 (0.81, 1.45) Quintile 4: 1.04 (0.78, 1.40) Quintile 5: 0.95 (0.70, 1.27)</p> <p>10-year lag, 0.98 (0.93, 1.04) Quintile 2: 1.15 (0.81, 1.63) Quintile 3: 1.08 (0.76, 1.54) Quintile 4: 0.90 (0.63, 1.28) Quintile 5: 0.99 (0.70, 1.42)</p>
<p>Results: Regression coefficient for modeled continuous PFOA is per ln y-ng/mL increase. Lowest quintile used as the reference group.</p> <p>Confounding: Age, sex, BMI, alcohol consumption, regular exercise, smoking status, education, insulin resistance, fasting status, history of working at DuPont plant, race</p>							
Jin et al., 2020, 6315720 Medium	United States 2007–2015	Cross-sectional	Children and adolescents diagnosed with	Plasma 3.42 (2.5–4.1)	Ballooning, Grade of steatosis, Liver fibrosis,	OR per IQR increase	Ballooning Few balloon cells: 0.99 (0.52, 1.86)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
			nonalcoholic fatty liver disease, Ages 7–19, N = 74		Lobular inflammation, Nonalcoholic steatohepatitis, Portal inflammation		<p>Many cells/prominent ballooning: 0.42 (0.07, 2.60)</p> <p>Grade of steatosis 34-66% steatosis: 1.41 (0.61, 3.23) >66% steatosis: 1.21 (0.60, 2.47)</p> <p>Liver fibrosis Mild (Stage 1): 1.68 (0.75, 3.73) Significant (Stages 2–4): 0.97 (0.33, 2.82)</p> <p>Lobular inflammation <2 foci: 0.90 (0.45, 1.81) 2-4 foci: 1.32 (0.52, 3.39)</p> <p>Nonalcoholic steatohepatitis 1.21 (0.67, 2.18)</p> <p>Portal inflammation Mild: 1.26 (0.65, 2.43) Moderate-to-severe: 0.65 (0.18, 2.39)</p>
<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.</p> <p>Confounding: Age, sex, ethnicity, BMI z-score</p>							
Girardi & Merler, 2019, 6315730 Low	Italy 1960–2018	Cohort	Male workers at a PFASs production plant N = 462	Serum T2: GM = 13,051 ng/mL-years T3: GM = 81,934 ng/mL-years	Liver cancer or cirrhosis mortality Liver cirrhosis mortality	SMR by tertiles Mortality risk ratio by tertiles	Liver cancer or cirrhosis mortality SMRs: T1: 0.44 (0.06, 3.15) T2: 2.76 (1.15, 6.63) T3: 2.86 (1.36, 6.00) RRs: T1: 1.17 (0.15, 9.42) T2: 7.26 (2.37, 22.3) T3: 6.68 (2.41, 18.5)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							Liver cirrhosis mortality SMRs: T2: 2.76 (0.89, 8.56) T3: 2.63 (0.85, 8.14) RRs: T2: 6.59 (1.57, 27.7) T3: 5.04 (1.19, 21.3)
							Results: Workers at nearby non-chemical factory used as reference. Tertile 1 used as the reference group. Confounding: For mortality risk ratio: age at risk, calendar period
Rantakokko et al., 2015, 3351439 Medium	Finland 2005–2011	Cross-sectional	Morbidly obese adults undergoing bariatric surgery, N = 160	Serum 2.56 (5 th –95 th percentile: 1.04–4.66)	Lobular inflammation	OR per log ₁₀ unit increase by level of lobular inflammation	<2 foci vs. none: 0.71 (0.10, 5.18) 2–4 foci vs. none: 0.02 (<0.01, 0.66), p-value = 0.027
							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 0.99 (IQR = 0.45)	Levels of ALT (u/L), AST (u/L)	Regression coefficient per 1-unit increase	ALT 1.64 (–8.68, 12.00) AST 0.14 (–4.73, 5.00)
							Confounding: Age, sex, race
Attanasio, 2019, 5412069 Medium	United States 2013–2016	Cross-sectional	Adolescents from NHANES, Ages 12–19, N = 305–354	Serum Boys: GM = 1.5 (SE = 0.06) Girls: GM = 1.22 (SE = 0.06)	Levels of ALT (ln-IU/L), AST (ln-IU/L)	Regression coefficient per ln-unit increase or by quartiles	ALT Boys, –0.07 (–0.13, –0.01) Q2: 0.02 (–0.16, 0.19) Q3: –0.01 (–0.13, 0.10) Q4: –0.11 (–0.21, –0.01), p-value = 0.03 p-trend=0.09 Girls, 0.09 (0.02, 0.17) Q2: 0.09 (0.01, 0.18) Q3: 0.16 (0.05, 0.28) Q4: 0.17 (0.05, 0.28) p-trend=0.02

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							AST Boys, –0.06 (–0.12, 0) Q2: –0.01 (–0.14, 0.12) Q3: 0.00 (–0.08, 0.08) Q4: –0.05 (–0.15, 0.04) Girls, 0.06 (0.00, 0.13) Q2: 0.04 (–0.02, 0.11) Q3: 0.10 (0.01, 0.19) Q4: 0.11 (0.01, 0.20)
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 = 508–630	Plasma Prenatal: 5.4 (3.9–7.6); Mid-childhood: 4.3 (3.0–6.0)	Levels of ALT (U/L)	Regression coefficient per IQR increase	Prenatal exposure: –0.5 (–1.3, 0.2) Mid-childhood exposure: –0.7 (–1.4, 0)
Confounding: For prenatal exposure maternal education, prenatal smoking, gestational age at blood draw, and child's sex, race/ethnicity, age at lipids/ALT measurements; For mid-childhood exposure maternal education, prenatal smoking, and child's sex, race/ethnicity, age at lipids/ALT measurements							

1 BMI = body mass index; GF = glomerular filtration; GM = geometric mean; HR = hazard ratio; IQR = interquartile range; OR = odds ratio; Q1 = quartile 1; Q2 = quartile 2; Q3 =
2 quartile 3; Q4 = quartile 4; SD = standard deviation; SE = standard error; SMR = standardized mortality ratio; T1 = tertile 1; T2 = tertile 2; T3 = tertile 3.

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 C.4 Immune

2 Table C-7. Associations between PFOA 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 (7 years)	Serum 4.4 (3.5–5.7)	Antibody concentrations (log2-IU/mL) for diphtheria or tetanus	Percent change per doubling of log2-unit PFOA	Anti-diphtheria, age 7 –25.4 (–40.9, –5.8) Anti-tetanus, age 7 –20.5 (–38.2, 2.1)
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: 2.0 (1.6–2.5) 7 years: 4.4 (3.5–5.7)	Levels of diphtheria antibody (log2- IU/mL), tetanus antibody (log2- IU/mL)	Percent change per doubling of PFOA	Diphtheria antibody Age 7: –4.1 (–25.4, 23.3) p-value = 0.742 Age 13: –17.5 (–35.6, 5.8) p-value = 0.129 Tetanus antibody Age 7: 9.4 (–24.7, 58.9) p-value = 0.637 Age 13: 3.3 (–27.3, 46.9) p-value = 0.856
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)	Cohort and Cross-sectional	Infants 2 weeks after expected term date, followed up at 18 months and 5 years All: N = 490, 18 months: N = 275, 5 years: N = 349	Serum 18 months: median = 2.8 (2.0–4.5) 5 years: median = 2.2 (1.8–2.8)	Age 5 levels of tetanus antibody (IU/mL), diphtheria antibody (IU/mL)	Percent change per doubling of PFOA	2007–2009 cohort Tetanus antibody Birth: –22.25 (–35.25, –6.63) p-value = 0.007 18 mo: –16.31 (–29.04, –1.31) p-value = 0.034 5 yr: –25.26 (–42.63, –2.64) p-value = 0.031 Diphtheria antibody: Birth: –18.93 (–33.16, –1.66) p-value = 0.033

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							18 months: 4.19 (–11.76, 23.02) p-value = 0.63 5 yr: 18.31 (–10.72, 56.78) p-value = 0.24
							Combined cohort Tetanus antibody: Birth: –17.59 (–28.38, –5.17) p-value = 0.007 18 mo: –16.47 (–28.84, –1.96) p-value = 0.028 5 yr: –18.75 (–31.79, –3.21) p-value = 0.020
							Diphtheria antibody: Birth: –17.82 (–29.11, –4.74) p-value = 0.009 18 mo: 5.44 (–10.28, 23.92) p-value = 0.52 5 yr: 3.38 (–14.16, 24.50) p-value = 0.73
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 = 3.8 ± 1.1 (range = 1.6–6.4) Breastfed: mean = 16.8 ± 6.6 (range = 2.6–36.7)	Levels of Hib antibody, tetanus antibody IgG, tetanus antibody IgG1, diphtheria antibody	Spearman correlation coefficient	Hib antibody: –0.32 p-value <0.05 Tetanus antibody IgG: –0.25 p-value <0.05 Tetanus antibody IgG: –0.22 p-value <0.05 Diphtheria antibody: –0.23 p-value <0.05
Confounding: Time since last vaccination							
	Guinea-Bissau 2012–2015	Cohort	Infants enrolled at 4–7 months	Maternal blood 0.68 (0.53–0.92)	Measles antibody	Percent difference per	Inclusion (no measles vaccination): –12 (–28, 7)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Timmermann et al., 2020, 6833710 Medium			old (inclusion), followed up at 9 months and 2 years Inclusion: N = 236 9-month Unvaccinated controls: N = 100 Intervention: N = 134 2-year Unvaccinated controls: N = 102 Intervention: N = 92		concentration (mIU/mL)	doubling of PFOA	9-month visit Control (no measles vaccination): -11 (-36, 22) Intervention (1 measles vaccination): 7 (-15, 35) 2-year visit Control (1 measles vaccination): -9 (-30, 18) Intervention (2 measles vaccinations): 12 (-11, 40)
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 1.22 (0.86–1.74)	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 PFOA	CA16 Cord blood: -16.3 (-25.3, 6.1) Girls: -8.7 (-22.6, 7.6) Boys: -22.0 (-33.1, -8.9) 3 months: -6.9 (-13.2, 0) Girls: -3.2 (-11.2, 5.5) Boys: -11.1 (-20.7, -0.3) CA16 below clinical protection Cord blood: 1.56 (1.13, 2.14); p-value = 0.007 Girls: 1.16 (0.72, 1.87) Boys: 1.95 (1.16, 3.27) p-value for interaction by sex = 0.181

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							3 months: 1.73 (1.08, 2.75); p-value = 0.022 Girls: 1.31 (0.71, 2.44) Boys: 2.49 (1.23, 5.04) p-value for interaction by sex = 0.263 EV71 Cord blood: -18.7 (-28.6, -7.4) Girls: -14.6 (-30.4, 4.6) Boys: -20.6 (-32.5, -6.6) 3 months: -7.2 (-13.2, -0.8) Girls: -4.9 (-13.7, 4.8) Boys: -8.2 (-16.2, 0.5) EV71 below clinical protection Cord blood: 1.49 (1.09, 2.05); p-value = 0.014 Girls: 1.27 (0.84, 1.93) Boys: 1.76 (1.07, 2.87) p-value for interaction by sex = 0.282 3 months: 2.11 (1.27, 3.48); p-value = 0.004 Girls: 1.52 (0.81, 2.85) Boys: 2.90 (1.34, 6.29) p-value for interaction by sex = 0.202
Outcome: Clinical protection threshold defined as titers $\geq 1:8$ in modified cytopathogenic effect assay. Confounding: Sex, age, parental education, parental occupation, family income, parity, and birth weight							
Adults and Adolescents							
Pilkerton et al., 2018, 5080265 Medium for youth Low for adult	United States 1999–2000	Cross-sectional	Adults and adolescents 12 years and older	Serum Women: mean = 4.3, SE = 0.2	Rubella IgA titers (log-IU)	Regression coefficient by quartiles or per quartile increase	Adolescents: Per quartile increase: : No associations. F-value = 0.34, p-value = 0.80

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			Youths: N = 1,012 Adults: N = 542 women, 613 men	Men: mean = 6.0 SE = 0.3			Adults: Per quartile increase: F-value = 6.60, p-value = 0.002 Women Q2: -0.25 (-0.52, 0.02) p-value = 0.064 Q3: -0.15 (-0.9, 0.6) p-value = 0.686 Q4: -0.17 (-0.97, 0.64) p-value = 0.677 Men Q2: -0.14 (-0.43, 0.15) p-value = 0.339 Q3: -0.55 (-0.81, -0.28) p-value = 0.0002 Q4: -0.45 (-0.84, -0.05) p-value = 0.028
Results: Lowest quartile used as reference group							
Confounding: Women: age, ethnicity, BMI, educational level, number of live births; men: age, ethnicity, BMI, educational level							
Zeng et al., 2020, 6315718 Low	China 2015–2016	Cross-sectional	Adults from the Isomers of C8 Health Project N = 605	Serum 5.12 (3.82–8.11)	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 PFOA	HBsAb concentration -0.38 (-0.79, 0.02); p-value = 0.061 HBsAb seronegative 1.89 (1.23, 2.90); p-value = 0.004 HBsAg concentration 0.41 (-0.42, 1.25); p-value = 0.33
Confounding: Age, gender, BMI, career, income, alcohol drinking, smoking, regular exercise; education for HBsAb concentration alone							

1 RR = risk ratio; CI = confidence interval; SE = standard error; BMI = body mass index; HAI = hemagglutinin inhibition; ICH = immunohistochemistry; Ab = antibody; Q2 =
2 quartile 2; Q3 = quartile 3; Q4 = quartile 4; T2 = tertile 2; T3 = tertile 3.

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-8. Associations between PFOA 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 = 1,558 (793 boys, 765 girls)	Maternal blood 2.01 (1.31–3.35)	Infectious diseases, total (including Otitis media, Pneumonia, RS virus, Varicella)	OR by quartiles	Girls Q2: 1.45 (0.92, 2.3) Q3: 1.37 (0.87, 2.19) Q4: 1.37 (0.86, 2.21) p-value for trend = 0.242 Boys Q2: 1.02 (0.67, 1.56) Q3: 1.34 (0.87, 2.11) Q4: 0.952 (0.61, 1.49) p-value for trend = 0.854 All Q2: 1.17 (0.87, 1.6) Q3: 1.32 (0.97, 1.82) Q4: 1.11 (0.81, 1.54) p-value for trend = 0.393
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 aged 1.5, 4, or 7 years Age 1.5: N = 1,188 Age 4: N = 1,184 Age 7: N = 1,068	Maternal blood 2.35 (1.63– 3.30)	LRTI	OR or RR per log2-unit increase in PFOA	OR 1.5 years: 0.92 (0.79, 1.07) 4 years: 1.11 (0.94, 1.31) 7 years: 0.69 (0.47, 1.01) RR, 1.5–7 years All: 0.96 (0.85, 1.08) Boys: 0.97 (0.82, 1.14) Girls: 0.99 (0.83, 1.18)
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 1.94 (1.30–2.95)	Chicken pox, RSV, otitis media, pneumonia, wheeze, eczema	OR or RR per ln-unit increase in PFOA	Pneumonia: OR: 1.17 (1.01, 1.37); p-value = 0.043 Otitis media: OR: 1.06 (0.92, 1.22); p-value = 0.45 Chicken pox: OR: 0.94 (0.81, 1.09); p-value = 0.381 RSV: OR: 0.96 (0.8, 1.17); p-value = 0.694 Wheeze: RR: 0.92 (0.84, 1.01); p-value = 0.089 Eczema: RR: 0.85 (0.77, 0.94); p-value = 0.001
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 1.68 (range = 0.32–10.12)	Fever, cough, nasal discharge, diarrhea, vomiting	OR (of proportion of days with symptoms) by tertiles	Fever T2: 1.55 (0.90, 2.95) T3: 1.97 (1.07, 3.62); p-value <0.05 Cough T2: 0.72 (0.42, 1.24) T3: 1.01 (0.42, 1.24) Nasal discharge T2: 1.19 (0.70, 2.04) T3: 1.37 (0.75, 2.51) Diarrhea T2: 1.10 (0.64, 1.89) T3: 0.94 (0.51, 1.74) Vomiting

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							T2: 1.05 (0.62, 1.78) T3: 0.95 (0.52, 1.72)
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 1.6 (1.2–2.1)	Common cold episodes from 0–2 years, LRTI episodes from 0–10 years	Regression coefficient per log2-unit increase in PFOA	Common cold 0–2 years –0.04 (–0.08, 0.01) p-value = 0.089 LRTI 0–10 years 0.28 (0.22, 0.35) 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 2.54 (1.86–3.30)	Common cold, bronchitis/pneumonia, throat infection with strep, pseudocroup, ear infection, diarrhea/gastric flu, urinary tract infection	OR per 1-IQR increase in PFOA	Common cold, 0–3 years: 0.96 (0.94, 0.99); p-value <0.05 Bronchitis/pneumonia 0–3 years: 1.27 (1.12, 1.43); p-value <0.05 6–7 years: 0.75 (0.45, 1.23) Throat infection with strep, 0–3 years: 1.14 (0.96, 1.35) Other throat infections, 0–3 years: 0.91 (0.80, 1.04) Pseudocroup, 0–3 years: 1.22 (1.07, 1.38); p-value <0.05 Ear infection 0–3 years: 1.00 (0.92, 1.08) 6–7 years: 1.12 (0.88, 1.41) Diarrhea/gastric flu

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							0–3 years: 1.00 (0.94, 1.06) 6–7 years: 1.48 (1.31, 1.67); p-value <0.05
							Urinary tract infection 0–3 years: 0.78 (0.69, 0.88); p-value <0.05 6–7 years: 0.66 (0.43, 1.01)
Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy							
Kvalem et al., 2020, 6316210 Low	Norway Enrollment: 1992–1993 Follow-up: 2002–2009	Cohort and cross-sectional	Children, 10 years 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: 4.36 (IQR: 1.77) Boys: 4.53 (IQR: 1.86) Girls: 4.13 (IQR: 1.63)	Common cold, LRTI	Colds: OR (reference: 1–2 colds) LRTI: RR per IQR-unit increase in PFOA	Colds, 10–16 years 3–5 colds All: 1.23 (0.33, 4.58) p-value = 0.76 Boys: 1.41 (0.29, 6.89) p-value = 0.67 Girls: 1.32 (0.19, 9.21) p-value = 0.78 >5 colds: All: 1.29 (0.36, 4.64) p-value = 0.7 Boys: 1.38 (0.29, 6.54) p-value = 0.69 Girls: 1.67 (0.26, 1.09) p-value = 0.59 LRTI 10–16 years All: 1.1 (1.02, 1.19) p-value = 0.01 Boys: 1.11 (0.97, 1.26) p-value = 0.12 Girls: 1.49 (1.15, 1.92) p-value = 0.002 16 years All: 1.14 (0.81, 1.59) p-value = 0.45

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							Boys: 1 (0.64, 1.59) p-value = 0.99 Girls: 1.61 (0.72, 3.58) p-value = 0.25
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 ^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.

6 Table C-9. Associations Between PFOA 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
Children							
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 = 0.97 (P5–P95: 0.45–2.34) Greenland: GM = 1.79 (P5–P95: 0.80–3.66)	Asthma	OR per SD increase in PFOA	Asthma ever (combined): 0.8 (0.62, 1.04) Ukraine: 0.93 (0.47, 1.84) Greenland: 0.79 (0.60, 1.03)
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 1.6 (1.2–2.1)	Asthma	OR per log2-unit increase PFOA	Current asthma (10y): 1.06 (0.82, 1.37); p-value = 0.649 Asthma ever (10y): 1.1 (0.78, 1.54); p-value = 0.589
Confounding: Sex							
Beck et al., 2019, 5922599 Medium	Denmark, Enrollment: 2010–2012	Cohort	Children, early pregnancy to 5 years	Maternal blood 1.68 (1.13–2.35)	Wheeze, self-reported asthma, doctor-	OR per doubling in	Wheeze All: 0.98 (0.78, 1.23) Boys 0.94 (0.71, 1.23)

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 PFOA	Girls: 1.08 (0.75, 1.55) Self-reported asthma All: 1.57 (0.93, 2.68) Boys: 2.17 (1.07, 4.42) Girls: 1.06 (0.49, 2.30) Doctor-diagnosed asthma All: 0.81 (0.53, 1.22) Boys: 0.72 (0.46, 1.12) Girls: 1.70 (0.63, 4.56)
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: 1.80 (Range: 0.56–5.03) Controls: 1.38 (Range: 0.36–4.28)	Asthma	OR per log-unit increase in PFOA	1.34 (0.55, 3.29)
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 2.54 (1.86–3.30)	Asthma	OR per IQR increase in PFOA	Current asthma: Total: 1.11 (0.69, 1.79); p-value = 0.657 Boys: 1.34 (0.70, 2.60); p-value = 0.38 Girls: 0.91 (0.46, 1.82); p-value = 0.799 Ever asthma: Total: 0.99 (0.70, 1.39); p-value = 0.933 Boys: 0.98 (0.63, 1.54); p-value = 0.945

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							Girls: 0.99 (0.58, 1.70); p-value = 0.982
Manzano-Salgado et al., 2019, 5412076 Medium	Spain, 2003–2008	Cohort	Children, 4 years, N = 1,184 7 years, N = 1,068	Maternal blood 2.35 (1.63–3.30)	Asthma	OR or RR per log2-unit increase in maternal PFOA	4-year follow-up: OR = 0.77 (0.50, 1.17) 7-year follow-up: OR = 0.77 (0.54, 1.10) 4 and 7 years Girls: RR = 1.01 (0.61, 1.68) Boys: RR = 0.74 (0.49, 1.13)
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: 7.13 (5.15–9.97) Girls: 6.51 (4.57–8.73)	Asthma	OR per log10-unit increase in PFOA	All: 0.98 (0.22, 4.49), p-value = 0.98 Boys: 0.32 (0.04, 2.36), p-value = 0.26 Girls: 5.6 (0.22, 145.87), p-value = 0.30
Confounding: Child weight at age 5, gestational age, breastfeeding during the first 6 months, maternal education, maternal pre-pregnancy BMI, and annual household income							
Jackson-Browne et al., 2020, 6833598 Medium	NHANES, United States, 2013–2014	Cross-sectional	Children, ages 3–11 years, N = 607	Serum GM = 1.9 (1.4–2.7)	Asthma	OR per ln-SD increase in PFOA	1.1 (0.9, 1.4) By age: 3–5 y: 1.6 (1.0, 2.7) 6–11 y: 1.0 (0.7, 1.3) p-value for interaction by age = 0.47 By sex: Females: 1.1 (0.6, 1.7) Males: 1.1 (0.9, 1.4) p-value for interaction by sex = 0.65

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							By race/ethnicity: White, non-Hispanic: 1.3 (0.9,2.0) Black, non-Hispanic: 0.9 (0.7, 1.3) Hispanic: 1.3 (0.9, 1.9) Other: 1.1 (0.6, 1.7) p-value for interaction by race = 0.41
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: 4.36 (IQR: 1.77) Boys: 4.53 (IQR: 1.86) Girls: 4.13 (IQR: 1.63)	Asthma	RR per IQR increase in PFOA	10 years All: 1.06 (0.93, 1.21) Boys: 0.99 (0.84, 1.16) 10–16 years All: 1.04 (0.88, 1.23) Boys: 0.95 (0.72, 1.26) Girls: 1.36 (0.98, 1.89) 16 years All: 1.04 (0.87, 1.24) Boys: 0.99 (0.76, 1.27) Girls: 1.21 (0.81, 1.82)
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., 2016, 3981296 Low	Taiwan 2009–2010	Case-control	Children with (cases) or without (controls) asthma ages 10–15 from the GBCA N = 456 Case boys: 158 Case girls: 73	Serum Case boys: 1.3 (0.5–2.3) Case girls: 0.8 (0.5–1.8) Control boys: 0.5 (0.4–1.4) Control girls: 0.5 (0.4–1.2)	Asthma	Asthma: Comparison of PFOA distributions (Wilcoxon rank-sum test)	Asthma: Increased PFOA among asthmatics, p-value <0.001

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			Control boys: 102 Control girls: 123 Confounding: Cases and controls were matched on age and sex				
Zhu et al., 2016, 3360105 Low	Taiwan 2009–2010	Case-control	Children with (cases) or without (controls) asthma ages 10–15 from the GBCA N = 456 Case boys: 158 Case girls: 73 Control boys: 102 Control girls: 123 Confounding: Age, BMI, parental education, ETS, parental asthma, month of survey	Serum Case boys: 1.26 Case girls: 0.81 Control boys: 0.52 Control girls: 0.54	Asthma	OR for highest vs. lowest quartiles of PFOA exposure	Boys: 4.24 (1.81, 9.42); p-value for trend = 0.001 Girls: 3.68 (1.43, 9.48); p-value for trend = 0.005
Zhou et al., 2017, 3858488 Low	Taiwan 2009–2010	Case-control	Children with (cases) or without (controls) asthma ages 10–15 from the GBCA N = 456 Case boys: 158 Case girls: 73 Control boys: 102 Control girls: 123	Serum Cases: 1.16 (0.48–2.16) Controls: 0.52 (0.44–1.27)	Asthma	OR per unit increase in PFOA	Females with high testosterone: 3.16 (1.47, 6.78) Females with low testosterone: 2.88 (1.39, 5.97) Males with high testosterone: 2.42 (1.47, 3.99) Males with low testosterone: 2.82 (1.60, 4.97) Females with high estradiol: 2.56 (1.27, 5.12) Females with low estradiol: 3.54 (1.61, 7.79) Males with high estradiol: 2.93 (1.64, 5.24)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			Sexes evenly divided into high/low hormone classifications				Males with low estradiol: 1.85 (1.12, 3.06) No statistically significant interactions for low/high hormone levels in either sex
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 3.3 (2.5–4.0)	Asthma	OR per doubling of maternal PFOA	Asthma (age 5): Total: 1.37 (0.81, 2.32) No MMR vaccine before age 5: 10.37 (1.06, 101.93) Yes MMR vaccine before age 5: 0.76 (0.41, 1.39) Asthma (age 13): Total: 1.12 (0.67, 1.88) No MMR vaccine before age 5: 9.92 (1.06, 93.22) Yes MMR vaccine before age 5: 0.65 (0.35, 1.20)
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 = 2.1 (IQR = 1.2) Boys: GM = 1.9 (IQR = 0.7)	Asthma, self-reported doctor diagnosed	OR by quartiles of PFOA	TFF1 Q4 vs. Q1: 2.07 (1.01, 4.23); p-value = 0.046 No other statistically significant associations
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 0.89 (Range: 0.16–7.1)	Recurrent wheezing episodes	Difference in prenatal PFOA levels for wheezing vs. no wheezing	No significant differences

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
						(Mann-Whitney test)	

Confounding: None reported

- 1 GM = geometric mean; IQR = interquartile range, OR = odds ratio, RR = risk ratio, CI = confidence interval, SD = standard deviation, BMI = body mass index, GBCA = Genetics
2 and Biomarkers Study for Childhood Autism, ETS = environmental tobacco smoke, MMR = measles, mumps, rubella, TFF1 = Tromsø Fit Futures.
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.

6 Table C-10. Associations Between PFOA 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
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 = 3.59 (2.46–5.36) 2007–2010: GM = 3.27 (2.43–4.47)	Food allergy or sensitization	OR by quartiles of PFOA exposure	Food allergy, 2007–2010 cycle Q2: 2.84 (0.83, 9.73) Q3: 1.70 (0.51, 5.65) Q4: 9.09 (3.32, 24.9) p-value for trend < 0.001 Food sensitization, 2005–2006 cycle Q2: 0.91 (0.47, 1.76) Q3: 1.28 (0.59, 2.76) Q4: 1.23 (0.57, 2.65) p-value for trend = 0.74
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 2.01 (1.31–3.35)	Allergic diseases, total	OR by quartiles of PFOA exposure	Q2: 1.07 (0.79, 1.47) Q3: 0.95 (0.70, 1.31) Q4: 0.83 (0.59, 1.16) p-value for trend = 0.208

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							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, ETS 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 3.3 (2.5–4.0)	Allergy, allergic rhino-conjunctivitis in past 12 months, positive skin prick test, IgE	OR per doubling of PFOA IgE: Percent change per doubling of PFOA	Allergy at age 5 0.92 (0.53, 1.57) Allergic rhino-conjunctivitis in past 12 months, at age 13 1.18 (0.65, 2.15) Positive skin prick test, age 13 1.16 (0.76, 1.77)
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 1.6 (1.2–2.1)	Rhinitis, rhino-conjunctivitis, SPT	OR per log2-unit increase in PFOA	Rhinitis, current, 10 y 1.30 (0.97, 1.74); p-value = 0.083 Rhinitis, ever, 10 y 1.29 (0.95, 1.74); p-value = 0.098 Rhino-conjunctivitis, ever, 10 y 1.32 (0.97, 1.79); p-value = 0.079 Rhinitis, ever, spes IgE > 0.35, 10 y 1.24 (0.90, 1.71); p-value = 0.185 SPT, any pos, 10 y 0.97 (0.75, 1.24); p-value = 0.788

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Confounding: Sex							SPT + and/pr sIgE > 0.35, 10 y 1.03 (0.81, 1.30); p-value = 0.815
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 2.54 (1.86–3.30)	Allergy, food or inhaled	OR per IQR- unit increase in PFOA	Allergy, food, current All: 1.32 (0.92, 1.90); p-value = 0.136 Boys: 1.49 (0.89, 2.50); p-value = 0.131 Girls: 1.15 (0.68, 1.94); p-value = 0.602 Allergy, food, ever All: 1.10 (0.77, 1.57); p-value = 0.613 Boys: 1.04 (0.63, 1.73); p-value = 0.867 Girls: 1.14 (0.68, 1.91); p-value = 0.626 Allergy, inhaled, current All: 0.96 (0.55, 1.67); p-value = 0.887 Boys: 1.0 (0.46, 2.15); p-value = 0.994 Girls: 0.88 (0.39, 2.01); p-value = 0.765 Allergy, inhaled, ever All: 1.25 (0.88, 1.78); p-value = 0.213 Boys: 1.13 (0.71, 1.80); p-value = 0.597 Girls: 1.44 (0.84, 2.47); p-value = 0.189
Confounding: Maternal age, maternal BMI, maternal education, parity, smoking during pregnancy, nurse attendance							

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Ait Bamai et al., 2020, 6833636 Medium	Hokkaido, Japan, 2003–2012	Cohort	Early pregnancy to 7 years, N = 2,689	Maternal blood 1.94 (1.30–2.95)	Rhino- conjunctivitis	RR per ln-unit increase in PFOA, from birth to 7 years old	0.95 (0.83, 1.09); p-value = 0.487
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: 4.36 (IQR: 1.77) Boys: 4.53 (IQR: 1.86) Girls: 4.13 (IQR: 1.63)	Rhinitis, skin prick test (SPT)	Change in RR per IQR increase in PFOA	<p>Rhinitis 10 years All: 0.84 (0.61, 1.15); p-value = 0.28 Boys: 0.77 (0.53, 1.11); p-value = 0.16 Girls: 0.84 (0.48, 1.49); p-value = 0.56</p> <p>16 years All: 1.08 (1.01, 1.14); p-value = 0.02 Boys: 1.06 (0.84, 1.32); p-value = 0.63 Girls: 1.16 (0.90, 1.50); p-value = 0.25</p> <p>SPT 10 years All: 1.11 (1.07, 1.15); p-value <0.0001 Boys: 1.02 (0.82, 1.27); p-value = 0.84 Girls: 1.19 (0.79, 1.80); p-value = 0.39</p> <p>16 years All: 1.07 (1.05, 1.08); p-value <0.0001</p>

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							Boys: 1.05 (1.03, 1.06); p-value <0.0001 Girls: 1.13 (0.86, 1.47); p-value = 0.38
							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; ETS = environmental tobacco smoke; 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.

6 Table C-11. Associations Between PFOA 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 2.01 (1.31–3.35)	Eczema	OR by quartiles of PFOA	Q2: 1.10 (0.76, 1.59) Q3: 0.92 (0.623, 1.34) Q4: 0.84 (0.56, 1.27) p-value for trend = 0.287 Girls Q2: 0.88 (0.50, 1.55) Q3: 1.16 (0.67, 2.03) Q4: 1.21 (0.68, 2.17) p-value for trend = 0.356 Boys Q2: 1.31 (0.80, 2.18) Q3: 0.74 (0.43, 1.27) Q4: 0.59 (0.32, 1.08) p-value for trend = 0.022

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							p-value for interaction by sex = 0.039
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, ETS 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: 3.3 (2.5–4.0) Age 5/7: 4.0 (3.3–5.0)	Atopic eczema at age 13	OR per doubling of PFOA at age 13	Age 5: 0.72 (0.42, 1.25) Age 13: 1.36 (0.85, 2.19) MMR vaccination before age 5 Yes: 4.48 (0.42, 47.69) No: 0.82 (0.49, 1.36)
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: 6.98 (Range = <0.09–29.97) Female: 7 (Range = 0.70–29.97) Male: 6.89 (Range = <0.09–25.99)	Atopic dermatitis	OR per log-unit increase in PFOA, or by quartiles	All: 1.35 (0.93, 1.97) Q2: 1.48 (0.87, 2.52) Q3: 1.16 (0.67, 2) Q4: 1.74 (1.02, 2.95) Female: 2.07 (1.13, 3.8) Q2: 1.23 (0.52, 2.93) Q3: 1.81 (0.79, 4.14) Q4: 2.52 (1.12, 5.68) Male: 0.98 (0.58, 1.64) Q2: 1.57 (0.76, 3.23) Q3: 0.81 (0.37, 1.78) Q4: 1.34 (0.64, 2.82)
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 1.6 (Q1–Q3 = 1.2–2.1)	Atopic dermatitis diagnosed anytime	OR per log2-unit increase PFOA	Ages 0–2: 1.18 (0.94, 1.5) Ages 0–10: 0.99 (0.59, 1.67)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					between 0–2 years old, or between 0–10 years old		
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 and 4 years, N = 1,071 at 7 years	Maternal plasma 2.35 (1.63–3.30)	Eczema	OR or RR per log2-unit increase in PFOA	Age 1.5: 1.1 (0.91, 1.31) Age 7: 0.96 (0.81, 1.14) Follow up at age 4: 0.97 (0.81, 1.17) Boys at ages 1.5, 4, and 7: 0.98 (0.81, 1.18) Girls at ages 1.5, 4, and 7: 0.9 (0.75, 1.07) From ages 1.5 to 7 years: 0.96 (0.85, 1.08) No statistically significant associations
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 0.65 (0.23–1.96)	Atopic dermatitis	OR by tertiles of PFOA	T2: 0.75 (0.26, 1.89) T3: 2.58 (1.27, 5.32); p-value <0.01
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	Infants followed from birth up to 5 years of age N = 863	Cord blood 0.65 (0.23–1.96)	Atopic dermatitis	Hazard ratio for PFOA ≥1.96 ng/mL vs. <1.96 ng/mL	1.89 (1.1, 3.16); p-value <0.05
Confounding: Sex, parental education, parental atopy, breast feeding, and maternal age at childbirth							

1 Q2 = Quartile 2, Q3 = Quartile 3, Q4 = Quartile 4; ETS = environmental tobacco smoke; INMA = Spanish Environment and Childhood (Infancia y Medio Ambiente).

2 ^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.3 ^bResults reported as effect estimate (95% confidence interval) unless otherwise noted.4 ^cConfounding indicates factors the models presented adjusted for.

1 **Table C-12. Associations Between PFOA Exposure and Autoimmune 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
Steenland et al., 2013, 1937218 Medium	West Virginia 1952–2011	Cohort	Males and females from C8 Health Project, Ages ≥20, N = 32,254	Serum 26 (13–68)	Occurrence of conditions with and without a 10-year lag: rheumatoid arthritis (RA), lupus, multiple sclerosis (MS), ulcerative colitis (UC), Crohn's disease (CD)	RR by quartiles of PFOA exposure	RA, no lag Q2: 1.24 (0.85, 1.79) Q3: 1.40 (0.96, 2.03) Q4: 0.99 (0.68, 1.43) p-trend = 0.84 RA, with lag Q2: 1.53 (0.61, 2.58) Q3: 1.73 (1.10, 2.71) Q4: 1.35 (0.87, 2.11) p-trend = 0.73 Lupus, no lag Q2: 1.49 (0.68, 3.34) Q3: 1.01 (0.44, 2.30) Q4: 0.71 (0.31, 1.65) p-trend = 0.94 Lupus, with lag Q2: 0.79 (0.27, 2.34) Q3: 1.26 (0.40, 4.03) Q4: 0.61 (0.19, 1.91) p-trend = 0.93 MS, no lag Q2: 0.85 (0.44, 1.63) Q3: 1.56 (0.81, 3.00) Q4: 1.26 (0.65, 2.42) p-trend = 0.22 MS, with lag Q2: 1.16 (0.54, 2.47) Q3: 1.62 (0.74, 3.52) Q4: 1.32 (0.61, 2.84) p-trend = 0.59

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							UC, no lag Q2: 1.76 (1.04, 2.00) Q3: 2.63 (1.56, 4.43) Q4: 2.86 (1.65, 4.96) p-trend < 0.0001
							UC, with lag Q2: 1.71 (0.89, 3.27) Q3: 2.05 (1.07, 3.91) Q4: 3.05 (1.56, 5.96) p-trend < 0.0001
							CD, no lag Q2: 1.25 (0.61, 2.58) Q3: 1.15 (0.55, 2.41) Q4: 1.00 (0.48, 2.09) p-trend = 0.73
							CD, with lag Q2: 0.80 (0.32, 1.99) Q3: 0.97 (0.36, 2.60) Q4: 0.69 (0.26, 1.82) p-trend = 0.79
							Results: Lowest quartile used as reference. Confounding: Sex, race/ethnicity, smoking, BMI, alcohol consumption ^c
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: 1.26 (IQR = 0.76) Controls: 0.99 (IQR = 0.51)	Celiac disease	OR per ln-unit change in PFOA	3.85 (0.71, 21.1) Girls: 20.6 (1.13, 375); p-value <0.05 Boys: 1.05 (0.11, 9.59)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Confounding: Genetic susceptibility score, albumin, BMI, age, race (non-Hispanic white vs. other race/ethnicity) and sex							
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 neither	Serum UC: 2.93 CD: 1.78 Controls: 1.33	UC	OR of UC vs. CD and/or neither per ln- unit increase in PFOA, or by quintiles	UC vs. CD: 1.68 (1.07, 2.23) UC vs. neither: 2.00 (1.08, 3.67) UC vs. CD & neither: 1.60 (1.14, 2.24) Q2: 0.81 (0.22, 2.93) Q3: 40.98 (11.67, 150.34) Q4: 33.36 (11.32, 119.36) Q5: 2.86 (0.94, 8.75)
Results: Lowest quintile used as reference. Confounding: Age, sex, ethnic group (white or non-white)							
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: 1.88 (1.34–2.32) Controls: 1.94 (1.38–3.01)	Relapsing remitting multiple sclerosis (RRMS)	Percent change in PFOA comparing MS cases vs. healthy controls	–12 (–24, 2); p-value = 0.099 Females: 7 (–13, 32); p-value = 0.526 Males: –28 (–42, –9); p-value = 0.006
Confounding: Age, sex, breastfeeding							

1 PFOA = perfluorooctanoic acid; RA = rheumatoid arthritis; MS = multiple sclerosis; UC = ulcerative colitis; CD = Crohn's disease; RR = risk ratio; BMI = body mass index; OR
2 = odds ratio; RRMS = relapsing remitting multiple sclerosis; CIS = clinically isolated serum syndrome.

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 C.5 Cardiovascular

2 C.5.1 Cardiovascular Endpoints

3 Table C-13. Associations Between PFOA 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 Levels not provided	DBP, SBP	Regression coefficient per log10- unit increase in PFOA	DBP Total cohort: 0.008 (–0.009, 0.026) Females: –0.005 (–0.027, 0.016) Males: 0.018 (–0.01, 0.046) SBP Total cohort: –0.003 (–0.01, 0.004) Females: –0.005 (–0.015, 0.004) Males: –0.004 (–0.014, 0.007)
Confounding: Age, gender, race, BMI, cotinine, dietary calcium, caloric intake, sodium consumption, potassium consumption, sampling year ^c							
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 2.3 (1.4–3.3) Plasma 1.5 (1.2–2.0)	DBP, SBP	Regression coefficient per log2-unit IQR increase PFOA	DBP Maternal PFOA: 0.29 (–0.55, 1.13) Childhood PFOA: 0.23 (–0.45, 0.91) SBP Maternal PFOA: –0.1 (–1, 0.8) Childhood PFOA: 0.39 (–0.34, 1.12)
Confounding: Cohort of inclusion, maternal age, maternal education level, maternal pre-pregnancy BMI, parity, parental country of birth, child age, child sex, child height							
Lin et al., 2013, 2850967	Taiwan 2006–2008	Cross-sectional	Adolescents and young	Serum	SBP, CIMT	Mean by PFOA exposure group	SBP: No associations CIMT: No associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Medium for CINT Low for Systolic BP			adults ages 12–30 N = 637	3.49 (75 th percentile = 6.54)			
Comparison: Groups were defined as follows: (1) up to 50 th percentile; (2) 50 th –75 th percentile; (3) 75 th –90 th percentile; (4) above 90 th percentile Confounding: Age, gender, smoking status, alcohol drinking, body mass index; for CINT, also includes SBP, low density lipoprotein cholesterol, triglyceride, high sensitivity CRP, homeostasis model assessment of insulin resistance							
Lin et al., 2016, 3981457 Medium	Taiwan 1992–2000	Cross-sectional	Adolescents and young adults ages 12–30 N = 848	Serum Geometric Mean = 3.21 (95% CI: 3.00–3.46)	8-OHDG (log-μg/g creatinine) CINT CD31+ / CD42a– (log count/μL) CD31+ / CD42a+ (log count/μL) CD62E (log count/μL) CD62P (log count/μL)	Mean by PFOA exposure level group	8-OHDG: Borderline statistically significant increase across exposure groups, 7.55–7.68 (Group 3); p-trend = 0.059 CINT: No associations across exposure groups; p-trend = 0.2868 CD31+ / CD42a–: Statistically significant decrease across exposure groups, 5.14–4.77; p-trend = 0.036 CD31+ / CD42a+, CD62E, CD62P: No statistically significant associations across exposure groups
Comparison: Groups were defined as follows: (1) up to 50 th percentile; (2) 50 th –75 th percentile; (3) 75 th –90 th percentile; (4) above 90 th percentile. Confounding: Age, gender, smoking status, BMI, systolic blood pressure, low density lipoprotein, triglyceride, homeostasis model assessment of insulin resistance, and high sensitivity CRP							
Khalil et al., 2018, 4238547 Low	United States 2016	Cross-sectional	Obese children ages 8–12 N = 48	Serum 0.99 (IQR = 0.45)	DBP, SBP	Regression coefficient per unit increase in PFOA	DBP: 7.75 (–0.25, 15.7) SBP: 7.99 (–2.29, 18.3)
Confounding: Age, race, sex							
Koshy et al., 2017, 4238478	United States 2011–2012	Cross-sectional	Children and adolescents	Serum	Augmentation Index (AI)	Regression coefficient	AI: –1.41 (–4.59, 1.78) BAD: 0.45 (0.04, 0.87)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Low			from the World Trade Center Health Registry (WTCHR) N = 308	1.81 (IQR = 0.90) Comparison: 1.39 (IQR = 0.75)	Brachial Artery Distensibility (BAD) Pulse Wave Velocity (PWV)	per ln-unit increase in PFOA	PWV: 0.05 (–0.17, 0.28)
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 = 2.32 (1.63–3.31)	Blood Pressure (BP) (z-score) Cardiometabolic Risk Score (CMR)	Regression coefficient per log2-unit increase in PFOA	BP All age 4: –0.06 (–0.16, 0.04) Girls: –0.04 (–0.18, 0.1) Boys: –0.08 (–0.23, 0.07) All age 7: –0.02 (–0.11, 0.07) Girls: –0.08 (–0.21, 0.04) Boys: 0.04 (–0.08, 0.16) CMR All age 4: 0.27 (–0.35, 0.89) Girls: –0.22 (–1.1, 0.66) Boys: 0.72 (–0.17, 1.62)
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 2.35 (1.63–3.30)	CRP (log10 mg/dL)	Percent median change by quartiles and per log10-unit increase in PFOA	CRP 2.86 (–8.12, 14.3) By quartile: Q2: –12.19 (–27.3, 6.18) Q3: –3.92 (–22.1, 17.3) Q4: 3.05 (–17.3, 28.4)
Results: Results by quartile use lowest quartile as the reference group.							
Confounding: Sub-cohort, country of birth, pre-pregnancy body mass index, previous breastfeeding, parity, gestational week at blood extraction, physical activity, relative Mediterranean Diet Score							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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 3.33 (2.13– 5.10)	DBP, SBP, hypertension	DBP and SBP: Regression coefficient per log10-unit increase in PFOA Hypertension: OR by tertiles or regression coefficient around inflection point (1.80 ng/mL)	DBP: –0.34 (–1.43, 7.55) SBP: 1.83 (0.40, 3.25) Hypertension T2: 1.03 (0.89, 1.2) T3: 1.32 (1.13, 1.54), p-value < 0.01, p-trend < 0.001 No significant interactions by age Females T2: 0.96 (0.77, 1.19) T3: 1.42 (1.12, 1.79), p-value < 0.001, p-trend = 0.003 Males: No statistically significant associations, or trends Ages >60 years T2: 0.84 (0.66, 1.06) T3: 1.32 (1.03, 1.68) p-trend = 0.003 Ages ≤60 years: No statistically significant associations or trends Levels ≤1.80 ng/mL: 0.56 (0.32, 0.99) Levels >1.80 ng/mL: 1.32 (1.03, 1.68)
<p>Outcome: Hypertension defined as average SBP > 140 mmHg and average DBP > 90 mmHg, or self-reported use of prescribed anti-hypertensive medication.</p> <p>Comparison: Tertiles are defined as follows (in ng/mL PFOA): T1 ≤2.5; 2.5 < T2 ≤4.4; 4.4 < T3.</p> <p>Results: Lowest tertile used as the reference group.</p> <p>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</p>							
Mattsson et al., 2015, 3859607 High	Sweden 1990–1991, 2002–2003	Case-control	Rural men N = 462	Serum Cases: 4.2 (IQR = 1.8) Controls: 4.0 (IQR = 2.0)	CHD	OR by quartiles	CHD Q2: 0.79 (0.44, 1.43) Q3: 1.18 (0.67, 2.06) Q4: 0.88 (0.5, 1.55)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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) = 3.59 (1.69)	Left Ventricular End-Diastolic Diameter (LVEDD) (mm) Left Ventricular Mass Index (LVMI) (g/m ^{2.7}) Relative Wall Thickness (RWT)	Regression coefficient per ln-unit increase in PFOA	LVEDD: 0.58 (–0.03, 1.18) LVMI: –0.65 (–1.94, 0.65) RWT: –0.12 (–0.22, –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 6.19 (4.08–9.31)	DBP, SBP, hypertension	Regression coefficient per ln-unit increase in PFOA Hypertension: OR per ln-unit increase PFOA	DBP Total: 2.18 (1.35, 2.98) SBP Total: 1.69 (0.25, 3.13) Females: 2.91 (0.1, 5.72) Males: No association Hypertension: No statistically significant associations
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	Plasma Females: 4.1 (2.8–5.6)	DBP, SBP	Partial Spearman correlation coefficient	DBP: 0.1; p-value < 0.05 SBP: 0.04

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			POUNDS Lost Study N = 621 (384 females, 237 males)	Males: 5.2 (3.9–6.8)			
			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: 4.9 (3.5–6.7) Year 2: 5.7 (4.0–8.0) Year 14: 2.8 (2.0–3.8)	DBP, SBP, pulse pressure (mmHg), and hypertension	Regression coefficient per log2-unit increase in PFOA or by quartiles Hypertension: HR or RR per log2-unit increase PFOA or by quartiles	DBP: No statistically significant associations by timepoint, by quartiles, or by sex (p-value for interaction by sex = 0.81) SBP: Baseline: 1.49 (0.29, 2.70) Baseline males: 2.36 (0.13, 4.60); p-value for interaction by sex = 0.28 No statistically significant associations by follow-up timepoint or by quartiles Pulse Pressure: No statistically significant associations by timepoint, by quartiles, or by sex (p-value for interaction by sex = 0.24) Hypertension Baseline males: 1.27 (1.06, 1.53); p-value for interaction by sex = 0.07165 No statistically significant associations by timepoint or by quartiles
			Outcome: Hypertension defined as SBP ≥ 140 mmHg and DBP ≥ 90 mmHg in those without diabetes, SBP ≥ 30 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				
Mi et al., 2020, 6833736 Medium	China 2015–2016	Cross-sectional	Shenyang residents ages 23-94	Serum 4.8 (3.6–7.4)	DBP, SBP, hypertension	DBP, SBP: egression coefficient per	DBP 1.49 (0.34, 2.64) Females: 0.38 (–0.75, 1.51)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			N = 1238 (559 women, 679 men)			In-unit increases in PFOA Hypertension: OR per ln-unit increase in PFOA	Males: 1.82 (–0.04, 3.67) p-interaction = 0.05 Ages >60: 1.96 (0.62, 3.31) Ages 23–60: No associations No statistically significant sex interactions within age groups SBP: No statistically significant associations or interactions by sex or age Hypertension 1.72 (1.27, 2.31) Females: 2.32 (1.38, 3.91) p-interaction = 0.22 Ages >60: 3.58 (2.14, 5.98) Ages 23–60: No associations No statistically significant sex interactions within age groups
Outcome: Hypertension defined as mean SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, or use of antihypertensive medicines for previous two weeks. Confounding: Age, sex, ethnicity, career, education, smoking, alcohol drinking, physical activity, annual household income, and seafood consumption							
Mitro et al., 2020, 6833625 Medium	United States 1999–2005	Cohort	Pregnant women and their children at age 3 from Project Viva N = 761 mothers (496 ages < 35, 265 ages ≥ 35)	Plasma 5.6 (4.0–7.6)	DBP, SBP, CRP (mg/L)	Percent difference per log2-unit increase in PFOA Regression coefficient per log2-unit increase in PFOA	DBP, SBP, CRP: No statistically significant associations
Population: For measurements of CRP, 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							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Liu et al., 2018, 4238514 Medium	United States 2013–2014	Cross-sectional	Adults ages 18+ from NHANES N = 1,871	Serum Geometric Mean (SE) = 1.86 (1.02)	Hypertension	OR per ln-unit increase in PFOA	Hypertension: 1.13 (0.81, 1.58)
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 2.8 (1.8–4.3)	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, PFOS, 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: 2.9 (2.2–4.2) Follow-up: 2.7 (1.9–3.6)	Hypertension	OR by tertiles or per SD-unit increase in PFOA	Hypertension Baseline: OR per increase: 1.12 (0.78, 1.59) Follow-up: OR for T3: 1.14 (0.51, 2.58)
Outcome: Hypertension defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, self-reported diagnosis, or use of antihypertensive drugs Results: Results by tertile use lowest tertile as the reference group. Confounding: Gender, age, education, sample year, body mass index, smoking habit, alcohol consumption, physical activity, healthy diet score							
Shankar et al., 2012, 2919176 Medium	United States 1990–2000, 2003–2004	Cross-sectional	Adults ages 40+ from NHANES N = 1,216 (623 females, 593 males)	Serum Female: 3.9 (2.9, 5.6) Male: 4.3 (3.0, 6.1)	CVD, cardiovascular heart disease (CVHD), peripheral arterial	OR by quartiles	CVD Q3: 1.77 (1.04, 3.02) Q4: 2.01 (1.12, 3.60) Increasing trend by quartiles; p-trend = 0.01 CVHD

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
					disease (PAD), stroke, CVD or PAD Cardiovascular Disease (CVD)		Q4: 2.24 (1.02, 4.94) Increasing trend by quartiles; p-trend = 0.007 PAD Q4: 1.78 (1.03, 3.08) Increasing trend by quartiles; p-trend = 0.04 Stroke Q2: 4.39 (1.44, 13.37) Q3: 3.94 (1.48, 10.05) Q4: 4.26 (1.84, 9.89) p-trend = 0.02 CVD or PAD: Q3: 1.72 (1.13, 2.64) Q4: 2.28 (1.40, 3.71) Increasing trend by quartiles; p trend <0.001 Females: Q4: 2.99 (1.53, 5.81) Increasing trend by quartiles; p-trend = 0.004 Males: Q3: 1.75 (1.04, 2.96) Q4: 1.83 (1.02, 3.28) Increasing trend by quartiles; p-trend = 0.04
Results: Lowest quartile used as reference.							
Confounding: Age, sex, race/ethnicity, educational level, smoking status, alcohol intake, body mass index, hypertension, diabetes mellitus, serum total cholesterol level; serum high-sensitivity CRP level and serum uric acid level for CVD and PAD outcomes only							
Fry and Power, 2017, 4181820 Medium	United States 2003–2006	Cohort	Adults ages 60+ from NHANES N = 1,023	Serum 23.7 ng/g (SE = 0.7 ng/g)	Mortality by cerebrovascul ar or heart diseases	HR per SD-unit increase in PFOA	Mortality 0.98 (0.81, 1.17)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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 3.3 (2.52–4.39)	CIMT, carotid artery intima- media complex grey scale median (CIM-GSM), carotid artery atheroscleroti c plaque	CIMT, CIM- GSM: Regression coefficient per ln-unit increase in PFOA Plaque: OR per ln-unit increase in PFOA	CIMT, CIM-GSM, atherosclerotic plaque: no statistically significant associations; all p-values >0.25
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 3.17 (1.97– 4.90)	CVD, angina pectoris, congestive heart disease, CHD, heart attack, stroke, CRP (mg/L)	OR by quartiles CRP: Spearman correlation coefficient	CVD: No association by quartiles, no significant trend; p-trend = 0.703 No associations, trend, or interaction by age groups Females Q2: 0.76 (0.49, 1.18) Q3: 1.04 (0.66, 1.66) Q4: 1.14 (0.75, 1.75) Males Q2: 1.49 (0.98, 2.26) Q3: 1.56 (1.02, 2.40) Q4: 1.45 (0.92, 2.28) No trend or interaction by sex Angina pectoris: No association by quartiles, no significant trend; p-trend = 0.391 Congestive heart disease: No association by quartiles, no significant trend; p-trend = 0.670

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							CHD: No association by quartiles, no significant trend; p-trend = 0.097
							Heart attack Q2: 1.57 (1.06, 2.34) Q3: 1.62 (1.04, 2.53) Q4: 1.47 (0.91, 2.37) p-trend = 0.231
							Stroke Q2: 1.01 (0.70, 1.44) Q3: 1.42 (0.94, 2.13) Q4: 1.37 (0.92, 2.05) p-trend = 0.045
							CRP: -0.068; p-value <0.001
Comparison: Age groups were defined as <50 years and ≥50 years. Results: Lowest quartile used as the reference group. Confounding: Age, sex, race/ethnicity, family poverty income ratio, education levels, physical activity levels, BMI, alcohol drinking status, smoking status, diabetes, hypertension, family history of CVD, total energy intake, log-transformed levels of serum cotinine, log-transformed levels of serum total cholesterol							
Cardenas et al., 2019, 5381549 Medium	United States 1996–2014	Controlled trial	Prediabetic adults ages 25+ from Diabetes Prevention Program (DPP) and Outcomes Study (DPPOS) N = 877	Plasma Geometric mean (IQR) = 4.82 (3.20)	Microvascular disease (MVD), neuropathy, retinopathy	OR per log2-unit increase in baseline PFOA	MVD, 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: 28.7 (12.9–73.6)	Stroke	OR per ln-unit increase in PFOA	0.96 (0.91, 1.01)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
				Without diabetes: 27.6 (13.4–70.4)			
Confounding: Age, BMI, CRP, 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) = 3.46 (0.04) Male Mean (SE) = 4.50 (0.06)	DBP, SBP	Percent difference per interquartile ratio increase in PFOA by quartiles	DBP: No associations in men or women. No significant trend (p-trend = 0.390 and 0.167 among females and males, respectively) SBP: No associations in men or women. No significant trend (p-trend = 0.096 and 0.642 among females and males, respectively)
Results: Lowest quartile used as the reference group. Interquartile ratio = 75 th /25 th percentiles of serum PFOA: 2.43 ng/mL.							
Confounding: None listed							
Yang et al., 2018, 4238462 Low	China Years not reported	Cross-sectional	Adult men N = 148	Serum 1.90 (Range: 0.6–5.0)	DBP, SBP, hypertension	Regression coefficient per log-unit increase in n-PFOA Hypertension: OR for elevated pressure (DBP ≥90 or SBP ≥140 mmHg) comparing above or below median	DBP: No statistically significant associations SBP: 12.94 (–1.46, 27.35) OR: 10.8 (1.31, 90)
Outcome: Hypertension evaluated by individual BP components.							
Confounding: Age							
Chen et al., 2019, 5387400 Low	Croatia 2007–2008	Cross-sectional	Adults aged 44–56 N = 122	Plasma Geometric mean (range) =	DBP, SBP	Regression coefficient per	DBP: –1.00 (–4.11, 2.11) SBP: –2.15 (–8.49, 4.18)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
				2.87 (1.03–8.02)		In-unit increase in PFOA	
				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 2.98 (1.94–4.69)	Cardiovascular conditions, self-reported	OR per log-unit increase in PFOA	Any condition 0.97 (0.9, 1.05)
				Confounding: Age, BMI			
Honda-Kohmo et al., 2019, 5080551 Low	United States 2005–2006	Cross-sectional	Adults ages 20+ from C8 Health Project N = 5,270 with diabetes and 49,191 without diabetes	Serum 28.4 (12.6–74.9)	CHD	OR per ln-unit increase in PFOA or by quintiles	CHD Diabetic adults: 0.9 (0.85, 0.96) Q2: 0.92 (0.71, 1.18) Q3: 0.86 (0.67, 1.11) Q4: 0.74 (0.58, 0.96) Q5: 0.73 (0.57, 0.94) Diabetic females: 0.88 (0.80, 0.96) Diabetic males: 0.93 (0.85, 1.00) Nondiabetic adults: 0.95 (0.92, 0.98)
				Results: Results by quintile use lowest quintile as the reference group.			
				Confounding: Age, BMI, CRP, diabetes duration, eGFR, HDL, LDL, hemoglobin, iron, sex, smoking history, uric acid, white blood cell count			
Occupational Populations							
Steenland et al., 2015, 2851015 Low	United States 2008–2011	Cohort	Current and former workers at a chemical plant N = 3,713	Serum Cumulative exposure IQR with or without 10-year lag: 0.8–7.04 or 3.03–11.42 µg/mL-year	CHD, hypertension, stroke	Incidence rate ratio (RR) by quartiles	CHD: No associations with or without lag; RRs ranging from 0.93 to 1.23. No significant trend. Hypertension: No association with or without lag; RRs ranging from 0.91 to 1.04 No significant trend. Stroke No lag Q2: 2.63 (1.06, 6.56)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							No associations with lag; RRs ranging from 2.63 to 2.07. No significant trend.
							Outcome: Hypertension was self-reported and only analyzed if participants reported taking medication for it.
							Results: Lowest quartiles used as the reference group.
							Confounding: Gender, race, education, BMI, smoking, alcohol consumption
Christensen et al., 2016, 3858533 Low	United States 2012–2013	Cross-sectional	Male anglers ages 50+ N = 154	Serum 2.50 (1.80–3.30)	Cardiovascular condition (any), CHD, hypertension	OR per unit increase of PFOA	Any condition: 0.96 (0.72, 1.29) CHD: 0.97 (0.61, 1.45) Hypertension: 0.74 (0.52, 1.01)
							Outcome: Hypertension was self-reported
							Confounding: Age, BMI, work status, and alcohol consumption
Girardi and Merler, 2019, 6315730 Low	Italy 1960–2018	Cohort	Male workers N = 154	Serum Geometric mean by tertiles = 1,700; 13,051; and 81,934 ng/mL-years	Mortality by circulatory disease, ischemic heart disease, or stroke (ictus)	Standardized Mortality Ratio by tertiles Mortality Risk Ratio (for PFAS plant workers vs. nearby metal factory workers)	Mortality: No statistically significant associations
							Exposure: Tertiles of cumulative serum PFOA were defined as follows (in ng/mL-years): T1 ≤4,034; 4,034< T2 ≤16,956; 16,956< T3
							Confounding: Age at risk, calendar period

PFOA = perfluorooctanoic acid; PFDE = perfluorodecanoic acid; PFOS = perfluorooctane sulfonate; PFHxS = perfluorohexane sulfonic acid; MPAH = 2-(N-methyl-PFOSA) 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); CRP = C-reactive protein; CHD = coronary heart disease; CVD = cardiovascular disease; CVHD = cardiovascular heart disease; PAD = peripheral arterial disease; CIM-GSM = carotid artery intima-media complex grey scale median; 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; OR = odds ratio; CI = confidence interval; SE = standard error; NHANES = National Health and Nutrition Examination Survey; IQR = 75th–25th percentiles.

^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.

1 **C.5.2 Serum Lipids**2 **Table C-14. Associations Between PFOA 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 = 0.5	Levels (ng/dL) of TC, LDL, HDL, and triglycerides	Regression coefficient per ln-unit increase PFOA	TC: 6.57 (2.72, 10.42) p-value = 0.001 LDL: 4.66 (1.67, 7.65) p-value = 0.002 HDL: -1.56 (-3.20, 0.08) p-value = 0.06 Triglycerides: 19.63 (14.82, 24.34) p-value < 0.001
Confidence: Results for triglycerides 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, ETS 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 age 9 = 9.7 (male) or 9.0 (female) Median at age 15 = 3.7 (male) or 3.4 (female) Median at age 21 = 3.1 (male) or 2.7 (female)	Levels (mmol/L) of triglycerides	Percent change in triglycerides at age 15 or 21 per 10 ng/mL increase in PFOA at age 9 or 15	Age 9 to 15: -1.46 (-17.84, 18.22) Age 9 to 21: -8.07 (-30.3, 20.9) Age 15 to 21: 2.54 (-31.18, 84.56)
Confounding: Sex, age, and triglycerides 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.							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
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 = 2.32	Levels (z-score) of TC, LDL, HDL, and triglycerides	Regression coefficient per log2-unit increase PFOA	TC: 0.02 (–0.10, 0.15) LDL: 0.03 (–0.08, 0.15) HDL: –0.04 (–0.15, 0.08) Boys: –0.20 (–0.37, –0.03) Girls: No association Triglycerides: 0.04 (–0.07, 0.15)
Confidence: Results for triglycerides 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 = 1.78	Levels (log10-mg/dL) of TC, HDL, and non-HDL	Regression coefficient per log10-unit increase linear PFOA	TC: –0.0085 p-value = 0.46 Non-HDL: –0.0016 p-value = 0.61 HDL: 0.0223 p-value = 0.45
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 ages 3–18 from Korea Environmental Health Survey in Children and Adolescents (KorEHS-C) N = 147	Serum Median = 1.88	Levels of TC (mg/dL), LDL (mg/dL), and triglycerides (ln-mg/dL)	Regression coefficient per ln-unit increase PFOA	TC: –2.26 (–11.49, 6.98) LDL: 3.90 (–4.81, 12.61) Triglycerides: 0.02 (–0.13, 0.18)
Results: LDL and triglycerides 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	Prenatal maternal plasma Median = 5.4	Levels (mg/dL) of TC, HDL, LDL, and triglycerides	Regression coefficient per IQR increase in PFOA	No statistically significant prenatal exposure associations Mid-childhood:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
			N = 512 prenatal, 596 mid- childhood	Mid- childhood plasma Median = 4.3			TC: 2.6 (–0.5, 5.7) Boys: 1.2 (–3.0, 5.4) Girls: 5.2 (0.4, 9.9) HDL: 1.5 (0.1, 2.9)
			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 = 1.62	Levels (standard deviation score) of TC, LDL, HDL, and triglycerides	Regression coefficient per unit increase in PFOA	Regression coefficients for all children were between –0.07 and 0.1, all with p-values > 0.05 LDL at 18 months Boys: –0.29 (–0.58, –0.003) p-value for interaction with sex = 0.01 Triglycerides at 18 months Boys: 0.43 (0.16, 0.70) p-value for interaction with sex < 0.01
			Confounding: Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI2, education, smoking, sex, and lipid outcome at 3 months				
Spratlen et al., 2020, 5915332 Medium	United States 2001–2002	Cross-sectional	Pregnant women and their children from the Columbia University World Trade Center birth cohort N = 222	Cord blood Median = 2.46	Levels (mg/dL) of TC, total lipids, and triglycerides in cord blood	Percent change per 1% increase in PFOA Geometric mean ratios (GMRs) by quartiles	TC: 0.038 (–0.032, 0.109) GMR p-trend = 0.39 Total lipids: 0.087 (0.021, 0.153) GMR p-trend = 0.04 Triglycerides: 0.256 (0.129, 0.383) GMR p-trend = 0.001

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
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 = 4.1	Levels (mmol/L) of TC	Regression coefficient per unit increase in PFOA by quintile	TC: Q2: 0.10 (–0.19, 0.39) Q3: 0.39 (0.10, 0.67) Q4: 0.24 (–0.05, 0.54) Q5: 0.45 (0.15, 0.75) p-trend = 0.003
Results: Lowest quintile used as for the reference group. Confounding: Age, parity, education, smoking and pre-pregnancy BMI, total caloric intake, and intake of vegetables, meat, and meat products							
Matilla-Santander et al., 2017, 4238432 Medium	Spain 2003–2008	Cohort	Pregnant women from the Spanish INMA birth cohort N = 1240	Plasma Median = 2.35	Levels of TC (mg/dL), triglycerides (log10- mg/dL), and C-reactive protein (log10-mg/dL)	Percent change in median lipid level per log10-unit increase in PFOA	TC: 1.26 (0.01, 2.54) Triglycerides: –2.78 (–6.15, 1.42) with inverted U-shaped dose-response
Confidence: Triglycerides 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 = 1.1	Levels of HDL (mg/dL) and triglycerides (ln- mg/dL)	Regression coefficient per ln-unit increase PFOA	HDL: 1.90 (0.22, 3.59) Triglycerides: –0.006 (–0.049, 0.036)
Confounding: Maternal age, race/ethnicity, pre-pregnancy body mass index, education, gravidity, smoking, and gestational age at blood draw							
General Population							
Liu et al., 2018, 4238514 Medium	United States 2013–2014	Cross-sectional	Adults ages 18+ from NHANES N = 1871	Serum Geometric mean = 1.86	Levels of TC (mg/dL), LDL (mg/dL), HDL (mg/dL), triglycerides (ln-mg/dL)	Regression coefficient per ln-unit increase in PFOA	TC: 5.58 (2.03) p-value < 0.05 LDL: 4.47 (2.47) HDL: 1.93 (0.64) p-value < 0.01

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
<p>Results: Coefficients are presented with standard error in parentheses.</p> <p>Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, waist circumference, and medications (anti-hypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)</p>							Triglycerides: -0.08 (0.04)
Dong et al., 2019, 5080195 Medium	United States 2003–2014	Cross-sectional	Adults age 20–80 from NHANES N = 8849	Serum Mean = 3.7	Levels (mg/dL) of TC, LDL, HDL	Regression coefficient per unit increase PFOA	TC all cycles: 1.48 (0.2, 2.8) Inconsistent associations with LDL or HDL across NHANES cycles.
<p>Confounding: Age, gender, race, family income index, BMI, waist circumference, physical activities, diabetes status, smoking status, number of alcoholic drinks per day</p>							
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 = 2.5 Male = 3.4	Levels (mg/dL) of TC, LDL, HDL, and triglycerides	Regression coefficient per log10-unit increase PFOA	TC OM: 0.0519 (0.0128, 0.0911) p-value = 0.01 No clear associations in NF, NM, or OF LDL OM: 0.0822 (0.0098, 0.1546) p-value = 0.03 No clear associations in NF, NM, or OF HDL: No clear associations Triglycerides: No clear associations
<p>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</p>							
Fan et al., 2020, 7102734 Medium	United States 2011–2014	Cross-sectional	Adults age 20+ from NHANES N = 1067	Serum Median = 2.05 ng/mL	Levels (mg/dL) of TC, LDL, HDL, and triglycerides	Regression coefficient per log10-unit increase in PFOA	TC: 6.74 (3.23, 10.2) p-value < 0.001 LDL: 4.67 (1.57, 7.77) p-value = 0.003

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
Confounding: Age, gender, race, education level, PIR, BMI, smoking status, alcohol use, energy intake levels, screen time							HDL: 2.23 (0.97, 3.49) p-value = 0.001 Triglycerides: 0 (–0.05, 0.04) p-value = 0.891
Donat-Vargas et al., 2019, 5080588 Medium	Sweden 1990–2013	Cohort	Non-diabetic adults ages 30–60 at baseline in Västerbotten Intervention Programme (VIP) N = 187	Plasma Baseline median = 2.9 Median at 10-year follow-up = 2.7	Levels (mmol/L) of TC and triglycerides	Regression coefficient per 1-SD increase in PFOA, or comparing tertiles	Per change in PFOA TC Baseline: –0.19 (–0.36, –0.02) Follow-up: –0.03 (–0.21, 0.15) Prospective: –0.12 (–0.23, 0) Triglycerides Baseline: –0.03 (–0.14, 0.07) Follow-up: –0.08 (–0.20, 0.04) Prospective: –0.07 (–0.13, –0.01) Overall non-significant inverse association using tertiles
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) and Outcomes Study (DPPOS)	Plasma Median = 4.9	Levels (mg/dL) of TC, LDL, HDL, triglycerides, non-HDL, and very low density lipids (VLDL); hypercholesterolemia, hypertriglyceridemia	Regression coefficient per doubling PFOA Hazard ratio (HR) or odds ratio (OR) for hypercholesterolemia or	Cross-sectional TC: 6.09 (3.14, 9.04); p < 0.01 LDL: 2.93 (0.22, 5.63); p-value < 0.05 HDL: –0.49 (–1.38, 0.40)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
			N = 940 (888 not on metformin)			hypertriglyceridemia per doubling of PFOA	Triglycerides: 17.75 (9.77, 25.74); p-value < 0.01 VLDL: 3.66 (2.18, 5.15); p-value < 0.01 Hypercholesterolemia at baseline OR: 1.29 (1.05, 1.57) Hypertriglyceridemia at baseline OR: 1.48 (1.21, 1.81) <u>Prospective</u> Hypercholesterolemia HR: 1.06 (0.94, 1.19) Greater effect in the placebo group Hypertriglyceridemia HR: 1.23 (1.04, 1.45) 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 = 35.8 Female = 22.65 Male = 58.3	Levels (mg/dL) of TC, LDL, HDL, non-HDL, and triglycerides	Regression coefficient per ln-unit increase PFOA by decile	TC 1.94 (1.48, 2.41) p-value for interaction with sex = 0.15 Associations for deciles 2–10 consistently increase from 2.83 to 9.10 LDL 1.12 (0.71, 1.52) p-value for interaction with sex = 0.577

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
							Associations for deciles 2–10 moderately increase from 1.4 to 5.3
							HDL 0.49 (0.32, 0.67) Male: 0.13 (–0.11, 0.37) Female: 0.83 (0.57, 1.1) p-value for interaction with sex < 0.001 Associations for deciles 2–10 moderately increase from 0.45 to 2.07
							Triglycerides 0.02 (0.01, 0.03) p-value for interaction with sex = 0.815 Associations for deciles 2–10 increase from 0.04 to 0.09
Results: Lowest decile used as the 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 = 4.6	Levels (mg/dL) of TC, triglycerides, and apolipoproteins log10- ApoB, ApoE, and ApoC-III	Least-squared means (LSM) by tertile PFOA	TC T1: 189.1 (7.9) T2: 189.3 (7.6) T3: 188.4 (7.7) p-trend = 0.67 Triglycerides T1: 111.1 (11.2) T2: 137.3 (10.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Select Results ^b
							T3: 131.8 (10.9) p-trend = 0.06
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							

1 TC = total cholesterol; LDL = low density lipids; HDL = high density lipids

2 ^aExposure reported as median (25th–75th percentile) in ng/mL unless otherwise specified.

3 ^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.

4 ^cConfounding indicates factors the models presented adjusted for.

5 C.6 Endocrine

6 **Table C-15. Associations Between PFOA 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	Fernald Community Cohort, Median age 38 years at enrollment, N = 122 for TSH measurements; 47 male and 75 female N = 144 for TT4 measurements;	Drinking water Serum 12.7	Levels of TSH (ln-μIU/mL) and TT4 (ln-μg/dL)	Percent change per IQR increase in PFOA	TSH –0.48 (–9.68, 9.65) p-value = 0.92 Males: 9.38 (–7.47, 29.3) p-value = 0.47 Females: –6.64 (–17.8, 5.97); p-value = 0.31 TT4 –1.18 (–5.12, 2.92); p-value = 0.57 Males: –2.71 (–9.05, 4.08); p-value = 0.43 Females: –1.62 (–6.88, 3.94); p-value = 0.56

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
			63 males and 81 females				
			Confounding: Age, year of measurement, sex, education, income, marital status, BMI ^c				
Jain and Ducatman, 2019, 6315816 Medium	United States 2007–2012	Cross-sectional	Adults from NHANES aged 20+ Glomerular filtration (GF) status: GF-1 = 1,653 GF-2 = 720 GF-3A = 114 GF-3B/4 = 62	Serum Levels not reported	Levels of TSH (log-μIU/mL), TGN (log-ng/mL), TT4 (log-μg/dL), FT4 (log-ng/dL), TT3 (log-ng/dL), FT3 (log-pg/mL)	Regression coefficient per log10-unit increase in PFOA	TSH GF-1: −0.004, p-value = 0.89 GF-2: 0.085, p-value < 0.01 GF-3A: −0.229, p-value = 0.04 GF-3B/4: 0.012, p-value = 0.88 FT4 GF-1: −0.010, p-value = 0.17 GF-2: −0.020, p-value = 0.08 GF-3A: 0.038, p-value = 0.07 GF-3B/4: −0.040, p-value = 0.15
			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 Lowest tertile T1 ≤3.3 Highest tertile T3 ≥5.1	Levels of TSH (μIU/L), FT3 (pg/L), TT3 (fg/dL), FT4 (pg/L), TT4 (pg/L), TGN	Regression coefficient per log-unit increase in PFOA, or by tertiles	TSH: Significantly increased levels (T3 vs. T1), p-value <0.01 TT3: 0.032, p-value = 0.01 FT3, FT4, 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	Children and adults from NHANES, aged 12–80 145 females 12 to <20	Serum Females 12–20: 1.53 Females 20–40: 1.49 Females 40–60: 1.62	Levels of TSH (μIU/mL), TT3 (ng/dL), FT3 (pg/mL), TT4 (μg/mL), FT4 (ng/dL)	Percent change per doubling of PFOA	TSH Females 12–20: 16.6 (2.6, 28.6) 20–80: No associations Males, all age groups: No associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
			680 females 20–80	Females 60–80: 2.55			TT3 Females 60–80: 3.3 (0.6, 6) Younger than 60: No associations Males, all age groups: No associations
			158 males 12 to <20	Males 12–20: 1.85			
			699 men	Males 20–40: 2.35 Males 40–60: 2.31 Males 60–80: 2.48			FT3 Females 60–80: 1.8 (0.2, 3.4) Younger than 60: No associations Males, all age groups: No associations
							TT4 Females 12–20: 4.1 (0.6, 8.9), p-value <0.1 20–80: No associations Males 40–60: –3.1 (–6.2, 0.1), p-value <0.10 12–40 or 60–80: No associations
							FT4 Females 20–40: 2.0 (0, 4.1) 12–20 or 40–80: no associations Males, all age groups: No associations
Confounding: Age, BMI, poverty income ratio, serum cotinine, and race/ethnicity							
Byrne et al., 2018, 5079678 Low	St. Lawrence Island, Alaska, USA 2013–2014	Cross-sectional	Alaska Natives, aged 18–45 N = 85	Serum 1.01 Male: 1.47 Female: 0.772	Levels of TSH (ln- μIU/mL), TT3 (pg/mL),	Regression coefficient per ln- unit increase in PFOA	TSH Total cohort: 0.63 (0.22, 1.03), p-value <0.005

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
			38 men 47 women		FT3 (ng/dL), TT4 (µg/dL), FT4 (ng/dL)		TT3 Total cohort: -7.67 (-18.61, 3.27), p-value = 0.17 Males: -14.24 (-26.24, -2.24), p-value = 0.02 Females: 11.29 (-5.25, 27.83) p-value for sex interaction = 0.18 FT3, TT4, FT4: No statistically significant associations
Confounding: Age, sex, smoking status							
Convertino et al., 2018, 5080342 Low	Scotland 2008–2011	Controlled trial	Adults, Solid-tumor cancer patients 49	Serum Median PFOA ranging from 9–1,530 nmol/mL	Levels of FT4 (mmol/L)	Regression coefficient per unit increase in PFOA Median and mean FT4 levels by exposure categories	0.003, p-value = 0.21 Increasing trend in FT4 by exposure categories
Confounding: None given							
Heffernan et al., 2018, 5079713 Low	United Kingdom 2015	Cross-sectional	Women aged 20–45 years, with (cases) or without (controls) polycystic ovarian syndrome (PCOS) N = 59	Serum Geometric mean = 2.49 for both cases and controls	Levels of TSH (mU/L), FT3 (ln-pmol/L), FT4 (ln-pmol/L)	Regression coefficient per ln-unit increase in PFOA	TSH PCOS cases: 0.86, p-value < 0.01 PCOS controls: -0.13, p-value = 0.75 FT3, FT4: No statistically significant associations
Confounding: Serum albumin							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
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: 11.10 Controls: 8.35	Levels (ng/mL) of TSH, FT3, FT4	Regression coefficient per log-unit increase in PFOA	TSH POI cases: 1.39 (0.18, 2.59) POI controls: 1.65 (0.86, 2.44) FT4: POI cases: –3.42 (–5.39, –1.46) POI controls: No association FT3 No statistically significant 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 = 2.37 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 PFOA	TSH :23.1 (1.9, 48.6) T4: 1.9 (–4.1, 8.3) FT3: 0.5 (–5.6, 6.9) FT4: 1.9 (–11.5, 17.2)
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 = 181–660	Serum Age 2: 4.39 Age 4: 3.65 Age 6: 3.83	Levels of TSH, FT4 (ng/dL), and T3 (ng/dL) at age 6	Regression coefficient per ln-unit increase in PFOA	FT4 at age 6 All: 0.07, p-value <0.05 Boys: 0.04, 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 PFOA	TSH, T3: No statistically significant associations between or within age groups
Confounding: Age, sex, dietary iodine intake							
Kang et al., 2018, 4937567 Medium	Korea 2012–2014	Cross-sectional	Children from Seoul and Gyeonggi aged 3–18 N = 147	Serum 1.88	Levels (ng/dL) of TSH, FT4	Regression coefficient per ln-unit increase in PFOA	TSH: –0.14 (–0.62, 0.34), p-value = 0.341 FT4: 0.04 (–0.01, 0.09), p-value = 0.075
Confounding: Age, sex, BMI z-score, household income, second-hand smoking							
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 7.57	Levels of TSH (ln-mIU/L), FT3 (pmol/L), FT4 (pmol/L)	Regression coefficient per ln-unit increase in PFOA	FT4 All children: 0.14 (0.02, 0.26) Boys: 0.25 (0.08, 0.42) Girls: 0.01 (–0.16, 0.18)
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 259 male children 240 female children	Plasma 2.00	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 PFOA	TgAb Boys, maternal TA negative: –0.13 (–0.27, –0.002), p-value = 0.047 All boys or maternal TA positive: no association Girls, maternal TA positive: 0.27 (0.95, 0.44), p-value = 0.007 All girls or maternal TA negative: no association TSH, FT3, FT4, TPOAb: No statistically significant associations
Confounding: Age at delivery, parity, educational level, alcohol consumption, smoking during pregnancy, pre-pregnancy BMI							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
Pregnant Women							
Xiao et al., 2020, 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 = 2.37 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 PFOA	TSH: 12.6 (–4.5, 32.8) T4: 0.7 (–5.5, 7.3) FT3: 3.1 (–1.2, 7.6) FT4: –0.4 (–5.4, 4.8)
Confounding: Child sex (in detailed results), parity, maternal BMI, maternal height, maternal education, maternal age, smoking and drinking alcohol during pregnancy, total PCB, mercury							
Preston et al., 2018, 4241056 Medium	United States 1999–2002	Cross-sectional	Pregnant women and their children N = 726 and 718 for free T4 and TSH measures, respectively	Maternal plasma 5.6	Levels of TSH (mIU/mL), FT4 µg/dL, TT4 (µg/dL)	Percent difference in hormone level per IQR increase in PFOA	FT4: –1.87 (3.4, –0.31) TSH: 0.28 (–9.26, 10.8) TSH TPOAb negative: 0.88 (–9.22, 12.1) TSH TPOAb positive: –19 (–35.1, 1.15) p-value for effect modification by TPOAb status = 0.08
Confounding: Maternal age, race/ethnicity, smoking status, fish intake, parity, and gestational week at blood draw							
Itoh et al., 2019, 5915990 Medium	Japan 2003–2005	Cross-sectional	Pregnant women and their children N = 499	Plasma 2.00	Levels of TSH (ln-µU/mL), FT3 (ln-pg/mL), FT4 (ln-pg/mL), TPOAb (ln-IU/mL),	Regression coefficient per ln-unit increase in PFOA	TPOAb: –0.23 (–0.44, –0.02), p-value = 0.033 TgAb: –0.01 (–0.21, 0.19), p-value = 0.929

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
					TgAb (ln-IU/mL)		
Confounding: Age at delivery, parity, pre-pregnancy BMI, educational level, alcohol consumption, and smoking habits							
Aimuzi et al., 2020, 6512125 Medium	Shanghai, China 2013–2016	Cross-sectional	Pregnant women prior to 16 weeks of gestation N = 1877 1615 TPOAb-negative 222 TPOAb-positive	Serum Total cohort: 12.32 TPOAb-negative: 12.32 TPOAb-positive: 12.3	Levels of TSH (ln-mIU/L), FT3 (pmol/L), FT4 (pmol/L)	Regression coefficient per ln-unit increase in PFOA	FT4 Total cohort: 0.12 (0.02, 0.23) TPOAb-negative: 0.11 (−0.01, 0.22) TPOAb-positive: 0.14 (−0.20, 0.48) TSH, FT3: All associations not statistically significant
Confounding: Pre-pregnancy BMI, gestational age at thyroid hormone (TH) measurement, fish intake, maternal age, hospital indicators, maternal education, difference between PFAS and THs measured gestational weeks							

TSH = thyroid stimulating hormone; T3 = triiodothyronine; T4 = thyroxine; FT3 = free triiodothyronine; FT4 = free thyroxine; TT3 = total triiodothyronine; TT4 = total thyroxine; TGN = thyroglobulin; TPOAb = thyroid peroxidase antibody; TgAb = thyroglobulin antibody; GF = glomerular filtration; GFR = glomerular filtration rate; 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 PFOA Exposure and Metabolic Effects in Recent Epidemiologic Studies

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Children and Adolescents							
Ashley-Martin et al., 2017, 3981371	Canada, Recruitment 2008–2011	Cohort	Pregnant women and their children,	Maternal blood 1.7	Adiponectin, leptin	Regression coefficient per log10-unit	Adiponectin, leptin: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
High			from the MIREC Study N = 1,176			increase in PFOA	
Confounding: Maternal age, pre-pregnancy body mass index, sex, and parity ^c							
Buck et al., 2019, 5080288 High	United States, 2003–2006	Cohort	Pregnant women and their children in the HOME study N = 230	Maternal serum 5.6	Adiponectin, leptin	Percent change per doubling of PFOA	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 6.74	BMI, WC, body fat, waist-to-height ratio	Regression coefficient per ln-unit increase in PFOA, or by tertile	BMI, WC, body fat, waist to height ratio: No statistically significant association
Confounding: Maternal age, maternal pre-pregnancy BMI, gestational week at delivery, maternal education, paternal smoking during pregnancy, and parity							
Jensen et al., 2020, 6833719 High	Denmark, 2010–2012	Cohort	Pregnant women and their infants assessed at birth, 3 months, and 18 months, Odense Child Cohort N = 593	Maternal serum 1.62	BMI z-score, WC	Regression coefficient per unit increase in PFOA	BMI z-score, WC: No statistically significant associations
Confounding: Maternal age, parity, pre-pregnancy BMI, pre-pregnancy BMI ² , education, smoking, sex, visit, adiposity marker at birth							
Minatoya et al., 2017, 3981691 High	Japan, 2002–2005	Cohort	Pregnant women and their children N = 168	Serum 1.4	Adiponectin, leptin	Regression coefficient per log10-unit increase in maternal serum PFOA	Adiponectin, leptin: No statistically significant associations
Confounding: Maternal BMI, parity, smoking during pregnancy, blood sampling period, gestational age, infant sex							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Alderete et al., 2019, Medium 5080614	United States, 2001–2012	Cohort	Obese Hispanic children, 8–14 years N= 39	Plasma GM = 2.78	Glucose (fasting, 2 hour, AUC), Insulin (fasting, 2 hour, AUC), HOMA-IR	Regression coefficient per ln-unit increase in PFOA	Glucose (2 hour): 30.6 (8.8, 52.4), p-value <0.05 Glucose (fasting, AUC), insulin, HOMA-IR: No statistically significant association
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, recruitment 2003–2006	Cohort	Pregnant women and their children N = 285	Serum 5.3	Overweight/obese, BMI z-score, WC, body fat percentage, weight-for-age	BMI z-score: Regression coefficient by Terciles Other outcomes: Mean change between 2 and 8 years by tercile	BMI z-score: 0.44 (0.13, 0.74) T2: 0.44 (0.23, 0.64) T3: 0.37 (0.14, 0.6) WC: 4.3 (1.7, 6.9) Body fat percent: 3.6 (1.8, 5.5) Weight-for-age T2: 0.49 (0.31, 0.67) T3: 0.43 (0.23, 0.64) Overweight/obese: No statistically significant association
Results: Lowest tercile used as the reference group. Tercile 1 (0.5–4.3 ng/mL), tercile 2 (4.4–6.7 ng/mL), tercile 3 (6.8–26 ng/mL) maternal PFOA. Confounding: Maternal age, race, education, income, parity, employment, marital status, depressive symptoms, BMI at 16 weeks gestation, fruit/vegetable consumption, fish consumption, prenatal vitamin use, maternal serum cotinine concentrations, child age in months.							
Conway et al., 2016, 3859824 Medium	United States, 2005–2006	Cross-Sectional	Children living in six PFOA-contaminated water districts with type 1 diabetes N = 39	Serum Mean = 68.4 ng/L	T1D, T2D, and uncategorized diabetes	OR per ln-unit increase in PFOA	T1D: 0.52 (0.54, 0.97) T2D and uncategorized diabetes: No statistically significant association
Confounding: Age, sex, race, BMI, eGFR, hemoglobin, iron							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Domazet et al., 2016, 3981435 Medium	Denmark, 1997–2009	Cohort	Children followed through ages 9, 15, and 21, N = 176	Blood, plasma, glucose Age 15 Males: 9.7 Females: 9.0 Age 21 Males: 3.1 Females: 2.7	WC (cm), HOMA-B, HOMA-IR, insulin, glucose, skinfold thickness, BMI	Percent change in WC at 21 years old in higher levels of PFOA at age 21 Percent change in HOMA-B at age 15 old per 10-unit increase in PFOA exposure at age 9	WC: –11.11 (–19.90, 1.36), p-value = 0.03 HOMA-B: –10.93 (–19.67, –1.11) HOMA-IR, insulin, glucose, skinfold thickness, BMI: No statistically significant association
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 the European Youth Heart Study aged 9 years N = 242	Plasma Boys: 9.5 Girls: 9.5	Leptin, fat mass, adiponectin	Percent change per 10% increase in PFOA	Body fat: –1.22 (–2.91, 0.5), p-value = 0.161 Adiponectin: 1.7 (–0.15, 3.59), p-value = 0.071 Leptin: –4.44 (–8.74, 0.06), p-value = 0.053
Confounding: age, sex, parity, maternal income level							
Gyllenhammar et al., 2018, 4238300 Medium	Sweden, 1996–2011, children followed up at age 5	Cohort	Mothers and their children from the POPUP Study N = 193	Maternal serum 2.3	BMI z-score	Regression coefficient per IQR increase in maternal PFOA	BMI z-score: Ages 36 and 48 months: Positive statistically significant associations. Age 60 months: Non-significant positive association (numeric results not provided)
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,	Cohort	Pregnant women and their daughters	Maternal serum 3.7	WC (cm), Trunk fat (%), BMI (kg/m ²)	Regression coefficient per	WC: –0.54 (–0.9, 0.11), p-value = 0.01

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
	recruitment 1991–1992		N = 319			unit increase in PFOA	Trunk fat: –0.27 (–0.55, 0.0), p-value = 0.05 BMI: –0.16 (–0.32, 0.0), p-value = 0.05 Body fat percentage: 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 PFOA	Blood glucose: 1.262 (–1.108, 3.633), p-value = 0.294
Confounding: Age, sex, BMI z-score, household income, second-hand smoking							
Kobayashi et al., 2017, 3981430 Medium	Japan, 2002–2005	Cross-sectional	Infants from Hokkaido Study on Environment and Children's Health N = 177	Maternal serum 1.4	Ponderal index at birth	Regression coefficient per ln-unit increase in PFOA	Ponderal index: –0.44 (–0.99, 0.12), p-value = 0.123
Confounding: Maternal age, pre-pregnancy BMI, parity, maternal education, maternal smoking during pregnancy, gestational age, infant sex, and maternal blood sampling period							
Karlsen et al., 2017, 3858520 Medium	Faroe Islands, recruited 2007–2009 (at birth)	Cohort	Mother-child pairs N = 444 follow up at child ages 18 months and 5 years	Serum Maternal 2-week serum: 1.40 Child 5-year serum: 2.20	BMI z-score, Overweight	Regression coefficient or RR per log10–unit increase in child or maternal PFOA, or by tertiles	BMI z-score at age 5: –0.27 (–0.52, –0.02), p-value <0.05 Overweight at age 5: RR: 1.5 (1.01, 2.24), p-value <0.05 T3: 1.88 (1.05, 3.35), p-value <0.05
Results: Lowest tertile used as reference. Confounding: Maternal nationality, age at delivery, pre-pregnancy BMI, smoking during pregnancy, child sex, exclusive breastfeeding duration, child's fish intake at age 5 years							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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: 1.64 Sweden: 2.33	BMI, triceps skin fold, subscapular skinfold, overweight	Regression coefficient or OR per ln-unit increase in maternal PFOA	BMI, triceps skin fold, subscapular skinfold, overweight: No statistically significant associations
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 N = 1123 (girls) N = 1169 (boys)	Serum Girls: 30.1 Boys: 34.8	Insulin-like growth factor-1 (IGF-1) (ln-ng/mL)	Percent difference by quartiles.	IGF-1 Girls: Q3: -3.6 (-6.6, -0.5) Boys Q3: -7.4 (-12.8, -1.6) No other statistically significant associations
Results: Lowest quartile used as the reference group. 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 = 2.32	BMI, WC, overweight, waist-to-hip ratio	Regression coefficient per-log2-unit increase in PFOA	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 3.1	Overweight	OR by quartiles	OW: No statistically significant associations
Results: Lowest quartile used as reference. Confounding: Risk strata, difference from strata-specific mean, sex							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Mora et al., 2017, 3859823 Medium	United States, 1999–2002	Cohort	Early childhood N = 992	Maternal Plasma 5.6	WC (cm), Skinfold thickness, BMI, waist-to-hip ratio, obesity, overweight, total fat mass index, total fat-free mass index	Regression coefficient per IQR-unit increase in PFOA	WC All: 0.31 (0.04, 0.57) Boys: 0.5 (0.06, 0.93) Skinfold thickness, BMI, waist-to-hip ratio, obesity, overweight, total fat mass index, total fat-free mass index: No statistically significant association
Confounding: maternal age, race/ethnicity, education, parity, pre-pregnancy BMI, timing of blood draw, household income, child sex, age at outcome assessment							
Pinney et al., 2019, 6315819 Medium	Greater Cincinnati and the San Francisco Bay Area, Recruitment 2004–2007, followed annually or semi-annually until 2014	Cohort	Girls, age 6–8 N = 667	Serum 6.4	BMI, waist-height ratio, waist-hip ratio	Regression coefficient by quintiles or per ln-unit increase in PFOA	BMI: Quintile 4 vs Quintile 1: –0.248 (–0.489, 0.007), p-value = 0.044 Quintile 5 vs. Quintile 1: –0.436 (–0.685, 0.187), p-value = 0.001 Per ln-unit increase –0.264 (–0.416, 0.112), p-value = 0.001 Waist-height Per ln-unit increase: –0.009 (–0.017, 0.002), p-value = 0.013 Waist-hip ratio: No statistically significant association
Results: Lowest quintile used as the reference group.							
Confounding (BMI): Race, parental education, average kcal, physical activity							
Confounding (Waist-height ratio): Age at exam, race, parental education, average kcal, physical activity							
Fleisch et al., 2017, 3858513 Medium for metabolic function	United States, Pregnant women recruited 1999–2002, outcome assessed at mid-	Cohort	Mid-childhood, 7.7 years N = 584	Plasma GM = 4.2	Leptin, Adiponectin, HOMA-IR	Percent change by quartiles	Leptin Q3: –23.3 (–37, –6.5) Q4: –20.1 (–35.1, –1.6) Adiponectin Q2: 16.3 (1.8, 32.9)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Low for HOMA-IR	childhood follow-up						Q3: 22.7 (6.9, 40.8) HOMA-IR: No statistically significant association Results: Lowest quartile used as reference 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							
Mitro et al., 2020, 6833625 High	United States, Recruitment 1999–2002	Cohort	Pregnant women N = 786	Plasma 5.6	WC(cm), BMI, Adiponectin, skinfold thickness, arm circumference, leptin	Percent change (%) or Regression coefficient per log2–unit increase in PFOA	WC: 1.1% (0.1, 2.2), p-value <0.05 BMI: 0.3 (0.0, 0.6), p-value <0.05 Adiponectin, skinfold thickness, arm circumference, hemoglobin, 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 the Project Viva cohort N = 1533	Plasma 5.9	GDM, impaired glucose tolerance, isolated hyperglycemia, blood glucose levels	Regression coefficient by quartiles OR by quartiles	Gestational diabetes, impaired glucose tolerance, isolated hyperglycemia, blood glucose levels: No statistically significant association
Confounding: Maternal age, pre-pregnancy BMI, prior history of gestational diabetes/parity, race/ethnicity, smoking, and education							
Starling et al., 2017, 3858473 High	United States, 2009–2014	Cohort	Pregnant women and their children N = 628	Maternal serum 1.1	Maternal glucose (ln(mg/dl))	Regression coefficient by tertiles	Maternal glucose: T3: –0.025 (–0.046, 0.004) Maternal glucose (continuous) and T2: No statistically significant association
Confounding: Maternal age, pre–pregnancy body mass index (BMI), race/ethnicity, education, smoking during pregnancy, gravidity, and gestational age at blood draw							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
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 15.2	GWG (kg)	Regression coefficient per log2-unit increase in PFOA	No statistically significant associations
Confounding: Age, income, parity							
Jaacks et al., 2016, 3981711 Medium	United States, 2005–2007	Cohort	Pregnant women N = 218	Serum Mean = 3.66	GWG (kg)	Regression coefficient and OR per SD-unit increase in PFOA	GWG 0.09 (–0.84 1.02) OR for excessive GWG: 1.06 (0.76, 1.47)
Confounding: Pre-pregnancy non-fasting serum lipids, BMI							
Jensen et al., 2018, 4354143 Medium	Denmark, recruitment 2010–2012, outcome assessed 12–20 weeks later	Cohort	Pregnant women, Odense Child Cohort N = 158	Serum 1.67	Blood glucose, insulin, c-peptide, 2-hour glucose, insulin resistance, beta cell function, insulin sensitivity	Percent change per log2-unit increase in PFOA	No statistically significant associations
Confounding: Age, parity, education level, pre-pregnancy BMI							
Liu et al., 2019, 5881135 Medium	China, 2013–2015	Case-control	Pregnant women without history or family history of diabetes N = 189	Serum 2.25	GDM, glucose homeostasis	Regression coefficient per ln–unit increase, or by tertiles of sum m–PFOA or L–PFOA	GDM: Per ln–unit increase sum m–PFOA: 1.23 (0.92, 1.64) T2: 0.91 (0.4, 2.07) T3: 2.01 (0.92, 4.37) Per ln–unit increase sum m–PFOA: 2.04 (0.99, 4.21) T2: 1.04 (0.47, 2.34) T3: 2.04 (0.94, 4.46) Per ln–unit increase sum L–PFOA:

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Glucose homeostasis (1 hour): 0.55 (0.01, 1.1), p-value = 0.049
							Glucose homeostasis (2 hours): 0.73 (0.27, 1.18), p-value = 0.002
							Glucose homeostasis (fasting, 1 hour, 2 hour) for m-PFOA and glucose homeostasis (fasting) for L-PFOA: No statistically significant association
Confounding: Maternal age, BMI in early pregnancy, fetal sex, serum triglyceride, total cholesterol							
Marks et al., 2019, 5381534 Medium	United Kingdom 1991–1992	Cohort	Mothers from ALSPAC N = 905	Serum Mothers of sons: 3.0 Mothers of daughters: 3.7	GWG (absolute)	Regression coefficient per 10% increase in log-unit PFOA	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 = 1.99	GDM	Risk Ratio per SD–unit increase in PFOA	GDM (family history of T2D): 1.27 (1.11, 1.45) Overall cohort, no family history of T2D, normal pre-pregnancy BMI, overweight pre-pregnancy BMI: No statistically significant association
Confounding: Maternal age, enrollment BMI, education, parity, race/ethnicity, serum cotinine							
Ren et al., 2020, 6833646 Medium	China, 2012	Cross-sectional	Pregnant women enrolled in the Shanghai–Minhang Birth Cohort Study N = 705	Plasma 20.2	Glucose (1 hour, fasting)	Regression coefficient per ln–unit increase in PFOA	Glucose (1 hour tolerance test): 0.31 (0.03, 0.52), p-value = 0.031 Glucose after fasting, glucose after 1 hour tolerance test by gestational weeks: No statistically significant association

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Confounding: maternal age at enrollment, pre-pregnancy BMI, per capita household income, education level, passive smoking, pregnancy complication, history of abortion and stillbirth, parity							
Shapiro et al., 2016, 3201206 Medium	Canada, 2008–2011	Cohort	Pregnant women N = 1,195	Urine Normal glucose GM = 1.68 Gestational impaired glucose tolerance GM = 1.70 Women with GDM GM = 1.64	GDM, gestational impaired glucose tolerance	OR per quartile PFOA	Gestational diabetes, gestational impaired glucose tolerance: No statistically significant association
Confounding: Maternal age, race, pre-pregnancy BMI, and education							
Valvi et al., 2017, 3983872 Medium	Faroe Islands, 1997–2000	Cohort	Pregnant women and their children N = 604	Maternal serum 3.31	Gestational diabetes	OR per doubling of PFOA, or by tertiles	Gestational diabetes: Per doubling: 0.79 (0.44, 1.41) T2: 1.01 (0.5, 2.06) T3: 0.66 (0.3, 1.48)
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 Cases: 1.38 Controls: 1.30	Fasting blood glucose, GDM	Fasting blood glucose: OR by tertiles of n-PFOA GDM: OR per unit increase in n-PFOA	Fasting blood glucose, GDM: No statistically significant associations
Confounding: Fasting blood glucose: BMI, age, GDM status; GDM: BMI, GWG, ethnic groups, maternal education, parity, maternal drinking during pregnancy, household income							
Wang et al., 2018, 5080352 Medium	China, 2013–2014	Cohort	Pregnant women aged 20–40	Serum 7.3	Fasting blood glucose, fasting insulin, HOMA-	LSM by tertiles	No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			N = 385		IR, gestational diabetes, oral glucose tolerance		
			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 = 4.82	Adiponectin (μg/mL), HbA1c (%), Insulin (fasting) (μU/mL), Glucose (fasting) (μU/mL), HOMA-IR, Insulin (30 min, μU/mL), Proinsulin (fasting, pM), HOMA-B (beta), Insulin (corrected response), Insulinogenic index, Diabetes, HOMA-IR, glucose (30 mins), glucose (2 hours), BMI	Regression coefficient per doubling of PFOA	Adiponectin: −0.29 (−0.54, −0.04), p-value = 0.02 HbA1c: 0.04 (0.001, 0.07), p-value = 0.05 Insulin (fasting): 2.26 (1.16, 3.35), p-value = 0.000056 Glucose (fasting): 0.66 (0.07, 1.24), p-value = 0.03 HOMA-IR: 0.64 (0.34, 0.94), p-value = 0.000031 Insulin (30 min): 7.85 (3.63, 12.07), p-value = 0.00028 Proinsulin (fasting): 1.17 (0.72, 2.71), p-value = 0.00070 HOMA-B: 15.93 (6.78, 25.08), p-value = 0.00066 Insulin (corrected): 0.04 (0.01, 0.07), p-value = 0.01

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Insulinogenic index: 0.08 (0.01, 0.15), p-value = 0.02
							Diabetes, HOMA-IR, glucose (30 mins), glucose (2 hours), BMI: 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 12.7	BMI	Percent change per IQR increase in PFOA	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 = 4.82	T2D	Hazard ratio per log2-unit increase in baseline PFOA and by PFOA tertiles	Diabetes: HR: 1.05 (0.94, 1.18) T2: 0.94 (0.75, 1.17) T3: 0.94 (0.75, 1.18)
Results: Lowest tertiles used as the reference group.							
Confounding: Sex, race/ethnicity, baseline age, marital status, education, income, smoking history, BMI, maternal diabetes, paternal diabetes, treatment assignment							
Christensen et al., 2019, 5080398 Medium	United States, 2007–2014	Cross-sectional	Adults from NHANES age 20+ N = 2,975	Serum 2.8 ng/L	Elevated waist circumference (Males: ≥102 cm. Females: ≥88 cm),	OR by quartiles	WC Q2: 0.66 (0.46, 0.92), p-value <0.05 Q3: 0.62 (0.39, 0.98), p-value <0.05

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
					metabolic syndrome, glucose		Metabolic syndrome, glucose level: No statistically significant association
							Confounding: PFDE, PFOS, PFHxS, MPAH, PFNA, PFUnDA, survey cycle, age, sex, race/ethnicity, family income, alcohol intake, and smoking status
Conway et al., 2016, 3859824 Medium	United States, 2005–2006	Cross-sectional	Adults working or living in six PFOA-contaminated water districts with diabetes N = 6,460	Serum All participants mean = 68.4 ng/L	T1D, T2D, Uncategorized Diabetes	OR per ln-unit increase in PFOA	All T1D: 0.76 (0.71, 0.8) T2D: 0.94 (0.92, 0.97) Uncategorized DM: 0.94 (0.9, 0.99) Adults Type 1 DM: 0.74 (0.7, 0.79) Type 2 DM: 0.91 (0.89, 0.94) Uncategorized DM: 0.92 (0.88, 0.96)
							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: 2.8 Controls: 3.0	Type 2 Diabetes, HOMA-IR, HOMA-Beta	OR per 1-log10 SD increase in baseline PFOA	T2D: 0.65 (0.43, 0.97) HOMA-IR, HOMA-Beta: No statistically significant association
							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 14.83	Fasting glucose (nmol/L), HbA1c	Regression coefficient per 1% increase in serum PFOA	Glucose (fasting): 0.018 (0.004, 0.033), p-value = 0.014 HbA1c: 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	Cross-sectional	Adults from NHANES, age 20+	Serum	Obesity	Comparison of geometric mean PFOA levels	Obesity: p-value = 0.02

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
			N = 2,883	GM = 2.2 (non-obese); 2.0 (obese)		non-obese vs obese	
			Confounding: Sex, race, age, poverty income ratio, physical activity, BMI, and serum cotinine				
Jeddy et al., 2018, 5079850 Medium	England, mothers recruited 1991–1002, outcome assessed at age 17	Nested case-control studies	Pregnant mothers and their 17-year-old daughters, ALSPAC N = 221	Maternal serum 3.8	Fat mass	Regression coefficient per unit increase in PFOA	105.88 (–621.59, 833.34)
			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 years N = 621	Plasma, glucose Males: 27.2 Females: 22.3	Leptin, HOMA-IR, insulin, resting metabolic rate, body weight, HbA1c, glucose, VAT fat mass, whole body fat, BMI, waist circumference	Partial Spearman correlation coefficient with baseline PFOA Regression coefficient log10-unit increase in PFOA, or by tertile	Spearman correlations Leptin: 0.09, p-value <0.05 HOMA-IR: 0.1, p-value <0.05 Resting metabolic rate, body weight, HbA1c, glucose, VAT fat mass, whole body fat, BMI, waist circumference: No statistically significant association
			Confounding: age, sex, race, education, smoking status, alcohol consumption, physical activity, menopausal status (women only), hormone replacement therapy (women only), and dietary intervention groups.				
Liu et al., 2018, 4238514 Medium	United States, 2013–2014	Cross-sectional	Adults from NHANES N = 1,871	Serum GM = 1.86	Fasting blood glucose, 2-hour glucose, HbA1c, insulin levels, HOMA-IR, beta cell function, metabolic syndrome, WC	Regression coefficient per ln-unit increase in PFOA	HbA1c: –0.12 (0.05), p-value <0.05 Beta cell function: 0.12 (0.05); p-value <0.05 Fasting blood glucose, 2-hour glucose, insulin levels, HOMA-IR, metabolic syndrome, WC: No statistically significant associations
			Results: Effect estimates are reported with SE in parentheses				

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, WC, and medications (anti-hypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)							
Mancini et al., 2018, 5079710 Medium	France, 1990–2012	Cohort	Women, 40–60 N = 71,294	Dietary Mean = 0.86 ng/kg body weight/day	T2D	Hazard ratio by deciles	T2D Decile 4: 1.21 (1.06, 1.46), p-value <0.05 Decile 5: 1.35 (1.15, 1.59), p-value <0.05 Decile 6: 1.19 (1.05, 1.41), p-value <0.05
Results: Lowest decile used as the reference group. Confounding: smoking status, physical activity, education level, hypertension, hypercholesterolemia, family history of diabetes, energy intake, alcohol intake, adherence to the Western diet and adherence to the Mediterranean diet, water consumption, dairy product consumption							
Su et al., 2016, 3860116 Medium	Taiwan, 2009–2011	Cross-sectional	Adults aged 20–60 living in Taiwan N = 571	Plasma 8.0	Diabetes, Fasting blood glucose (ng/mL), blood glucose (120 mins) (ln) (ng/mL), glucose AUC (ng/mL), HbA1c (ln) (%)	OR by quartiles, per doubling of PFOA Geometric mean ratio (GMR) by quartiles, or per doubling of PFOA	Diabetes: Q2: 0.39 (0.16, 0.96) Q3: 0.2 (0.07, 0.58) Q4: 0.16 (0.05, 0.5) Total: 0.56 (0.43, 0.75) Glucose (Fasting): Q2: 0.96 (0.93, 0.99) Q3: 0.95 (0.92, 0.97) Q4: 0.95 (0.92, 0.98) Per doubling PFOA: 0.98 (0.97, 0.99) Glucose (120 min) Q2: 0.87 (0.82, 0.94) Q3: 0.9 (0.94, 0.95) Q4: 0.85 (0.79, 0.91) Per doubling PFOA: 0.96 (0.94, 0.98) Glucose AUC: Q2: 0.9 (0.85, 0.95) Q3: 0.9 (0.86, 0.95) Q4: 0.88 (0.84, 0.93)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
							Per doubling PFOA: 0.97 (0.95, 0.99)
							HbA1c: Q2: 0.98 (0.96, 1.0) Q4: 0.97 (0.95, 1.0) Per doubling PFOA: 0.99 (0.98, 1.0)
							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, 1989–2011 ^d	Case-control	Female nurses drawn from the Nurses' Health Study II cohort N = 1586	Plasma Cases: 4.96 Controls: 4.57	Type 2 Diabetes, HbA1c, fasting insulin, adiponectin	Regression coefficient per SD log10-unit increase in PFOA OR by tertiles	T2D Per increase: 1.24 (1.06, 1.45), p-value = 0.009 OR for T3: 1.54 (1.04, 2.28) HbA1c, fasting insulin, adiponectin: No statistically significant association
							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 = 2.87 (Range: 1.03–8.02)	BMI, fasting insulin (μIU/mL), fasting plasma glucose (mmol/L), glycated HbA1c (%), hip circumference (cm), homeostatic model assessment of	Metabolic syndrome: OR per ln-unit increase in PFOA All other outcomes: regression coefficient per ln-unit increase in PFOA	Metabolic syndrome: 2.19 (0.88, 4.42); p-value = 0.09 All other outcomes: No statistically significant associations

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels ^a	Outcome	Comparison	Results ^b
					beta-cell function (HOMA-β), homeostatic model assessment of insulin resistance (HOMA-IR), metabolic syndrome defined by the ATP III criteria, waist circumference (cm)		
Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity							
Occupational Populations							
Steenland et al., 2013, 1937218 Medium	United States 2005–2006	Retrospective Occupational Cohort	Adult residents and workers from the C8 Health Project N = 32,254	Serum 26	Type 1 diabetes, with and without a 10-year lag	RR by quartiles	T1D, validated and self-reported No lag: No statistically significant associations or trends by quartiles With lag Q2: 0.42 (0.09, 2.00) Q3: 0.70 (0.14, 0.35) Q4: 0.38 (0.08, 1.93) p-trend = 0.84 T1D, validated cases only: No statistically significant associations or trends by quartiles
Confounding: Sex, race/ethnicity, smoking, BMI, alcohol consumption							

- 1 PFOA = Perfluorooctanoic acid; 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

1 Type 2 Diabetes; HOME = Health Outcomes and Measures of the Environment; MIREC = Maternal Infant Research on Environmental Chemicals; POPUP = Persistent Organic
 2 Pollutants in Uppsala Primiparas.

3 ^aExposure levels are reported as median in ng/mL unless otherwise noted.

4 ^bResults are reported as effect estimate (95% confidence interval) unless otherwise noted.

5 ^cConfounding indicates factors the models presented adjusted for.

6 ^dRecruitment 1989, blood sample collection 1995–2000, outcome assessed during biennial follow up through June 2011.

7 C.8 Nervous

8 **Table C-17. Associations Between PFOA 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: 5.6 (4.1–7.7) Child: 4.4 (3.1– 6.0)	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	Mean differences by quartiles of PFOA exposure	Visual-motor Early childhood Q2: 1.0 (–1.0, 2.9) Q3: 0.5 (–1.6, 2.6) Q4: 2.3 (0.1, 4.5) Mid-childhood (maternal plasma) Mid-childhood (child plasma) Q2: –4.1 (–8.0, –0.2) Q3: –0.4 (–4.5, 3.7) Q4: –6.1 (–10.5, –1.6) 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 (–3.3, 4.1) Q3: –1.5 (–5.4, 2.3) Q4: –3.2 (–7.4, 1.0) Verbal IQ Mid-childhood (maternal plasma)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					and picture memory		Q2: -3.3 (-5.7, -1.0) Q3: -2.7 (-5.2, -0.2) Q4: -2.4 (-5.1, 0.2) Mid-childhood (child plasma) Q2: -1.0 (-3.9, 2.0) Q3: -2.0 (-5.11, 1.1) Q4: -2.8 (-6.2, -0.6) Design memory Mid-childhood (maternal plasma) Q2: 0.2 (-0.3, 0.8) Q3: 0.3 (-0.3, 0.8) Q4: 0.7 (0.1, 1.3) Mid-childhood (child plasma) Q2: 0 (-0.6, 0.6) Q3: -0.4 (-1.1, 0.2) Q4: -0.4 (-1.1, 0.3) Picture memory Mid-childhood (maternal plasma) Q2: -0.6 (-1.2, 0) Q3: 0.1 (-0.5, 0.7) Q4: -0.1 (-0.7, 0.5) Mid-childhood (child plasma) Q2: -0.3 (-1.0, 0.4) Q3: 0.2 (-0.5, 1.0) Q4: 0 (-0.8, 0.7) PPVT-III: 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	China,	Cohort	Pregnant women and	Maternal plasma 19.9 (15.3–27.4)	ASQ-3 skill scales:	RR per ln-unit increase in	Communication 0.84 (0.59, 1.19)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
High	Recruitment: 2012; Follow-up at age 4 years		their children from the Shanghai-Minhang Birth Cohort N = 533 (236 Females; 297 Males)		communication, gross motor, fine motor, problem solving, personal-social	PFOA and by tertiles	<p>Females: 0.64 (0.34, 1.19) T2: 0.86 (0.49, 1.50) T3: 0.55 (0.28, 1.10) p-trend <0.10 Males: 1.07 (0.70, 1.62) T2: 1.02 (0.65, 1.6) T3: 0.96 (0.61, 1.52) p-value for interaction by sex = 0.255</p> <p>Gross Motor 0.86 (0.47, 1.58) Females: 2.31 (0.75, 7.10) T2: 1.08 (0.33, 3.57) T3: 1.90 (0.66, 5.44) Males: 0.47 (0.25, 0.89); p-value <0.05 T2: 0.51 (0.23, 1.11) T3: 0.45 (0.19, 1.04) p-trend <0.10 p-value for interaction by sex = 0.002</p> <p>Fine Motor 0.99 (0.53, 1.84) No statistically significant associations, trends, or interactions by sex</p> <p>Problem Solving 1.26 (0.73, 2.15) No statistically significant associations, trends, or interactions by sex</p> <p>Personal-Social Skills</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							1.67 (0.89, 3.14) Females: 9.00 (3.82, 21.21); p-value <0.05 Males: 1.03 (0.53, 2.01) T2: 1.60 (0.80, 3.19) T3: 1.50 (0.77, 2.93) p-value for interaction by sex = 0.002
<p>Outcome: Neuropsychological problems defined as scores $\leq 10^{\text{th}}$ percentile.</p> <p>Results: Lowest tertile used as reference. For personal-social skills, no cases of neuropsychological problems were present among the lowest tertile of PFOA exposure among girls; as a result, the Poisson regression model did not converge.</p> <p>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 sex</p>							
Oulhote et al., 2016, 3789517 High	Faroe Islands, Recruitment: 1997–2000, Follow-up at ages 5 and 7 years	Cohort	Children at 5 years (n = 508) and 7 years (n = 491)	Serum Maternal: 3.34 (2.56–4.01) 5 years: 4.06 (3.33–4.98) 7 years: 4.37 (3.53–5.66)	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 PFOA	SDQ total score Prenatal exposure: –0.37 (–1.34, 0.61), p-value = 0.46 5-year serum: 1.03 (0.11, 1.95), p-value = 0.03 7-year serum: 0.1 (–0.83, 1.03), p-value = 0.84 Hyperactivity/Inattention Prenatal exposure: 0.93 (0.76, 1.13), p-value = 0.43 5-year serum: 1.1 (0.91, 1.32), p-value = 0.33 7-year serum: 0.97 (0.8, 1.16), p-value = 0.71 Conduct Prenatal exposure: 0.86 (0.71, 1.04), p-value = 0.12 5-year serum: 1.19 (0.99, 1.44), p-value = 0.06 7-year serum: 1.01 (0.84, 1.22), p-value = 0.92

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							<p>Peer Relationship</p> <p>Prenatal exposure: 0.99 (0.71, 1.38), p-value = 0.96</p> <p>5-year serum: 1.54 (1.16, 2.06), p-value <0.01</p> <p>7-year serum: 1.23 (0.92, 1.65), p-value = 0.17</p> <p>Emotional</p> <p>Prenatal exposure: 1.04 (0.84, 1.3), p-value = 0.7</p> <p>5-year serum: 1.09 (0.88, 1.34), p-value = 0.45</p> <p>7-year serum: 0.98 (0.8, 1.21), p-value = 0.85</p> <p>Prosocial</p> <p>Prenatal exposure: 1.02 (0.95, 1.1), p-value = 0.58</p> <p>5-year serum: 0.97 (0.9, 1.04), p-value = 0.4</p> <p>7-year serum: 1 (0.93, 1.07), p-value = 0.95</p> <p>Internalizing</p> <p>Prenatal exposure: 0 (–0.55, 0.55), p-value = 0.99</p> <p>5-year serum: 0.59 (0.06, 1.13), p-value = 0.03</p> <p>7-year serum: 0.19 (–0.34, 0.72), p-value = 0.49</p> <p>Externalizing</p> <p>Prenatal exposure: –0.37 (–0.99, 0.24), p-value = 0.24</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							5-year serum: -0.09 (-0.69, 0.5), p-value = 0.15 7-year serum: -0.09 (-0.69, 0.5), p-value = 0.76 Autism screening Prenatal exposure: -0.22 (-0.67, 0.23), p-value = 0.35 5-year serum: 0.68 (0.25, 1.11) 7-year serum: 0.18 (-0.25, 0.6), p-value = 0.42
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 age 4–5 years	Cohort	Pregnant women and their children from the HOME study N = 175 (80 Females; 95 Males)	Maternal Serum 5.5 (3.8–7.6)	Social Responsiveness Scale (SRS) total score	Regression coefficient per log10-unit/2SD increase in PFOA	SRS -0.9 (-3.1, 1.4) Females: -1.8 (-4.6, 1.0) Males: 0.7 (-2.5, 3.8) p-value for interaction by sex = 0.22
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 = 2.6 (SD = 2.5)	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 PFOA	Cognitive: -0.3 (-3.3, 2.7) Fine-Motor: -0.1 (-3.1, 2.9) Gross-Motor: -1.1 (-4.7, 2.3) Language: 0.8 (-2.4, 3.9) Self-Help: -1.7 (-5.6, 2.2) Social: 0.8 (-3.2, 4.9) Whole Test: -0.6 (-3.7, 2.4)
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							
	United States, 2008–2010	Cohort	Children aged 7 years from	Blood	SDQ scores: total behavioral	Regression coefficient (total	Total Behavioral Difficulties (β) -0.01 (-0.06, 0.05)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Ghassabian et al., 2018, 5080189 Medium			Upstate KIDS Study N = 788	1.12 (IQR = 0.96)	difficulties – total score, borderline problems; hyperactivity, conduct, peer, or emotional problems; difficulties in prosocial behavior	behavioral difficulties, problem scores) and OR (borderline behavioral difficulties, problem scores, difficulties in prosocial behavior) per log-SD increase in PFOA and by quartiles	Q2: -0.05 (-0.19, 0.10) Q3: 0.03 (-0.12, 0.17) Q4: -0.05 (-0.21, 0.12) Difficulties in Prosocial Behavior (OR) 1.35 (1.03, 1.75) Q2: 2.63 (0.97, 7.14) Q3: 2.93 (1.03, 8.28) Q4: 3.23 (1.04, 10.07) All other outcomes: No statistically significant associations
Outcome: Borderline behavioral difficulties were defined as having SDQ Total Difficulties Score within the borderline/abnormal range. Results: Lowest quartile used as reference. Confounding: Child's age and sex, maternal age, pre-pregnancy BMI, race/ethnicity, education, marital status, history of smoking in pregnancy, having private insurance, parity, and infertility treatment							
Goudarzi et al., 2016, 3981536 Medium	Japan, 2002–2005	Cohort	Pregnant women and their infants at 6 and 18 months from the Hokkaido Study on Environment and Children's Health N = 90 Females; 83 Males	Maternal serum 1.2 (0.8–1.7)	Bayley Scales of Infant Development, Second Edition (BSID-II) mental development index (MDI), psychomotor development index (PDI)	Regression coefficient log10-unit increase in PFOA and by quartiles, least square means by quartiles	MDI Females (6 months) -0.296 (-11.96, -0.682) Q1: 89.25 (82.03, 96.47) Q2: 89.68 (82.14, 97.23) Q3: 89.03 (81.35, 96.72) Q4: 84.19 (76.11, 92.28), p-trend = 0.045 β Q2: 0.43 (-4.39, 5.25) β Q3: -0.21 (-5.29, 4.86) β Q4: -5.05 (-10.66, 0.55) Males (6 months) 0.110 (-3.31, 7.14) No statistically significant trend by quartiles, p-trend = 0.615 β Q2: 0.23 (-5.29, 5.77) β Q3: 2.44 (-2.39, 7.29)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							β Q4: 0.44 (−4.91, 5.81)
							PDI
							6 months: −0.006 (−5.93, 5.50)
							Females: 0.055 (−8.37, 12.93)
							Males: 0.068 (−5.56, 9.26)
							18 months: 0.002 (−7.66, 7.85)
							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 age 15 and 18 months	Cohort	Mothers and daughters aged 15 and 38 months from ALSPAC N = 353	Maternal serum 3.7 (2.8–4.8)	MacArthur Communicative Development Inventories (MCDI): communicative, intelligibility, language, nonverbal communication, social development, verbal comprehension, and vocabulary comprehension scores	Regression coefficient	Nonverbal, 15 mo.: 0.10 (−0.07, 0.27) Social, 15 mo.: −0.06 (−0.36, 0.23) Verbal, 15 mo.: 0.24 (0.12, 0.36) Maternal age ≤30: No statistically significant associations Maternal age >30: 0.35 (0.15, 0.55) Vocabulary, 15 mo.: 0.29 (−2.07, 2.64) Maternal age <25: −11.39 (−22.76, −0.02) Maternal age ≥25: No statistically significant associations Communicative, 38 mo.: −0.02 (−0.08, 0.04) Maternal age <25: 0.29 (0.03, 0.54)

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
							Intelligibility, 38 mo.: -0.04 (-0.08, -0.01)
							Maternal age ≤ 30 : No statistically significant associations
							Maternal age > 30 : -0.06 (-0.11, -0.01)
							Language, 38 mo.: -0.83 (-2.21, 0.54)
							Nonverbal, social, 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 Factor
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: 4.06 (3.08–5.50) Controls: 4.00 (3.01–5.42)	ADHD, ASD	RR and OR (stratified by quartile or by sex) per ln-unit increase in PFOA or by quartiles	ADHD: 0.98 (0.82, 1.16) ASD: 0.98 (0.73, 1.31) No 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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 4.28 (3.15–5.49)	Wechsler Primary and Preschool Scales of Intelligence-Revised (WPPSI-R) full scale IQ, performance IQ, verbal IQ	Regression coefficient for mean difference per ln-unit increase in PFOA and by quartiles	Full Scale IQ: –0.1 (–2.7, 2.4) Performance IQ: 0.5 (–2.1, 3.0) Verbal IQ: –1.1 (–3.7, 1.6) No statistically significant associations or trends by quartiles
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: 0.29 (Range: 0.10–0.78) Controls: 0.32 (Range: 0.10–1.86)	ASD	OR per unit increase in PFOA	0.164 (0.013, 2.216), p-value = 0.167 Females: 0.001 (0, 192.7), p-value = 0.275 Males: 0.270 (0.020, 3.634), p-value = 0.536
Confounding: Child's birth year, child sex, mother's age at delivery, father's 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 N = 985 (553 Cases; 432 Controls)	Maternal serum Cases: GM = 3.58 (95% CI = 3.41–3.76) Controls: GM = 3.67 (95% CI = 3.49–3.86)	ASD measured by Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR), intellectual disability	OR per ln-unit increase in PFOA and by quartiles	ASD: 0.78 (0.60, 1.01) Q2: 0.56 (0.39, 0.81) Q3: 0.58 (0.40, 0.86) Q4: 0.62 (0.41, 0.93), p-trend = 0.05 Intellectual Disability: 0.63 (0.43, 0.92) Q2: 0.44 (0.26, 0.76)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							Q3: 0.67 (0.39, 1.14) Q4: 0.48 (0.26, 0.88), p-trend = 0.06
Results: Lowest quartile used as reference.							
Confounding: Matching factors, parity, maternal age, race/ethnicity, weight at sample collection, and maternal birthplace							
Oulhote et al., 2019, 6316905 Medium	Faroe Islands, Recruitment: 1997–2000; Follow-up at age 7 years	Cohort	Children N = 419	Maternal blood 3.25 (2.54–3.99)	Boston Naming Test with and without cues, SDQ total score	Regression coefficient per IQR increase in PFOA	Boston Naming Test With Cues Prenatal: –0.14 (–0.26, 0.05) 5-year serum: –0.01 (–0.07, 0.05) Without Cues Prenatal: –0.07 (–0.16, 0.00) 5-year serum: –0.01 (–0.07, 0.05) SDQ Prenatal: 0.11 (0.02, 0.26) 5-year serum: 0 (–0.06, 0.06)
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 LINC 54 (20 Females; 34 Males)	Cord blood 870.0 ng/L (Range: 200– 2,300 ng/L)	Child Behavior Checklist 1.5–5 (CBCL 1.5–5) measures of ADHD, externalizing behavior	Regression coefficient by tertiles	ADHD Slightly negative, not statistically significant associations for overall population and males. Slightly positive for females. No interactions reported by sex. Externalizing Behavior T2: –3.33 (–7.65, 0.29), p-value = 0.12 T3: –2.30 (–6.88, 1.55), p-value = 0.31 Females T2: –5.24 (–12.82, 0.00), p-value = 0.10 T3: 0.71 (–3.83, 5.21), p-value = 0.74

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							<p>Males</p> <p>T2: -5.87 (-10.76, -0.43), p-value = 0.05</p> <p>T3: -5.54 (-11.57, -0.29), p-value = 0.09</p> <p>Results: Lowest tertile used as reference.</p> <p>Confounding: Alcohol use, smoking, family history of ADHD, education</p>
Shin et al., 2020, 6507470 Medium	United States, Recruitment: 2002–2009; Follow-up: 2009–2017	Case-Control	Mother-child pairs from the CHARGE study, with children aged 2–5 years 453 (239 Cases; 214 Controls; 88 Females; 365 Males)	Maternal serum 2.33 (1.59–3.32)	ASD measured by Autism Diagnostic Interview-Revised (ADI-R)	OR per increase (ln-transformed or linear scale) in modeled, maternal, prenatal PFOA or measured, maternal, postnatal PFOA and by quartiles	<p>By modeled prenatal exposure ln-transformed: 0.94 (0.59, 1.49) Linear: 1.01 (0.89, 1.14)</p> <p>By measured postnatal levels ln-transformed: 1.09 (0.71, 1.67) Linear: 1.06 (0.84, 1.33)</p> <p>No statistically significant associations, trends, or interactions by quartiles or by sex</p> <p>Results: Lowest quartile used as reference.</p> <p>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</p>
Skogheim et al., 2019, 5918847 Medium	Norway, Recruitment: 1999–2008; Follow-up: 2007–2011	Cohort	Mother-child pairs from MoBa N = 943	Maternal plasma 2.50 (1.77–3.21)	Nonverbal and Verbal Working Memory measured by Stanford Binet Intelligence Scales	Regression coefficient per unit increase in PFOA and by quintiles	<p>Nonverbal Working Memory Q2: -0.12 (-0.32, 0.09) Q3: -0.19 (-0.41, 0.03) Q4: -0.18 (-0.41, 0.05) Q5: -0.38 (-0.61, -0.15), p-value <0.01</p> <p>Verbal Working Memory Q2: 0.17 (-0.05, 0.40) Q3: 0.32 (0.07, 0.56) Q4: 0.24 (-0.01, 0.49) Q5: 0.24 (-0.01, 0.50)</p> <p>Results: Lowest quintile used as reference.</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 = 2.31 (Range: 0.18–8.14)	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 PFOA	MDI Year 1: –1.10 (–3.83, 1.63) Year 2: 1.26 (–2.64, 5.16) Year 3: 3.93 (0.08, 7.77) PDI Year 1: –1.05 (–6.02, 3.92) Year 2: 0.23 (–3.27, 3.74) Year 3: 2.35 (–2.84, 7.54) Full IQ Year 4: 2.50 (–1.15, 6.15) Year 6: 0.87 (–3.89, 5.63) Performance IQ Year 4: 0.64 (–4.12, 5.4) Year 6: –1.37 (–6.25, 3.51) Verbal IQ Year 4: 3.99 (–0.34, 8.32) Females: 5.97 (0.34, 11.6) Males: 1.92 (–4.76, 8.60) Interaction p-value = 0.29 Year 6: 3.02 (–2.49, 8.53) No other statistically significant associations or interactions by sex
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							
Stein et al., 2013, 2850964 Medium	United States, Recruitment: 2005–2006, Follow-Up: 2009–2010	Cohort	Pregnant mothers and their children aged 6–12 years	Modeled in utero exposure 43.7 (11.7–110.8)	NEPSY-II scores: comprehension of instructions, design copying, narrative memory	Regression coefficient per ln-unit increase in PFOA and by quartiles	Comprehension of instructions Prenatal: 0.14 (–0.08, 0.36) By serum: 0.03 (–0.22, 0.28) Design copying

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
			from the C8 Health Project Modeled = 284 Measured = 319	Serum 35.0 (15.3–93.2)	free and cued recall, word generation semantic/initial letter Wechsler Abbreviated Scale of Intelligence: Full-scale IQ, performance IQ, verbal IQ Conners' Continuous Performance test scores: clinical confidence index, commissions T-score, hit reaction time T-score, omissions T-score Wechsler Individual Assessment Test II (WIAT-II) scores: word reading/pseudoword decoding, numeral operations		Prenatal: 0.21 (–0.06, 0.48) Q4: 1.02 (0, 2.04) By serum: 0.26 (–0.04, 0.55) Narrative memory free and cued Recall Prenatal: –0.14 (–0.36, 0.08) By serum: –0.07 (–0.31, 0.17) Word generation semantic/initial letter Prenatal: 0.10 (–0.09, 0.30) By serum: 0.03 (–0.19, 0.25) Full-scale IQ Prenatal: 0.83 (–0.13, 1.79) Q4: 4.61 (0.68, 8.54) By serum: 0.99 (–0.06, 2.04) Performance IQ Prenatal: 0.58 (–0.39, 1.55) By serum: 0.94 (–0.14, 2.01) Verbal IQ Prenatal: 0.41 (–0.60, 1.42) By serum: 0.29 (–0.83, 1.40) Clinical confidence index Prenatal: –2.37 (–4.24, –0.50) Q2: –2.14 (–9.86, 5.57) Q3: –7.68 (–15.32, –0.04) Q4: –8.49 (–16.14, –0.84) By serum: –2.15 (–4.19, –0.10) Q2: –5.62 (–12.52, 1.27) Q3: –3.23 (–10.37, 3.91)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							Q4: -6.90 (-14.04, 0.25)
							Commissions t-score Prenatal: -0.17 (-0.89, 0.55) Q2: 1.52 (-1.46, 4.51) Q3: 0.16 (-2.79, 3.12) Q4: 0.03 (-2.93, 2.99) By serum: 0.12 (-0.66, 0.91) Q2: 0.95 (-1.71, 3.61) Q3: -0.32 (-3.08, 2.44) Q4: 0.60 (-2.16, 3.36)
							Hit reaction time t-score Prenatal: -0.37 (-1.22, 0.49) Q2: -1.69 (-5.24, 1.86) Q3: -1.88 (-5.40, 1.63) Q4: -1.38 (-4.90, 2.14) By serum: -0.70 (-1.63, 0.24) Q2: -1.67 (-4.84, 1.49) Q3: -1.76 (-5.04, 1.52) Q4: -1.73 (-5.01, 1.55)
							Omissions t-score Prenatal: -0.02 (-1.06, 1.03) Q2: 0.10 (-4.21, 4.42) Q3: -0.40 (-4.68, 3.88) Q4: 0.10 (-4.19, 4.38) By serum: 0.12 (-0.66, 0.91) Q2: -2.20 (-5.95, 1.55) Q3: 0.07 (-3.82, 3.95) Q4: -0.57 (-4.46, 3.31)
							Word reading Prenatal: 0.50 (-0.40, 1.41) Q2: 1.72 (-2.05, 5.48) Q3: 0.61 (-3.07, 4.30)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							Q4: 2.27 (–1.43, 5.96) By serum: –0.02 (–1.01, 0.98) Q2: –1.32 (–4.70, 2.06) Q3: –1.91 (–5.34, 1.52) Q4: –1.09 (–4.54, 2.36) Numerical operations Prenatal: 0.65 (–0.48, 1.78) Q2: 4.45 (–0.25, 9.14) Q3: 4.75 (0.13, 9.36) Q4: 3.12 (–1.51, 7.76) Females: –0.6 (–5.0, 3.9) Males: 4.4 (0.4, 9.2) p-value for interaction by sex = 0.14 By serum: 0.15 (–1.17, 1.46) Q2: 0.36 (–4.17, 4.88) Q3: 1.11 (–3.51, 5.73) Q4: –0.41 (–5.06, 4.25) Females: –4.1 (–8.6, 0.3) Males: 3.9 (0.2, 9.6) p-value for interaction by sex = 0.01 No other statistically significant interactions by sex
Results: Lowest quartile used as reference. For brevity, only statistically significant associations by quartiles are included for NEPSY-II and Wechsler Abbr. Confounding: Child age at neuropsychological assessment, child sex, test examiner, HOME score, maternal Full-Scale IQ, child BMI at neuropsychological assessment							
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 3.7 (IQR = 2.0)	Depression, ADHD, scholastic achievement	Depression, ADHD: Hazard ratio (depression and ADHD) by tertile	Depression T2: 1.37 (0.85, 2.21) T3: 1.03 (0.61, 1.73) p-value for trend = 0.28 ADHD

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
						Scholastic achievement: Regression coefficient per unit increase in PFOA and by tertiles	T2: 0.48 (0.18, 1.28) T3: 0.74 (0.29, 1.87) p-value for trend = 0.45 Scholastic Achievement: -0.07 (-0.15, 0.001), p-value = 0.18 T3: -0.25 (-0.64, 0.14), p-value = 0.21
							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	Maternal serum 5.4 (3.6–7.5)	Behavior Rating Inventory of Executive Function (BRIEF) scores for behavioral regulation index, metacognition index, global executive composite, inhibit, shift, emotional control, working memory, plan/organize, initiate, organization of materials, monitor	All outcomes: OR for score ≥60 per unit increase in PFOA Index and composite scores only: Regression coefficient per ln-unit increase in PFOA and by quartiles	Behavioral Regulation: 1.11 (-1.22, 3.44) Metacognition: 0.58 (-1.77, 2.93) Global Executive Function: 1.06 (-1.33, 3.45) No statistically significant associations or interactions by age; no statistically significant associations or trends by quartiles Inhibit: 1.45 (0.76, 2.77) Shift: 1.01 (0.51, 1.98) Emotional control: 1.33 (0.62, 2.84) Working memory: 0.84 (0.47, 1.47) Plan/organize: 1.43 (0.74, 2.76) Initiate: 2.13 (0.89, 5.10) Organization: 1.83 (0.81, 4.16) Monitor: 1.80 (0.86, 3.78)
							Confounding: Maternal age, race, education, income, maternal serum cotinine, maternal depression, HOME score, maternal IQ, marital status, child sex

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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: 5.4 (3.7–7.4) 8 years: 2.4 (1.8–3.2)	BRIEF measures of behavioral regulation, metacognition, global executive composite indices	OR per ln-unit increase in PFOA	Behavioral Regulation 3 years: 1.01 (0.29, 3.53) 8 years: 1.56 (0.49, 4.92) Metacognition 3 years: 1.30 (0.47, 3.57) 8 years: 3.18 (1.17, 8.60) Global Executive Function 3 years: 1.39 (0.45, 4.24) 8 years: 2.69 (0.92, 7.90)
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 age 3 and 8 years	Cohort	Mother-child dyads from the HOME study N = 204	Serum Prenatal: 5.2 (3.6–7.6) 3 years: 5.4 (3.7–7.4) 8 years: 2.5 (1.7–13.2)	Conners' Continuous Performance Test II commissions t-score, omissions t-score, hit reaction time, tau (ms) Virtual Morris Water Maze (VMWM) scores for visual-spatial learning distance (pool units), learning time (s), memory retention distance (%), and memory retention time (s)	Regression coefficient per ln-unit increase in PFOA	Conners' Commissions Prenatal: –2.0 (–3.8, –0.3) 3 years: –0.1 (–2.3, 2.1) 8 years: –0.01 (–2.4, 2.4) Omissions Prenatal: –2.3 (–7.1, 2.6) 3 years: –1.9 (–7.8, 3.9) 8 years: 1.0 (–5.8, 7.8) Hit reaction time Prenatal: –0.7 (–3.5, 2.2) 3 years: 0.2 (–3.5, 4.0) 8 years: –2.3 (–6.8, 2.3) Tau Prenatal: –10.6 (–43.6, 22.3) 3 years: 22 (–16.5, 60.6) 8 years: 14.6 (–21.9, 51.1) Visual-spatial scores (VMWM) Learning distance Prenatal: –0.1 (–1.7, 1.5) 3 years: 0.5 (–1.2, 2.2) 8 years: 0.1 (–1.8, 2.0)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							Learning time Prenatal: 0.5 (–2.0, 3.0) 3 years: 1.4 (–1.4, 4.2) 8 years: –0.1 (–3.5, 3.3) Memory retention distance Prenatal: 2.8 (–1.7, 7.4) 3 years: –0.9 (–7.1, 5.4) 8 years: 1.1 (–5.8, 8.0) Memory retention time Prenatal: –0.3 (–2.0, 1.3) 3 years: –1.5 (–3.3, 0.2) 8 years: –0.1 (–2.4, 2.1)
							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 age 3 and 8 years	Cohort	Pregnant women and their children from the HOME study N = 221	Serum Maternal: GM = 5.2 8 years: GM = 2.4	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 PFOA	Full Scale IQ Prenatal: 3.3 (–0.4, 6.9) 3 Years: 2.4 (–1.5, 6.4) 8 Years: 2.3 (–3.3, 7.9) Perceptual Reasoning Prenatal: 0.7 (–3.2, 4.6) 3 Years: 1.2 (–3.0, 5.4) 8 Years: 2.3 (–3.7, 8.2) Processing Speed Prenatal: 3.3 (–0.8, 7.5) 3 Years: 1.7 (–2.6, 6) 8 Years: 2.8 (–3.0, 8.5) Verbal Comprehension Prenatal: 2.3 (–1.1, 5.6) 3 Years: 1.0 (–2.9, 4.8) 8 Years: –1.8 (–6.9, 3.2) Working Memory Prenatal: 4.1 (0.3, 8.0)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							3 Years: 2.9 (−1.0, 6.7) 8 Years: 4.3 (−0.7, 9.3)
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 = 6.1 (SD = 3.8)	Wide Range Achievement Test 4 (WRAT-4) reading composite score	Regression coefficient per log10-unit increase in PFOA	12.6 (3.0, 22.2)
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 age 5 years	Cohort	Pregnant women and their children aged 5 and 8 years from TMICS N = 120	Serum 5 years: 2.50 (1.54–3.35) 8 years: 2.50 (1.54–3.33)	Full Scale IQ, Performance IQ, Verbal IQ	Regression coefficient per log2-unit increase in PFOA	Full Scale IQ 5 Years: 1.2 (−1.0, 3.5) 8 Years: −0.4 (−2.5, 1.7) Performance IQ 5 Years: 1.0 (−1.4, 3.4) 8 Years: −1.1 (−3.2, 1.0) Verbal IQ 5 Years: 0.9 (−1.4, 3.3) 8 Years: 0.5 (−1.5, 2.5)
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 age 3, 5, and 7 years	Cohort	Pregnant women and their children aged 3, 5, and 7 years from the HOME study N = 167	Serum Maternal: 5.4 (3.6–7.3) 3 years: 5.5 (3.9–7.7) 8 years: 2.4 (1.8–3.2)	Basic reading, brief reading, letter word identification, passage comprehension measured by Woodcock Johnson Test of Achievement-III (WJ-III)	Basic Reading Maternal Serum: 0.7 (−4.9, 6.2) Year 3 Serum: 6.4 (−1.6, 14.1) Brief Reading Maternal Serum: 3.7 (−1.8, 9.3) Year 3 Serum: 10.4 (2.8, 18.1) Letter Word Identification Maternal Serum: 2.0 (−3.1, 7.1) Year 3 Serum: 9.2 (2.1, 16.3)	

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							Reading composite, word reading, sentence comprehension measured by Wide Range Achievement Test 4 (WRAT-4) Passage Comprehension Maternal Serum: 3.8 (0.1, 7.7) Year 3 Serum: 8.5 (3.3, 13.7) Word Attack Maternal Serum: 0.5 (–5.1, 6.1) Year 3 Serum: 4.9 (–2.0, 11.8) Reading Composite Maternal Serum: 3.5 (–1.1, 8.2) Year 3 Serum: 2.8 (–3.1, 8.8) Year 8 Serum: 2.6 (–3.1, 8.2) Word Reading Maternal Serum: 2.3 (–2.1, 6.7) Year 3 Serum: 1.0 (–4.7, 6.7) Year 8 Serum: 6.1 (0.9, 11.3) Sentence Comprehension Maternal Serum: 3.7 (–1.6, 9.0) Year 3 Serum: 3.1 (–4.1, 10.1) Year 8 Serum: –0.1 (–6.6, 6.4)
Confounding: Maternal age, race, education, household-income, parity, smoking (serum cotinine concentration, ng/mL), maternal IQ, breastfeeding duration (weeks), 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 2.0 (1.3–2.9)	High and low frequency hearing impairment (HFHI and LFHI)	OR per log2- unit increase in PFOA and ≥90 th percentile vs. <90 th percentile	HFHI OR (per doubling): 0.97 (0.82, 1.14) OR (90 th percentiles): 1.05 (0.61, 1.81) LFHI OR (per doubling): 0.98 (0.73, 1.32)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							OR (90 th percentiles): 1.40 (0.48, 4.07)
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: 0.25–22,412	Memory impairment (self-reported)	OR per doubling of log-unit PFOA and by quartiles	OR: 0.96 (0.94, 0.98) Q2: 0.88 (0.79, 0.97) Q3: 0.83 (0.75, 0.92) Q4: 0.79 (0.71, 0.88) Q5: 0.79 (0.71, 0.88) 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 40.000 ng/L (26.809–61.256 ng/L)	ADHD	OR per IQR increase in ln-unit PFOA	1.35 (0.87, 2.11), p-value = 0.183
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, 1999–2006	Cross-sectional	Adults aged 20+ years from NHANES N = 2,525	Serum 2.25 (Range: 0.07–51.1)	Hearing threshold >25 dB by frequency	OR by quartiles	2,000 Hz Q2: 1.41 (0.95, 2.10) Q3: 1.26 (0.85, 1.87) Q4: 1.76 (1.20, 2.60), p-trend <0.01 3,000 Hz Q2: 1.39 (0.98, 1.98) Q3: 1.38 (0.98, 1.96) Q4: 1.64 (1.16, 2.34), p-trend = 0.02 4,000 Hz Q2: 1.31 (0.95, 1.83) Q3: 1.12 (0.81, 1.56)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
							Q4: 1.41 (1.01, 1.98), p-trend = 0.11
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 8.1 (5.9–11.9)	Affective state: Beck Depression Inventory (BDI) total score, State-Trait Anxiety Inventory state and trait t-scores Attention: Trail making test Part A (ln-transformed time to complete) Executive function: Stroop color word test t-score, Trail making test part B (ln-transformed time to complete), Wisconsin card sorting test preservative ln-transformed error and response Memory and learning: California Verbal Learning Test total and subscores,	Regression coefficient per IQR increase in ln-transformed PFOA	Depression: 0.08 (–0.85, 1.02), p-value = 0.86 CVLT Total Score: 2.63 (0.20, 5.06), p-value = 0.03 Wisconsin card-sorting test Perseverative Error: –0.18 (–0.34, –0.01), p-value = 0.04 Perseverative Response: –0.20 (–0.38, –0.02), p-value = 0.03 Wechsler Memory Scale Logical Memory Immediate Recall: 0.28 (–0.85, 1.42), p-value = 0.62 Delayed Recall: 0.09 (–0.98, 1.15), p-value = 0.87 Visual Reproduction Immediate Recall: –0.11 (–0.79, 0.56), p-value = 0.74 Delayed Recall: –0.12 (–0.83, 0.59), p-value = 0.74 No statistically significant associations: State-Trait Anxiety Inventory, Stroop color word test, trail-making tests, motor

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
					Wechsler Memory Scale logical memory and visual reproduction immediate and delayed recall scores		function outcomes, visuospatial outcomes
					Motor function (dominant and non-dominant hands): finger tapping test average scores, grooved pegboard test ln-transformed times to completion, static motor steadiness test ln-transformed total numbers of contacts and times touching, dominant hand reaction time		
					Visuospatial function: Wechsler Adult Intelligence Scale-Revised total scores for block design and digit symbol coding		
Confounding: Age, sex, education, serum total PCB							
Pregnant Women							

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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 = 300	Maternal serum 5.4 (3.6–7.6)	Beck Depression Inventory-II (BDI-II)	Relative risk per In-unit increase in PFOA	Medium Score Trajectory: 1.3 (0.8, 2.0) High Score Trajectory: 0.9 (0.5, 1.9) OR for score >13 from pregnancy to 8 years postpartum: 1.1 (0.7, 1.6)

ADHD = Attention deficit hyperactivity disorder; ASD = Autism spectrum disorder; CDI = Comprehensive Developmental Inventory; ID = Intellectual disability; ASQ = Ages and Stages Questionnaire; SDQ = Strengths and Difficulties Questionnaire; PFOA = Perfluorooctanoic 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 PFOA Exposure and Renal 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							
Dhingra et al., 2016, 3981521 High	United States, 1951–2011	Cohort	Adults from C8 Health Project/C8 Science Panel, >20 years, Main cohort = 28,240,	Serum Cumulative PFOA exposure at failure or end of follow-up: Mean = 3.32 ng/mL-yr (SD = 7.26)	CKD	HR by PFOA quintiles, at 0-, 5-, 10-, and 20-year lags	Main cohort 0-year lag: Quintile 2: 1.26 (0.9, 1.75), p-value = 0.18 Quintile 3: 1.12 (0.8, 1.55), p-value = 0.52 Quintile 4: 1.12 (0.81, 1.56), p-value = 0.49

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
			prospective cohort = 27,952				<p>Quintile 5: 1.24 (0.88, 1.75), p-value = 0.21 p-value for trend = 0.80</p> <p>5-, 10-, and 20-year lag: No statistically significant associations or trends</p> <p>Prospective cohort Quintile 2: 1.36 (0.89, 2.09), p-value = 0.16 Quintile 3: 0.94 (0.62, 1.45), p-value = 0.79 Quintile 4: 1.12 (0.72, 1.75), p-value = 0.6 Quintile 5: 1.08 (0.7, 1.66), p-value = 0.74 p-value for trend = 0.77</p> <p>Outcome: CKD was self-reported then confirmed by medical records or presence in United States Renal Data System renal failure registry (non-neoplastic, non-genetic, and diagnosed after age 20). Results: Lowest quintile used as reference group. Confounding: Gender, time-varying self-reported hypertension, time-varying self-reported diabetes diagnosis, time-varying self-reported high cholesterol diagnosis, time-varying smoking, category of BMI, and education category^c</p>
Dhingra et al., 2017, 3981432 Medium	United States, 2005–2006	Cross-sectional	Women from C8 Science Panel, 30–65 years, N = 29641	<p>Serum Measured: 60th percentile = 36.3 µg/mL (20th–80th percentile = 11.1–88.0 µg/mL)</p> <p>Modeled: 60th percentile = 26.8 µg/mL (20th–80th</p>	eGFR	Regression coefficient per ln-unit increase in PFOA, or by quintiles, or by deciles	<p>Modeled serum PFOA Per ln increase: 0.05 (0.01), p-value = 0.43 Quintile 2: –0.08 (0.27), p-value = 0.77 Quintile 3: 0.37 (0.27), p-value = 0.17 Quintile 4: 0.21 (0.27), p-value = 0.44 Quintile 5: 0.23 (0.27), p-value = 0.41</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
				percentile = 5.8–82.4 µg/mL)			Dose-response by deciles: decreased until the 4th decile and remained approximately flat thereafter Measured serum PFOA Per ln increase: -0.14 (0.07), p-value = 0.03 Quintile 2: -0.64 (0.27), p-value = 0.018 Quintile 3: -1.03 (0.27), p-value = 0.0001 Quintile 4: -0.84 (0.27), p-value = 0.0019 Quintile 5: -0.98 (0.27), p-value = 0.0003 Results: Lowest quintile used as reference group. Effect estimates are provided with standard deviation in parentheses. Confounding: Smoking status, BMI, education level, race, sex, and birth year
Lin et al., 2013, 2850967 Low	Taiwan, 2006– 2008	Cross-sectional	Adolescents and young adults from YOTA study, 12–30 years, N = 644	Serum 3.49 (75 th percentile = 6.54)	Uric acid (mg/dL)	Mean concentration by PFOA percentiles	≤50 th percentile: 6.08 (0.1) 50 th –75 th : 6.08 (0.11) 75 th –90 th : 6.11 (0.14) >90 th : 6.13 (0.17) p-value for trend = 0.983 Results: Effect estimates are provided with standard error in parentheses. Confounding: Age, gender, smoking status, alcohol drinking, BMI
Blake et al., 2018, 5080657 Medium	United States, 1991–2008	Cohort	Adults and children, Fernald Community Cohort (FCC) N = 192 (115 females, 77 males)	Serum 12.7 (7.83–19.5)	eGFR	Percent change per IQR increase in PFOA	All: Repeated measures model: -0.83 (-2.44, 0.77); p-value = 0.31 Latent model: -0.74 (-2.45, 0.96); p-value = 0.39 Female: -1.38 (-3.41, 0.65), p-value = 0.18 Male: 0.95 (-3.08, 4.98), p-value = 0.21

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							p-value for interaction by sex = 0.38
Confounding: Age, year of measurement, sex, education, income, marital status, and 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: 28.6 (12.6–72.7) Non-diabetic: 28.0 (13.6–71.4)	CDK (eGFR of <60 mL/min/1.73 m ²)	OR per ln-unit increase in PFOA	Diabetics: 0.92 (0.86, 0.98) Non-diabetic: 0.99 (0.96, 1.03)
Confounding: Age, sex, BMI, HDL, LDL, white blood cell count, CRP, hemoglobin, and iron							
Covertino et al., 2018, 5080342 Low	United Kingdom, 2008–2011	Controlled trial	Adults, solid-tumor cancer patients N = 49	Plasma Exposure levels non reported	Creatinine (μmol/L), urea (μmol/L)	Regression coefficient per 1-μM increase in PFOA	No observable differences with measured plasma PFOA concentrations
Confounding: None reported							
Arrebola et al., 2019, 5080503 Low	Spain, 2009–2010	Cross-sectional	Adults, BIOAMBIENT. ES study N = 342	Serum 1.83 (1.34–2.53)	Uric acid (mg/dL), hyperuricemia	OR(hyperuricemia) or regression coefficient per log-unit increase in PFOA	Uric acid Wet-basis and lipid-basis models: 0.04 (–0.06, 0.14); p-value = 0.425 Wet-basis model with adjustment for serum lipids: 0.04 (–0.06, 0.14); p-value = 0.459 Hyperuricemia (OR) Wet-basis and lipid-basis models: 1.83 (0.93, 3.68); p-value = 0.083 Wet-basis model with adjustment for serum lipids: 1.78 (0.90, 3.45); p-value = 0.095
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							

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 = 1,871	Serum GM = 1.86 (SE = 1.02)	Total protein (g/dL)	Regression coefficient per ln-unit increase in PFOA	0.05 (SE = 0.03)
Confounding: Age, gender, ethnicity, smoking status, alcohol intake, household income, waist circumference, and medications (anti-hypertensive, anti-hyperglycemic, and anti-hyperlipidemic agents)							
Chen et al., 2019, 5387400 Low	Croatia, 2007–2008	Cross-sectional	Adults, 44–56 years, N = 122	Plasma GM = 2.87 (range = 1.03–8.02)	Uric acid (μmol/L), creatinine (μmol/L)	Regression coefficient per ln-unit increase in PFOA	Uric acid: 5.02 (–22.09, 32.09) Creatinine: 0.46 (–5.60, 6.52)
Confounding: Age, sex, education, socioeconomic status, smoking, dietary pattern, and physical activity							
Jain and Ducatman, 2019, 5381566 Low	United States, 2005–2014	Cross-sectional	Adults from NHANES, ≥20 years, N = 8,220	Serum Levels not reported	Levels of albumin in urine (log10-μg/mL), creatinine in urine (log10-mg/dL), albumin-to-creatinine ratio in urine (log10-mg/g), albumin in serum (log10-mg/dL), creatinine in serum (log10-mg/dL)	Regression coefficient per log10-unit increase in PFOA, or percent change per 10% increase in PFOA	Albumin in urine Per log10-unit increase: –0.17 p-value <0.01 Negative associations across GF stages Percent change per 10% increase: –1.59 p-value <0.05 p-value for gender and race/ethnicity interaction = 0.15 Creatinine in urine Per log10-unit increase: 0.02 p-value = 0.2 No significant associations across eGFR stages Percent change per 10% increase: 0.22 p-value for gender and race/ethnicity interaction = 0.02 Albumin- to-creatinine ratio in urine Per log10-unit increase: –0.19 p-value <0.01

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							<p>Negative associations across GF stages</p> <p>Percent change per 10% increase: -1.82</p> <p>p-value <0.05</p> <p>p-value for gender and race/ethnicity interaction = 0.88</p> <p>Albumin in serum</p> <p>Per log10-unit increase: 0.02</p> <p>p-value <0.01</p> <p>Positive associations across eGFR stages</p> <p>Percent change per 10% increase: 0.17</p> <p>p-value <0.05</p> <p>p-value for gender and race/ethnicity interaction = 0.74</p> <p>Creatinine in serum</p> <p>Per log10-unit increase: 0.01</p> <p>p-value = 0.19</p> <p>Positive associations in GF-1</p> <p>Negative associations in GF-3B/4</p> <p>Percent change per 10% increase: 0.07</p> <p>p-value for gender and race/ethnicity interaction <0.01</p>
<p>GF Stages: GF-1: GFR ≥ 90 mL/min/1.73m²; GF-2: GFR between 60 and 90 mL/min/1.73m²; GF- 3A: GFR between 45 and 60 mL/min/1.73m²; GF- 3B/4: GFR between 15 and 45 mL/min/1.73m²</p> <p>Confounding: Gender, race/ethnicity, age, log10(BMI), log10(serum cotinine), poverty income ration, NHANES survey period</p>							
Jain et al., 2019, 5080378 Low	United States, 2007–2014	Cross-sectional	Adults from NHANES, ≥ 20 years, Males = 3330, females = 3506	Serum Males: GM = 2.36 (2.24–2.48)	Uric acid (mg/dL) by glomerular filtration (GF) stage	Regression coefficient per log10-unit increase in PFOA	<p>Males</p> <p>GF-1: 0.04, p-value <0.01</p> <p>GF-2: 0.05, p-value <0.01</p> <p>GF-3A: 0.03, p-value = 0.27</p> <p>GF-3B: -0.07, p-value <0.01</p>

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
				Females: GM = 3.19 (3.06–3.32)			Females GF-1: 0.03, p-value = 0.01 GF-2: 0.02, p-value = 0.11 GF-3A: 0.09, p-value <0.01 GF-3B: 0.004, p-value = 0.91
							GF Stages: GF-1: eGFR >90 mL/min per 1.73 m ² , GF-2: 60 < eGFR ≤90 mL/min per 1.73 m ² , GF-3A: 45 < eGFR ≤60 mL/min per 1.73 m ² ; GF-3B/4: 15 < eGFR ≤45 mL/min per 1.73 m ² Confounding: Gender, race/ethnicity, age, log10(BMI), log10(serum cotinine), poverty income ration, NHANES survey period
Wang et al., 2019, 5080583 Low	China, 2015–2016	Cross-sectional	Adults, Isomers of C8 Health Project N = 1,612 (males = 1,204, females = 408)	Serum 6.19 (4.08–9.31)	CKD, eGFR	OR(CKD), or regression coefficient per ln-unit increase in PFOA, or by quartiles	CKD (OR) Per ln-unit increase: 0.73 (0.57, 0.95), p-value = 0.019 Q2: 0.72 (0.45, 1.13) Q3: 0.83 (0.52, 1.31) Q4: 0.60 (0.36, 1.01) p-value for trend = 0.234 eGFR Per ln-unit increase: All: 1.23 (0.30, 2.17), p-value = 0.008 Males: 1.29 (0.21, 2.36), p-value = 0.019 Females: 1.54 (–0.36, 3.44), p-value = 0.111 p-value for interaction by sex = 0.999 Q2: 1.00 (–0.8, 2.81) Q3: 0.63 (–1.2, 2.46) Q4: 2.07 (0.22, 3.91) p-value for trend = 0.050
							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

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
Zeng et al., 2019, 5918630 Low	China, 2015–2016	Cross-sectional	Adults, Isomers of C8 Health Project N = 1,612 (males = 1,204, females = 408)	Serum 6.19 (4.08–9.31)	Uric acid (mg/dL), hyperuricemia	OR (hyperuricemia) or regression coefficient (uric acid) per log10-unit increase in PFOA	Hyperuricemia (OR) All: 1.29 (1.08, 1.54) Males: 1.21 (1, 1.46) Females: 1.76 (1.06, 2.94) p-value for interaction by sex = 0.183 Uric acid All: 0.18 (0.09, 0.26), p-value <0.001 Males: 0.17 (0.06, 0.27) Females: 0.14 (0.01, 0.27) p-value for interaction by sex = 0.988
Outcome: Hyperuricemia defined as serum uric acid levels >7.0 mg/dL in males or >6.0 mg/dL in females.							
Confounding: Age, sex, BMI, income, drinking, smoking, career, exercise, offal consumption, fish and seafood consumption, serum creatinine							
Lee et al., 2020, 6833761 Low	United States, 1999–2016	Cross-sectional	Adults from NHANES, 18+ years, N = 46,748	Serum Exposure levels not reported	Albuminuria	OR per SD–unit increase in log-PFOA	Discovery data set: 0.69 (0.57, 0.83). FDR=0.006 Validation data set: 0.68 (0.58, 0.80), p-value = 0.029
Outcome: Albuminuria defined as urine albumin-to-creatinine ratio ≥30 mg/g.							
Confounding: Age, age-squared, sex, diabetes mellitus, hypertension, BMI, race/ethnicity, smoking, and socioeconomic status							
Scinicariello et al., 2020, 6833670 Low	United States, 2009–2014	Cross-sectional	Adults from NHANES N = 4915 (no CKD = 4103; CKD = 874)	Serum GM = 2.37 (SE = 0.06)	Uric acid (mg/dL), hyperuricemia, gout	OR (hyperuricemia, gout), or regression coefficient (uric acid) by quartiles	Uric acid Overall population Q2: 0.17 (0.06, 0.29) Q3: 0.24 (0.11, 0.37) Q4: 0.42 (0.26, 0.57) p-value for trend = 0.0001 Participants with CKD Q2: 0.14 (–0.38, 0.65) Q3: –0.05 (–0.63, 0.53) Q4: 0.6 (–0.04, 1.24) p-value for trend = 0.02 Participants without CKD Q2: 0.08 (–0.03, 0.2)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							Q3: 0.31 (0.17, 0.46) Q4: 0.16 (0.01, 0.31) p-value for trend = 0.001
							Hyperuricemia (OR) Overall population Q2: 1.05 (0.77, 1.44) Q3: 1.21 (0.87, 1.69) Q4: 1.81 (1.29, 2.55) p-value for trend = 0.004 Participants with CKD Q2: 1.15 (0.69, 1.92) Q3: 0.95 (0.53, 1.69) Q4: 1.82 (0.96, 3.47) p-value for trend = 0.21 Participants without CKD Q2: 0.96 (0.64, 1.44) Q3: 1.19 (0.75, 1.88) Q4: 1.65 (1.1, 2.46) p-value for trend = 0.02
							Gout (OR) Overall population Q2: 1.75 (0.9, 3.31) Q3: 2.34 (1.32, 4.15) Q4: 3.17 (1.68, 5.98) p-value for trend = 0.01 Participants with CKD Q2: 1.83 (0.79, 4.19) Q3: 3.02 (1.28, 7.15) Q4: 2.73 (1.28, 5.84) p-value for trend = 0.04 Participants without CKD Q2: 2.11 (0.72, 6.23) Q3: 2.57 (1, 6.59) Q4: 3.88 (1.46, 10.33)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							p-value for trend = 0.05
Outcomes: CKD defined as eGFR <60 mL/min per 1.73 m ² and/or albuminuria. Hyperuricemia defined as serum uric acid levels ≥7.0 mg/dL in males or ≥6.0 mg/dL in females. Gout was self-reported diagnosis from a health professional.							
Results: Lowest quartile used as reference group.							
Confounding: Race/ethnicity, age, sex, education, alcohol, smoking, serum cotinine, BMI, diabetes, hypertension, CKD							
Children and Adolescents							
Geiger et al., 2013, 2919148 Low	United States, 1999–2000; 2003–2008	Cross-sectional	Children and adolescents from NHANES, 12–18 years, N = 1,772	Serum Mean = 4.3 (SE = 0.1)	Uric acid (mg/dL), hyperuricemia	OR (hyperuricemia) or regression coefficient (uric acid) per ln-unit increase in PFOA or by quartiles	Hyperuricemia (OR) Per ln increase: 1.59 (1.19, 2.13) Q2: 0.94 (0.58, 1.53) Q3: 1.01 (0.62, 1.63) Q4: 1.62 (1.1, 2.37) p-value for trend = 0.007 Uric acid Per ln increase: 0.2 (0.11, 0.29) Q2: 0.02 (–0.10, 0.14) Q3: 0.03 (–0.11, 0.17) Q4: 0.3 (0.17, 0.43) p-value for trend = 0.0001
Outcome: Hyperuricemia defined as serum uric acid levels ≥6 mg/dL.							
Results: Lowest quartile as reference group.							
Confounding: Age, sex, race/ethnicity, BMI, annual household income, moderate activity, total cholesterol, serum cotinine							
Kataria et al., 2015, 3859835 Low	United States, 2003–2010	Cross-sectional	Children and adolescents from NHANES, 12–19 years, 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: –2.63 (–7.07, 1.81) Q3: –5.42 (–11.46, 0.61) Q4: –6.61 (–11.39, –1.83), p-value <0.01 Uric acid Q2: 0.17 (–0.033, 0.37) Q3: 0.13 (–0.03, 0.28) Q4: 0.21 (0.056, 0.37), p-value <0.01 Creatinine

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
							Q2: 0.007 (–0.012, 0.027) Q3: 0.021 (–0.008, 0.05) Q4: 0.029 (0.004, 0.054), p-value <0.05
Results: Lowest quartile used 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: 0.5 (0.4–1.3) Boys: 0.5 (0.4–1.4) Girls: 0.5 (0.4–1.2)	Uric acid (mg/dL), hyperuricemia	Regression coefficient per ln-unit increase in PFOA (uric acid), and by quartiles; OR scaled with increasing quartiles (hyperuricemia)	Uric acid All: 0.15 (0.01, 0.28), p-value = 0.032 Boys: 0.24 (0.06, 0.42), p-value = 0.011 Increasing trend in mean uric acid levels by quartiles; Q1 = 4.85 (4.53, 5.17) vs. Q4 = 5.65 (5.33, 5.96); p-value for trend = 0.033 Girls: 0.01 (–0.19, 0.22), p-value = 0.892 No trend in mean uric acid levels by quartiles; Q1 = 4.64 (4.43, 4.94) vs. Q4 = 4.73 (4.41, 5.06); p-value for trend = 0.756 Hyperuricemia (OR) All: 2.16 (1.29, 3.61), p-value <0.05 Boys: 2.76 (1.37, 5.56), p-value <0.05 Girls: 1.64 (0.69, 3.85)
Outcome: Hyperuricemia defined as uric acid level ≥ 6 mg/dL. Results: Lowest quartile used as the reference group. Confounding: Age, gender, BMI, parental education level, exercise, environmental tobacco smoke exposure, and serum creatinine							
Khalil et al., 2018, 4238547 Low	United States 2016	Cross-sectional	Obese children, 8–12 years N = 40	Serum 0.99 (IQR = 0.45)	Creatinine (mg/dL)	Regression coefficient per unit increase in PFOA	–0.02 (–0.15, 0.11)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
Confounding: Age, sex, race							
Pregnant Women							
Gyllenhammar et al., 2018, 4238300 Medium	Sweden; 1996–2011	Cohort	Mothers and infants follow up to 5 years of age, POPUP study N = 381	Maternal serum 2.3 (1.6–3.0)	Cystatin C (GFRcc) (mL/min/1.73 m ²)	Regression coefficient per IQR increase in maternal PFOA	0.004 (SD = 0.002), p-value = 0.022
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							
Nielsen et al., 2020, 6833687 Low	Sweden, 2009–2014	Cohort	Pregnant women, PONCH study N = 73	Serum Early pregnancy: 1.8 (5 th –95 th percentile = 0.8–4.4) Late pregnancy: 1.5 (5 th –95 th percentile = 0.7–3.1)	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: 0.002, p-value = 0.99 CKD-EPI(creatinine): 0.03, p-value = 0.83 CAPA: 0.06, p-value = 0.64 CKD-EPI(cystatin C): 0.03, p-value = 0.83 mean of LMrev and CAPA: 0.04, p-value = 0.76 mean of CKD-EPI(creatinine) and CKD-EPI(cystatin C): 0.002, p-value = 0.98 Glomerular pore size: CAPA/LMrev: 0.09, p-value = 0.47 CKD-EPI(cystatin C)/CKD-EPI(creatinine): –0.003, p-value = 0.98

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
Outcome: Glomerular pore size is estimated as the ratio between eGFR(cystatin C) and eGFR(creatinine) and was calculated by the two ratios provided.							
Confounding: Number of days between sampling, pregnancy-induced change in BMI							
Occupational Populations							
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 4.2 (range = 0.3–18)	Uric acid (μmol/L)	Regression coefficient per log10-unit increase in PFOA	0.021 (SE = 0.032), p-value = 0.508
Confounding: Age, sex, BMI, smoking status, total serum protein, PFOS, 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; POPUP = Persistent Organic Pollutants in Uppsala Primiparas; 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 PFOA Exposure and Hematological 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							
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 = 3.9 (2.6–5.5)	Vitamin D deficiency (<50 ng/mL), 25-hydroxy Vitamin D	Regression coefficient or prevalence OR (POR) per doubling of	Per doubling of PFOA: Vitamin D deficiency POR: 1.02 (0.93, 1.11) 25-hydroxy Vitamin D –0.3 (–1.0, 0.4)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
					([25(OH)D], nmol/L)	PFOA, or by quintiles	No 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 period ^c							
Chen et al., 2019, 5387400 Medium	Croatia 2007–2008	Cross-sectional	Adults, 44–56 years of age, N = 122	Plasma GM = 2.87 (min = 1.03, max = 8.02)	Calcium in serum (mmol/L)	Regression coefficient per ln-unit increase PFOA	–0.02 (–0.07, 0.03)
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	Adult serum non-anemic males: GM = 3.3 (95 % CI: 3.2, 3.4) non-anemic females: GM = 2.5 (95 % CI: 2.4, 2.6) anemic males: GM = 2.4 (95 % CI: 2.1, 2.7) anemic females: GM = 1.6 (95 % CI: 1.4, 1.7)	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: 0.026, p-value <0.01 Anemic females: 0.034, p-value <0.01
Outcome: Anemia defines as whole blood hemoglobin concentrations <12 g/dL (females) and <13 g/dL (males). Confounding: Age, BMI, poverty income ratio, serum cotinine, survey year, daily alcohol intake							
Convertino et al., 2018, 5080342 Low	United Kingdom, 2008–2011	Controlled trial	Solid-tumor cancer patients ≥18 years of age, N = 49	Plasma Range = 0–~633,527 μM	aPTT (s) Fibrinogen (g/L) PPT (s)	Regression coefficient per unit increase in PFOA	“Almost no observable differences” (statistical results not provided)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL) ^a	Outcome	Comparison	Select Results ^b
Confounding: By design, randomly assigned exposure levels and excluded patients with life expectancy <3 months, anticancer therapy within the last 4 weeks, HIV infection, hepatitis B or hepatitis C, inadequate hematologic function, inadequate renal function, abnormal liver function tests, lack of physical integrity of the gastrointestinal tract, uncontrolled cardiac disease, or use of warfarin, phenytoin, or tolbutamide.							
Khalil et al., 2018, 4238547 Low	United States, 2016	Cross-sectional	Children with obesity, 8–12 years of age, N = 47	Serum, median = 0.99 (IQR = 0.45)	25-hydroxy Vitamin D (ng/mL)	Regression coefficient(per unit increase in PFOA	1.90 (–5.49, 9.30)
Confounding: Age, sex, race							

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 PFOA 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 2019	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 = 2.4 (IQR = 2) Postnatal (child) Median = 1.5 (IQR = 0.8)	FEV1	Regression coefficient per log2-unit increase in PFOA	Prenatal: –1.4 (–2.7, –0.1), p-value = 0.03 Postnatal: 0.5 (–0.6, 1.5), p-value = 0.33

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, and 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	Adolescents and young adults' serum Comparison group: median = 1.38 (min = 0.36, max = 4.28) WTCHR group: median = 1.80 (min = 0.56, max = 5.03)	FEV1 FVC FEV1/FVC TLC RV FRC Resistance at an oscillation frequency of 5Hz, 5–20Hz, 20Hz	Regression coefficient per log-unit increase in PFOA	No statistically significant differences observed between exposure groups for the measured outcomes, p-value >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 years, N = 641	Cord blood, Median = 1.6 (1.2, 2.1)	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 PFOA	1.43 (1.03, 1.98), p-value = 0.033 1.25 (0.83, 1.89, (p-value = 0.276 1.08 (0.56, 2.07), p-value = 0.819
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 children followed up at ages 1.5, 4, and 7 years, N = 503 (4 years) N = 992 (7 years)	Maternal blood, Median = 2.35 (1.63, 3.30)	FEV1, FVC FEV1/FVC, FEF25–75%	Regression coefficient per log2-unit increase PFOA	FVC (4 years): –0.17 (– 0.34, –0.01) p-value not reported FEV1, FEV1/FVC, FEF25–75%: No

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							statistically significant associations
Qin et al., 2017, 3869265 Medium	Taiwan, 2009–2010	Case-control	Children with asthma and without asthma, aged 10–15, N = 132 (with asthma) N = 168 (without asthma)	Serum, Children with asthma: Median = 1.02 (0.48, 2.13) Children without asthma: Median = 0.50 (0.43, 0.69)	FEV1 FVC FEF25–75% PEF	Regression coefficient per ln-unit increase PFOA, or by quartiles	Children with asthma: FEV1: –0.10 (–0.19, –0.02), p-value <0.05 Quartile analysis: p-value for trend=0.002 FEF25–75%: –0.22 (–0.40, –0.05), p-value <0.05 Quartile analysis p-value for trend = 0.014 FVC, PEF: No statistically significant associations Children without asthma: No statistically significant associations for any outcomes
Confounding: age, sex, BMI, parental education level, exercise, environmental tobacco smoke exposure, and month of survey							
Steenland et al., 2015, 2851015 Low	United States, 2008–2011	Cohort	Adult workers and former workers at a chemical plant, N = 146	No lag cumulative exposure, 3.03–11.42 ug/mL-year 10-year lag cumulative exposure, 0.8–7.04 ug/mL-year	COPD no lag and 10-year lag	Rate ratio (RR) by quartiles	No lag: Q2: 1.2 (0.64, 2.27) Q3: 1.25 (0.65, 2.37) Q4: 1.13 (0.59, 2.17) 10-year lag: Q2: 0.75 (0.38, 1.48) Q3: 1.16 (0.6, 2.26) Q4: 0.77 (0.38, 1.57)
Results: Lowest quartile used as reference group Confounding: Gender, race, education, BMI, smoking, alcohol consumption							

- 1 FEF25–75% = Forced Expiratory Flow at 25–75%; FEV1 = Forced Expiratory Volume in 1s; FRC = Functional Residual Capacity; FVC = Forced Vital Capacity; PEF = Peak
2 Expiratory Flow rate; RV = Residual Volume; TLC = Total Lung Capacity; WTCHR = World Trade Center Health Registry; BMI = body mass index.

- 1 ^aExposure levels reported as median (25th–75th percentile) unless otherwise noted.
 2 ^bResults reported as effect estimate (95% confidence interval), unless otherwise noted.
 3 ^cConfounding indicates factors the models presented adjusted for.

4 C.12 Musculoskeletal

5 **Table C-21. Associations Between PFOA Exposure and Musculoskeletal Health 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 3.8 (2.9–4.9)	Area adjusted BMC (g), bone area (cm ²), BMC (g), BMD, cortical bone area (cm ²), cortical BMC (mg), cortical BMD (mg/cm ²), cortical thickness (mm), endosteal circumference (mm), height (cm), periosteal circumference (mm), total femoral neck BMD (g/cm ²), total hip BMD (g/cm ²), total lean mass (g)	Regression coefficient per unit increase in PFOA	Height: –0.6 (–1.06, –0.14) Bone area: –15.48 (–29.40, –1.55) No other statistically significant associations
Confounding: Maternal pre-pregnancy BMI, maternal education, maternal age at delivery, gestational age at sample collection, ever breastfed status at 15 months ^c							
Cluett et al., 2019, 5412438 Medium	United States, 1999–2010	Cross-sectional	Children from Project Viva, Ages 6–10,	Plasma Overall: 4.4 (IQR = 3.2)	Areal bone mineral density (aBMD) z-score, log2-unit	Regression coefficient per log2-unit	aBMD z-score –0.16 (–0.25, –0.06) Males: –0.11 (–0.23, 0.00)

			Overall N = 531 Male N = 296 Female N = 280		bone mineral content (BMC) z-score	increase in PFOA	Females: -0.24 (-0.4, -0.07) p-value for interaction by sex = 0.27 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 0.99 (IQR = 0.45)	BMD measured as broadband ultrasound attenuation (dB/MHz) and speed of sound (m/s), stiffness index (%)	Regression coefficient per unit increase in PFOA	BMD (broadband ultrasound attenuation) -0.08 (-24.2, 24) BMD (speed of sound) -31.2 (-64, 1.54) Stiffness index -8.79 (-28.1, 10.5)
Confounding: Age, sex, race							
Di Nisio et al., 2019, 5080655 Low	Italy 2017–2018	Cross-sectional	Male high school students N = 100 (50 controls, 50 exposed)	Serum Controls: 4.70 (3.5–6.6) Exposed: 7.35 (4.7–14.9) Semen Controls: 0.1 (0.08–0.11) Exposed: 0.24 (0.11–0.99)	Arm span (cm)	Mann-Whitney test (Exposed vs Controls)	Arm span Controls: 182.75 (178.0–185.8) Exposed: 179.00 (174.2–187.0) Adjusted p-value for comparison of medians = 0.738
Results: Values for each outcome are reported as median (25 th –75 th percentile).							
Confounding: None reported							
General Population							
Uhl et al., 2013, 1937226 Medium	United States, 2003–2008	Cross-sectional	Females from NHANES, Ages 20–84, N = 1,921 Ages 20–49 N = 1,104	Serum Females 20–84: Weighted mean = 4.22 Females 20–49: Weighted mean = 4.83	Osteoarthritis	OR per ln-unit increase in PFOA and by quartiles	Females ages 20–84 1.35 (1.02, 1.79), p-value <0.05 Q2: 1.44 (0.80, 2.62) Q3: 1.18 (0.67, 2.08) Q4: 1.98 (1.24, 3.19), p-value <0.01 Females ages 20–49 2.23 (0.81, 6.12)

			(All adults N = 3,809)				Q2: 2.71 (0.93, 7.91) Q3: 1.52 (0.36, 6.39) Q4: 4.95 (1.27, 19.4), p-value <0.05
							All adults ages 20–49 Q4: 3.76 (1.25, 11.4)
							No other statistically significant associations
			Results: Lowest quartiles 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, Females N = 842, Females in menopause N = 305	Serum GM = 3.96 (SD = 3.86)	Total BMD (g/cm ²) in hip or lumbar spine; fractures in hip, wrist, spine, or all types	OR per ln-unit increase in PFOA	All fracture types Males: 0.84 (0.67, 1.07) Females: 0.98 (0.75, 1.28) Females in menopause: 1.53 (0.63, 3.74) Other outcomes: no statistically significant associations
			Confounding: Age, race/ethnicity, BMI, smoking, drinking, treatment for osteoporosis, use of prednisone or cortisone daily				
Khalil et al., 2016, 3229485 Medium	United States, 2009–2010	Cross-sectional	Adults from NHANES, Ages 12–80, N = 958 females, 956 males	Serum Mean = 3.7 (SE = 0.18)	BMD (g/cm ²) of total femur, femoral neck, lumbar spine; Osteoporosis among females	BMD: Regression coefficient per ln-unit increase in PFOA and by quartiles Osteoporosis: OR per ln-unit increase in PFOA and by quartiles	Total femur Females: −0.017 (−0.038, 0.003) Q2: −0.02 (−0.04, −0.001), p-value <0.05 Q3: −0.002 (−0.038, 0.034) Q4: −0.03 (−0.063, 0.003) Males: Not statistically significant Femoral neck Females: −0.017 (−0.033, −0.001) No statistically significant associations by quartiles Males: Not statistically significant Osteoporosis: 1.84 (1.17, 2.90), p-value = 0.008 Q2: 1.25 (0.38, 4.06) Q3: 1.23 (0.37, 4.05)

						Q4: 2.59 (1.01, 6.67), p-value = 0.049	
						Lumbar spine: No statistically significant associations	
Results: Lowest quartile used as the reference group.							
Confounding: Age, ethnicity, BMI, serum cotinine, physical activity, milk consumption, blood lead concentration							
Hu et al., 2019, 6315798 Medium	United States, 2004–2007	Cohort and cross-sectional	Adults from the POUNDS-LOST study, Ages 30– 70, N = 294	Plasma	BMD and 2-yr	Regression	Spine BMD analyses
				Cross-sectional:	Δ BMD (g/cm ²)	coefficient per	Cross-sectional: –0.021 (–0.038,
				Mean = 5.2 (3.5–	of spine, total	SD increase in	–0.004)
				6.5)	hip, femoral	PFOA	2-yr Δ BMD: –0.002 (–0.007, 0.004)
				Cohort:	neck, hip		Total hip BMD analyses
				Mean = 5.4 (3.7–	trochanter, hip		Cross-sectional: –0.015 (–0.029,
				6.6)	intertrochanteric		–0.001)
					area, and Ward’s		2-yr Δ BMD: –0.004 (–0.008, 0.000)
					triangle area		
							Femoral neck BMD analyses
							Cross-sectional: –0.016 (–0.03,
							–0.002)
							2-yr Δ BMD: –0.001 (–0.007, 0.004)
							Hip trochanter BMD analyses
							Cross-sectional: –0.015 (–0.029,
							–0.002)
							2-yr Δ BMD: –0.003 (–0.007, 0.001)
							Hip intertrochanteric area BMD analyses
							Cross-sectional: –0.016 (–0.032, 0.000)
							2-yr Δ BMD: –0.006 (–0.011, –0.001), p-value <0.05
							Ward’s triangle area BMD analyses
							Cross-sectional: –0.015 (–0.033, 0.003)
							2-yr Δ BMD: –0.004 (–0.012, 0.005)

							No statistically significant associations or interactions by sex
Confounding: For cross-sectional, age, sex, race, alcohol consumption, physical activity, BMI, dietary intervention group; For cohort, age, sex, race, alcohol consumption, physical activity, BMI, dietary intervention group, baseline BMD, 2-yr weight change							
Occupational Populations							
Steenland et al., 2015, 2851015 Low	United States 2008–2011	Retrospective occupational cohort	DuPont plant workers from the C8 Health Project N = 3,713	Drinking water/serum Median = 113; Cumulative exposure, 25th–75th percentiles with or without 10-year lag: 0.8–7.04 or 3.03–11.42 µg/mL-year	Osteoarthritis	Incidence rate ratio by quartiles	Osteoarthritis no lag Q2: 0.88 (0.58, 1.34) Q3: 0.97 (0.71, 1.54) Q4: 0.97 (0.59, 1.59) p-trend logPFOA cumulative exposure = 0.92 p-trend via quartiles = 0.48 Osteoarthritis with lag Q2: 0.74 (0.49, 1.10) Q3: 0.56 (0.34, 0.93) Q4: 0.67 (0.39, 1.14) p-trend logPFOA cumulative exposure = 0.13 p-trend via quartiles = 0.15

Results: Lowest quartile used as the reference group.

Confounding: Gender, race, education, BMI, smoking, alcohol consumption

- 1 aBMD = areal bone mineral density; ALSPAC = Avon Longitudinal Study of Parents and Children; BMD = bone mineral density; BMI = body mass index; GM = geometric
 2 mean; IQR = interquartile range; NHANES = National Health and Nutrition Examination Survey; OR = odds ratio; POUNDS-LOST = Prevention of Obesity Using Novel Dietary
 3 Strategies Lost clinical trial; Q1 = quartile one; Q4 = quartile four; SD = standard deviation; SE = standard error; SES = socioeconomic status.
 4 ^aExposure levels reported as median (25th–75th percentile) unless otherwise specified.
 5 ^bResults reported as effect estimate (95% confidence interval) unless otherwise specified.
 6 ^cConfounding indicates factors the models presented adjusted for.

7 C.13 Gastrointestinal

8 **Table C-22. Associations Between PFOA Exposure and Gastrointestinal 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
Timmerman et al., 2020, 6833710	Guinea-Bissau 2012–2015	Cohort	Children aged <2 years previously	Serum 0.68 (0.53–0.92)	Diarrhea	OR per doubling of PFOA at	At inclusion: 1.09 (0.56, 2.09) At 9 months: 1.54 (0.72, 3.29)

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
Medium			enrolled in a RCT for measles vaccination N = 236 (113 girls, 123 boys)			inclusion or 9-month visit	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 1.68 (Range: 0.32–10.12)	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 PFOA exposure	Diarrhea Number of days with symptom T2: 1.07 (0.61, 1.89) T3: 1.08 (0.55, 2.13) Proportion of days under/above median T2: 1.10 (0.64, 1.89) T3: 0.94 (0.51, 1.74) Vomiting Number of days with symptom T2: 0.89 (0.61, 1.32) T3: 0.95 (0.62, 1.47) Proportion of days under/above median T2: 1.05 (0.62, 1.78) T3: 0.95 (0.52, 1.72)
Results: Lowest tertile used as reference. Confounding: Maternal age, maternal educational level, parity, and child age							
Hammer et al., 2019, 8776815 Low	Faroe Islands Enrollment: 1986–2009; follow-up until 2017	Cohort	Children and adults from CHEF N = 2,843	Blood Low exposure: GM = 0.95 (0.76–1.34) High exposure: GM = 4.42 (3.55–4.98)	Inflammatory bowel disease	Incidence rate ratio for highest vs. lowest tertile of PFOS exposure	0.60 (0.23, 1.56)
Confounding: Age, calendar period							

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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: 20 (11–29) Ronneby resampling: 16 (9–23) Karlshamn: 2 (1–2)	Inflammatory bowel disease (ln-ng/mL levels of calprotectin or zonulin)	Regression coefficient per unit increase in PFOA	Calprotectin Panel study: –0.006 (–0.03, 0.02) Resampling: –0.01 (–0.03, 0.005) Karlshamn: –0.15 (–0.84, 0.55) Zonulin Panel study: –0.002 (–0.02, 0.02) Resampling: –0.01 (–0.02, 0.01) Karlshamn: –0.29 (–0.85, 0.27)

Confounding: Age, BMI, gender

1 PFOA = perfluorooctanoic acid; 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.

5 C.14 Dental

6 **Table C-23. Associations Between PFOA Exposure and Dental 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
Ramesh et al., 2018, 5080517 Medium	United States 1999–2002	Cross-sectional	Adolescents from NHANES aged 12–19 years N = 2,869	Serum Median = 3.5 (2.3–4.9)	Dental caries	OR per log2-unit increase in PFOA and by quartiles	1.00 (0.91, 1.12) Q2: 0.95 (0.74, 1.20) Q3: 1.04 (0.82, 1.32) Q4: 0.95 (0.74, 1.21)
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 = 1.92 (95% CI: 1.74, 2.11)	Dental caries experience	OR per IQR increase in PFOA	1.33 (0.70, 2.53); p-value = 0.352

Reference, Confidence	Location, Years	Study Design	Population, Ages, N	Exposure Matrix, Levels (ng/mL) ^a	Outcome	Comparison	Results ^b
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							

PFOA = perfluorooctanoic acid; 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 PFOA 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 = 6.06 (3.97–9.12)	Visual impairment, synechia, macula disorder, corneal pannus, shallow anterior chamber, vitreous disorder, retinal disorder, lens opacity, conjunctival disorder, combined eye disease	OR per ln-unit increase in PFOA	Visual impairment 1.8 (1.37, 2.37); p-value <0.05 Eye disease, combined ≤65 years: 1.25 (1.01, 1.56); p-value <0.05 >65 years: 1.19 (0.71, 1.98) All other outcomes: No statistically significant associations
Confounding: Age, sex, BMI, education, income, career, exercise time, drinking, smoking ^c							

PFOA = perfluorooctanoic acid; 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.

1 C.16 Dermal

2 **Table C-25. Associations Between PFOA 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: 4.8 (2.7–8.2) Girls Sample 2: 4.1 (2.3–6.4) Boys Sample 1: 5.1 (2.8–8.3) Boys Sample 2: 4.3 (2.2–6.7)	Acne, age at occurrence (months)	Regression coefficient per log2-unit increase in PFOA, and by tertiles	Girls: –5.16 (–8.50, –1.82) T3: –6.09 (–12.10, –1.70) Boys: –1.06 (–3.62, 1.49); p-value = 0.58
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							

3 PFOA = perfluorooctanoic acid; DNBC = Danish National Birth Cohort.

4 ^aExposure levels reported as median (10th–90th percentile).

5 ^bResults reported as effect estimate (95% confidence interval).

6 ^cConfounding indicates factors the models presented adjusted for.

7 C.17 Cancer

8 **Table C-26. Associations Between PFOA Exposure and Cancer in Recent Epidemiologic Studies**

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL)	Outcome	Comparison	Select Results ^a
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 = 40.22	Prostate-specific antigen (PSA) level	Regression coefficient (β) and geometric mean ratio (GMR) (PSA < 4.0 ng/mL vs.	Age 20–49 β = 1, p-value = 0.9 GMR = 1.15 (0.67, 1.98) Age 50–69

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL)	Outcome	Comparison	Select Results ^a
						PSA \geq 4.0 ng/mL)	$\beta = 1$, p-value = 0.72 GMR = 0.96 (0.77, 1.2)
Confounding: Age, smoking status, average alcohol intake, and body mass index ^b							
Ghisari et al., 2017, 3860243 Medium	Denmark 1996–2002	Nested case-control	Adult women, 283	Serum Median = 4.87 (cases), 4.90 (controls)	Breast cancer	Relative risk ratio (RR) per ln-unit increase in PFOA, compared across genotypes: CYP1A1 (Ile462Val), CYP1B1 (Leu432Val), COMT (Val158Met), CYP17 (–34T > C), CYP19 (C > T)	Cohort RR = 1.17 (0.63, 2.17) CYP19 CC RR = 7.24 (1.00, 52), 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							
Wielsøe et al., 2017, 3858479 Medium	Greenland 2000–2003, 2011–2014	Case-control	Adult women, 158	Serum Median = 2.08 (cases), 1.48 (controls)	Breast cancer	OR by tertiles OR per unit increase in PFOA	T2: 1.86 (0.8, 4.31), p-value = 0.149 T3: 2.64 (1.17, 5.97), p-value = 0.019 Per increase: 1.26 (1.01, 1.58), p-value = 0.039
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 = 2.350 (cases), 2.475 (controls)	Breast cancer (invasive)	OR per log10-unit increase in PFOA, or by tertiles	T2: 0.901 (0.705, 1.152) T3: 0.925 (0.715, 1.197)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL)	Outcome	Comparison	Select Results ^a
							Per change: 0.733 (0.496, 1.081), p-value = 0.11
Results: Lowest tertile used as the reference group 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 1959–1967	Nested case-control	Adult daughters of women in CHDS cohort, 310 controls, 102 cases	Perinatal serum Median = 30.5 (cases), 0.4 (controls)	Breast cancer	OR per log2-unit increase in PFOA	“found no associations”; No results reported
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 = 6.64	Breast cancer	ORs by quartiles ORs by estrogen (ER) or progesterone receptor (PR) status	Overall: Q2: 1.69 (0.89, 3.21) Q3: 0.88 (0.43, 1.8) Q4: 0.92 (0.43, 1.98) p-trend = 0.43 ER negative: ORs of 3–7 p-trend = 0.59 PR negative: ORs of 1–4 p-trend = 0.90
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 1993–2014	Nested case-control	Adults, 55–74, 648 Ages 55–59, 190 Ages 60–65, 224	Serum Median = 5.5	Renal cell carcinoma	ORs per log2-unit increase in PFOA or by	Q2: 1.47 (0.77, 2.8) Q3: 1.24 (0.64, 2.41) Q4: 2.63 (1.33, 5.2)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL)	Outcome	Comparison	Select Results ^a
			Ages 65+, 234 Males 432 Females 216			quartiles (total cohort only)	p-trend = 0.007 Per unit increase: 1.71 (1.23, 2.37) 55–59: 2.1 (1.21, 3.34) 60–65: 1.6 (1, 2.45) 65+: 1.79 (1.21, 2.77) p-heterogeneity = 0.66 Males: 1.7 (1.31, 2.35) Females: 1.79 (1.1, 2.95) p-heterogeneity = 0.87
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+, 1,032	Serum Median = 23.7 ng/g	Cancer mortality	Hazard ratio per SD unit increase in PFOA	0.94 (0.8, 1.11), p-value = 0.45
Confounding: Age, gender, race/ethnicity, and smoking status							
Steenland et al., 2015, 2851015 Low	United States 2008–2011	Retrospective occupational cohort	Adult workers, 3,713	Drinking water/occupational, serum Median = 113; Cumulative exposure, 25th–75th percentiles with or without 10-year lag: 0.8–7.04 or 3.03–11.42 µg/mL-year	Cancers with and without a 10-year lag: bladder, colorectal, melanoma, prostate	Incidence rate ratio by quartiles	Bladder cancer no lag: Q2: 0.32 (0.08, 1.33) Q3: 0.95 (0.28, 3.14) Q4: 0.23 (0.05, 0.93)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL)	Outcome	Comparison	Select Results ^a
							<p>p-trend logPFOA cumulative exposure = 0.04 p-trend via quartiles = 0.19</p> <p>Bladder cancer with lag: Q2: 0.55 (0.12, 2.61) Q3: 0.47 (0.1, 2.21) Q4: 0.31 (0.06, 1.54) p-trend logPFOA cumulative exposure = 0.06 p-trend via quartiles = 0.03</p> <p>Colorectal, melanoma and prostate cancers report p-trends of 0.10 or greater</p>
<p>Results: Lowest quartile used as the reference group Confounding: Gender, race, education, BMI, smoking, alcohol consumption</p>							
Christensen et al., 2016, 3858533 Low	Wisconsin, US, 2012–2013	Cross-sectional	Male anglers, 50+, 154	Serum Median = 2.50	Cancer (any)	OR per unit increase in PFOA	1.5 (1.08, 2.17)
Confounding: Age, BMI, work status, alcohol consumption							
Girardi & Merler, 2019, 6315730 Low	Italy 1960–2018	Occupational Retrospective Cohort	Male workers, 154	Occupational, serum Geometric mean by tertiles = 1,700; 13,051; and 81,934 ng/mL-years	Mortality: Liver cancer, liver cancer or cirrhosis, lung cancer, malignant neoplasm, malignant neoplasms of lymphatic and	Mortality risk ratio (RR) by tertiles for PFAS plant workers vs. nearby metal factory workers	Malignant neoplasms of lymphatic and haematopoietic tissues RR T1: 1.44 (0.18, 11.8)

Reference, Confidence	Location, Years	Design	Population, Ages, N	Exposure Method, Levels (ng/mL)	Outcome	Comparison	Select Results ^a
					haematopoietic tissues	Standardized mortality ratio in each cumulative PFOA tertile	RR T2: 1.8 (0.22, 14.6) RR T3: 5.06 (1.61, 16) p-trend < 0.001 Any malignant neoplasm p-trend = 0.008 All other mortalities not significant
Confounding: Age at risk, calendar period							
Lin et al., 2020, 6835434 Low	China 2014–2017	Case-control	Children, <16, 84	Serum Median = 13.89	Germ cell tumors	OR per unit increase in PFOA	1.03 (0.99, 1.08)
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 = 2.15	Breast cancer	OR per ln-unit increase in PFOA	Total cohort: 1.14 (0.66, 1.96) Age 50 or younger: 0.78 (0.4, 1.51) Age over 50: 0.89 (0.59, 1.34)
Confounding: Pregnancy history, oral contraception use, abortion, BMI, menopause, and education level							

1 OR = odds ratio

2 ^aResults reported as effect estimate (95% confidence interval), unless otherwise noted.3 ^bConfounding indicates factors the models presented adjusted for.

Appendix D. Detailed Toxicokinetics

D.1 Absorption

D.1.1 Cellular Uptake

Several studies using cell lines transfected with specific transporters or vector controls support cellular accumulation of PFOA through facilitated transport. Several transporters classically considered specific to renal or enterohepatic resorption have also been found to be expressed in tissues relevant to absorption. Specifically, OAT2 transcripts have been identified in several tissues in addition to kidney including the small intestine {Cropp, 2008, 9641964}. OATP1A2 expression has also been identified in intestine {Kullak-Ublick, 1995, 9641965}.

A single study in immortalized intestinal Caco-2 cells found that uptake was fast and saturable, supporting a carrier-mediated uptake process. The K_m for PFOA uptake was calculated to be $8.3 \pm 1.2 \mu\text{M}$ and uptake clearance (V_{\max}/K_m) was $55.0 \mu\text{L}/\text{mg protein}/\text{min}$. Uptake was found to be independent of sodium ions, while concentration, temperature, and pH all influenced uptake. Substrates or inhibitors of OATPs significantly decreased the uptake of PFOA, suggesting that the uptake of PFOA from the apical membranes of Caco-2 cells was at least partially mediated by OATPs (Kimura et al., 2017, 3981330).

Lipid binding may influence PFOA accumulation in various cell types relevant to absorption as well as distribution. Sanchez-Garcia et al. (2018, 4234856) compared PFOA and PFOS in their ability to accumulate and be retained in cells including lung epithelial cells (NCI-H292). Cellular accumulation and retention of PFOS was observed in lung cells at higher levels compared to azithromycin-dihydrate, 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 pH7.4 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 pH7.4. 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 PFOA 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	$313 \pm 101^*$	26 ± 4	$39 \pm 3^*$	$2.33 \pm 0.11^*$	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 PFOA into cells. This is consistent with observations that cells transfected with vector only could take up PFOA, albeit at lower levels than cells transfected with PFOA-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 PFOA 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 $\log(K_{\text{mem/w}}/[LW/\text{kgmem}])$ for PFOA was 3.93, similar to the experimentally determined value of 3.52 ± 0.08 . $K_{\text{mem/w}}$ values increase with increasing chain length, reflecting increased surface area for van der Waals interactions. The authors observed that perfluoroalkanesulfonic acids (PFSA) 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 PFOA ranged from -6.89 to -7.45 , 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

Based on animal data, PFOA is well absorbed following oral exposure. Studies on male rats administered PFOA by gavage using a single dose (11.4 mg/kg, CD rats) or daily doses over 28 days (5 or 20 mg/kg/day, Sprague-Dawley rats) all estimated dose absorption of at least 92.3% {Gibson, 1979, 9641813; Cui et al., 2010, 2919335}.

Toxicokinetic parameters informing absorption were derived by comparing oral to IV dosing in two studies conducted in rats (Kim et al., 2016, 3749289; Dzierlenga et al., 2019, 5916078). In the study by Kim and colleagues, rapid differences in absorption based on sex were observed for PFOA but not PFOS (Kim et al., 2016, 3749289). Male and female Sprague-Dawley rats were administered 1 mg/kg by either the IV or oral route. Urine and feces were collected daily for both males and females, and blood was collected at 11 time points on the first day (females) or 3 time points on the first day and then up to 12 days after exposure (males). The time to reach the maximum PFOA plasma concentration (T_{max}) following oral exposure in females was 1.44 hours versus 2.07 days in males. Dzierlenga et al. (2019, 5916078) administered a single bolus IV (6 mg/kg) or gavage dose (6, 12, or 48 mg/kg) to adult male Sprague Dawley rats and a single bolus IV (40 mg/kg) or gavage dose (40, 80, or 320 mg/kg) to adult female Sprague Dawley rats. Blood and urine were collected for up to 8 time points during the first 24 hours and then up to 12 (females) or 50 (males) days post-dosing. T_{max} in rats administered these doses via gavage ranged from 2.33 to 3.22 hours in females and 4.86 to 8.33 hours in males for PFOA. In females, C_{max} per dose (mM/mmol/kg) decreased with increasing dose suggesting saturation of absorption kinetics at higher doses. Similar to the Kim et al. study (2016, 3749289), shorter T_{max} values were observed in females compared with males at all doses.

The data from studies of adverse effects on monkeys, rats, and mice receiving PFOA in capsules, food, or drinking water demonstrate gastrointestinal absorption. In cynomolgus monkeys, steady-state serum and urine PFOA levels were reached 4–6 weeks after dosing with capsules containing 3, 10, or 20 mg/kg PFOA for 6 months (Butenhoff et al. 2004b, 3749227). Serum

PFOA concentrations in male Crl:CD BR rats fed diets containing 0.06, 0.64, 1.94, or 6.5 mg PFOA/kg for 90 days were 7.1, 41, 70, and 138 µg/mL, respectively (Perkins et al. 2004, 1291118). Peak blood levels of PFOA were attained 1–2 hours following a 25 mg/kg dose to male and female rats {Kennedy, 2004, 724950}. Studies on same-sex rats found no differentiation in blood or plasma levels of PFOA when comparing single and repeated daily PFOA dose administrations {Kennedy, 2004, 724950; Elcombe, 2010, 2850034}.

In rats, marked sex differences in serum and tissue PFOA levels exist following PFOA administration. Males consistently have much higher levels than females with differences maintained and becoming more pronounced over time. Female rats show much greater urinary excretion of PFOA than do male rats with serum half-life values in hours for females compared with days for males. These differences account for variability in postexposure serum PFOA concentrations between males and females.

D.1.3 Inhalation Exposure

Data on exposure to PFOA by inhalation remains unchanged since Hinderliter et al. (2006, 135732) measured the serum concentrations of PFOA following single and repeated nose-only aerosol inhalation exposures of 0, 1, 10, or 25 mg/m³ PFOA in Sprague-Dawley rats, which found that PFOA plasma concentrations increased proportional to aerosol exposure concentrations. The male plasma C_{max} values were approximately 2–3 times higher than the female plasma C_{max} values. The female C_{max} occurred approximately 1 hour after the exposure period with plasma concentrations then declining. In males, C_{max} was observed immediately after the exposure period ended and persisted for up to 6 hours. These data demonstrate absorption of PFOA via inhalation and provide evidence of the sex differences consistent with rate of excretion.

D.1.4 Dermal Exposure

Evidence that PFOA is absorbed following dermal exposure remains unchanged since 2005, with in vitro percutaneous absorption studies of PFOA through rat and human skin allowing calculation of permeability coefficients for PFOA in rat skin to be 3.25×10^{-5} cm/hr, and that of human skin to be 9.49×10^{-7} cm/hr (Fasano et al., 2005, 3749187). Previously, O'Malley and Ebbens (1981, 4471529) utilized mortality as an indicator of dermal uptake across groups of two male and two female New Zealand white rabbits receiving 0, 100, 1,000, or 2,000 mg/kg PFOA; after 14 daily dermal doses, all of the animals died at the highest dose, 3 of 4 animals died in the mid-dose group, and no animals died in the low-dose group. Kennedy (1985, 3797585) detected elevated blood organofluorine levels in male New Zealand white albino rabbits and male and female Crl:CD rats that were dermally treated with a total of 10 applications of PFOA at doses of 0, 20, 200, or 2,000 mg/kg. Treatment resulted in elevated blood organofluorine levels that increased in a dose-related manner.

D.1.5 Developmental Exposure

The literature contains no studies on PFOA absorption following developmental exposure. Additional information on PFOA distribution during reproduction and development is found in Section D.2.4.

D.1.6 Bioavailability

The Kim and Dzierlenga studies discussed above also observed very high bioavailability in rats (Table D-2) (Kim et al., 2016, 3749289; Dzierlenga et al., 2019, 5916078). At a lower dose of 1 mg/kg, Kim et al. (2016, 3749289) found that C_{max} values after oral administration were 85% and 92% of values obtained after IV administration (bioavailability values were not reported in this study). In the Dzierlenga et al. (2019, 5916078) study, bioavailability (calculated by dividing the dose-adjusted gavage area under the curve [AUC] by the IV AUC) was 140% in males administered 6 mg/kg and 182% in females administered 40 mg/kg. The authors suggested that the high bioavailability of PFOA may be attributed to increased reabsorption by intestinal transporters by the oral route.

Table D-2. PFOA Parameters from Toxicokinetic Studies Informing Bioavailability in Sprague-Dawley Rats

Study	Dose (mg/kg)	Route	Sex	C_{max} (µg/mL)	T_{max} (hours) ^a
Kim et al., 2016, 3749289	1	Oral	Male	7.55 ± 0.51	49.68 ± 5.04
		IV	Male	8.92 ± 2.34	NA
	1	Oral	Female	5.41 ± 0.38	1.44 ± 0.096
		IV	Female	5.84 ± 0.38	NA
Dzierlenga et al., 2019, 5916078	6	Oral	Male	36.85 ± 2.90	4.86 ± 0.81
		IV	Male	52.59 ± 2.5	NA
	40	Oral	Female	240.16 ± 24.84	3.22 ± 0.32
		IV	Female	369.76 ± 81.16	NA

C_{max} = maximum serum concentration; T_{max} = time to C_{max} ; IV = intravenous; NA = not applicable.

^aConverted published T_{max} (days) to T_{max} (hours) for Kim et al. 2016 (3749289).

Li (2015, 2851033) examined bioavailability from food sources in female BALB/c mice and using in vitro methods. In mice, PFOA was mixed with foods of different nutritional compositions (e.g., meat, seafood, milk, and fruits/vegetables) and fed to mice over a 7-day period. By comparing PFOA administration via food mixtures to administration in water, relative bioavailability was assessed by measuring accumulation in liver. PFOA bioavailability relative to water ranged from 4.30 ± 0.80 to $69.0 \pm 11.9\%$ and was negatively correlated with lipid content ($r = 0.76$). The authors suggest that sorption by free fatty acids in foods could limit PFOA access to intestinal transporters. Another possibility is cations in the gastrointestinal tract, such as Ca^{2+} and Mg^{2+} , can complex PFOA promoting partitioning to the lipid phase. Three different in vitro methods were used to measure bioavailability using these food mixtures including the in vitro digestion method (IVD) (James et al., 2011, 6718854), the unified BARGE method (UBM) (Smith et al., 2012, 6702349), and the physiologically based extraction test (PBET) (Tilston et al., 2011, 5120687). Instead of soil, 0.3 g of food was used at sample/solution ratios of 1:97.5 for UBM, 1:100 for PBET, and 1:150 for IVD. PFOA bioaccessibility varied by the method (8.7–73% for UBM, 9.8–99% for PBET, and 21–114% for IVD). As observed in the in vivo study, bioaccessibility was negatively correlated with lipid content for the UBM method ($r = 0.82$) but not for other in vitro methods ($r = 0.11$ – 0.22). The authors suggest that the UBM method can be used to model bioaccessibility, possibly because this method is associated with higher lipolysis and better mimics cations in gastrointestinal fluid of UBM. This may lower the potential to form stable micelles using this method compared to PBET and IVD methods.

Together, these findings suggest PFOA bioavailability is strongly influenced by diet, with high fat diets associated with reduced absorption, and that an important factor influencing PFOA bioaccessibility is colloidal stability in intestinal solutions.

D.2 Distribution

D.2.1 Protein Binding

Kerstner-Wood et al. (2003, 4771364) used in vitro methods to evaluate PFOA binding to protein in plasma from humans, cynomolgus monkeys, and rats. In all species, plasma proteins were able to bind 97–100% of the PFOA added at concentrations ranging from 1 to 500 ppm. In humans, serum albumin carried the largest portion of PFOA among the protein components of human plasma. Serum albumin is a common carrier of hydrophobic materials in the blood (Fasano et al. 2005) and approximately 60% of the serum protein in humans and rats is albumin {Harkness, 1983, 9641985} {Saladin, 2004, 9642161}.

Han et al. (2003, 5081471) investigated the binding of PFOA to rat and human plasma proteins in vitro and determined that the primary PFOA binding protein in plasma was serum albumin. No significant differences in binding to the serum albumin were found between humans and rats. Calculation of disassociation constants (K_d) for PFOA, conducted using purified rodent and human serum albumin binding using labeled ^{19}F nuclear magnetic resonance (NMR) and micro-size exclusion chromatography and the estimated number of binding sites from this study are presented in Table D-3.

Table D-3. Dissociation Constants of Binding Between PFOA and Albumin

Parameter	Method	Rat Serum Albumin	Human Serum Albumin
K_d (mM)	NMR ^a	0.29 ± 0.10^b	ND
K_d (mM)	micro-SEC ^c	0.36 ± 0.08^b	0.38 ± 0.04
Number of Binding Sites	micro-SEC ^c	7.8 ± 1.5	7.2 ± 1.3

K_d = dissociation constant; NMR = nuclear magnetic resonance; ND = not determined; micro-SEC = micro-size exclusion chromatography.

^aAverage of the two K_d values (0.31 ± 0.15 and 0.27 ± 0.05 mM) obtained by NMR.

^bOn the basis of the result of unpaired t-test at 95% confidence interval, the difference of K_d values determined by NMR and micro-SEC is statistically insignificant.

^cValues were obtained from three independent experiments and their standard deviations are shown.

Several studies have examined the interactions between PFOA and human serum albumin {Wu, 2009, 536376; MacManus-Spencer et al., 2010, 2850334; Qin et al., 2010, 3858631; Salvalaglio et al., 2010, 2919252; Weiss et al., 2009, 534503; Kerstner-Wood et al., 2003, 4771364; Luebker et al., 2002, 1291067; L. Zhang et al., 2013, 5081488; Cheng and Ng, 2018, 5024207; Gao et al., 2019, 5387135; Yue et al., 2016, 3479514}. Wu et al. (2009, 536376) examined whether PFOA, after absorption, was transported bound to albumin by dialyzing PFOA solutions in the presence and absence of human serum albumin. The authors found that, in the absence of albumin, 98% of the dissolved PFOA crossed the dialysis membrane into the dialysate within 4 hours. In the presence of albumin, the amount of PFOA found in the dialysate decreased in direct proportion to the albumin concentration, demonstrating binding to the protein. No albumin was identified in the dialysate. Circular dichroism measurements of the albumin/PFOA complex suggested a

conformational change in the protein as a result of the PFOA binding. These conformational changes could interfere with the functional properties of serum albumin or other serum proteins impacted by surface monolayers of PFOA. For example, albumin's ability to transport its natural ligands could be decreased by the presence of PFOA on the protein surface {Wu, 2009, 536376}.

MacManus-Spencer et al. (2010, 2850334) used a variety of approaches to quantify the binding of PFOA to serum albumin (e.g., surface tension measurements, ¹⁹F NMR spectroscopy, fluorescence spectroscopy) using bovine serum albumin. Taken together, the results from these analyses suggested the presence of primary and secondary binding sites on albumin. The results of the fluorescence spectroscopy suggested a conformational change in albumin following binding of PFOA that moved tryptophan residue 214 from a slightly polar region of the protein to a less polar region. Qin et al. (2010, 3858631) also used fluorescence spectroscopy quenching analysis to study PFOA binding to bovine serum albumin and reported that albumin underwent a conformational change following the binding of PFOA. They also suggested that van der Waals forces and hydrogen bonds were the dominant intermolecular binding forces. Similar findings were observed more recently (Chen et al., 2020, 6324256) for human serum albumin. This study used infrared spectroscopy to examine PFOA-mediated effects on albumin secondary structure and found that PFOA binding led to a decrease in the β -sheet and α -helix conformations.

Salvalaglio et al. (2010, 2919252) conducted a modeling study to determine the binding sites of PFOA on human serum albumin and classify them by their interaction energy using molecular modeling. They estimated a maximum number of nine PFOA binding sites on human serum albumin and determined that these site locations were common to the natural binding sites for fatty acids, thyroxine (T4), Warfarin, indole, and benzodiazepine. The binding site closest to tryptophan residue 214 had the highest binding affinity.

Beesoon and Martin (2015, 2850292) examined differences in the binding of the linear and branched chain PFOA isomers to calf serum albumin and human serum proteins. The linear PFOA isomer bound more strongly to calf serum albumin than the branched chain isomers. When arranged in order of increasing binding, the order was 4m < 3m < 5m < 6m (iso) < linear. In the isomer-specific binding to spiked total human serum protein, the linear molecule clearly had the strongest binding potential with about 7–10% free. The relationship for the other isomers was 5m > 6m > 4m > 3m (15–30% free).

Weiss et al. (2009, 534503) screened PFOA and 29 other perfluorinated compounds—differing by carbon chain length (C4–18), fluorination degree, and functional groups—for potential binding to the serum thyroid hormone transport protein, transthyretin (TTR), using a radioligand-binding assay. The natural ligand of TTR is T4. Human TTR was incubated overnight with ¹²⁵I-labeled T4, unlabeled T4 (reference), and 10–10,000 nmol PFOA as a competitor for the T4 binding sites. The authors concluded that the binding affinity for TTR was highest for the fully fluorinated compounds tested and those having at least a carbon chain length of 8, characteristics that apply to PFOA. PFOA demonstrated a high binding affinity for TTR with 949 nmol, causing a 50% inhibition of T4 binding to TTR.

Binding to albumin and other serum proteins may affect transfer of PFOA from maternal blood to the fetus. Gao et al. (2019, 5387135) correlated placental transfer with experimentally measured K_d to human serum binding proteins, serum albumin, and L-FABP. For PFOA, K_{ds} were calculated to be $115 \pm 16 \mu\text{M}$ for albumin, $166 \pm 10 \mu\text{M}$ for serum binding proteins, and

197 ± 13 µM for L-FABP. These K_{ds} 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. Since there is effectively a competition between PFOA 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% per 1 g/L albumin). However, maternal serum albumin concentration was associated with reduced transfer efficiency (decrease of 2.5% 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.

In contrast to serum proteins, little is known regarding PFOA binding to proteins in the gut. Yue et al. (2016, 3479514) examined whether PFOA that enters the digestive tract binds to gastric enzymes, specifically pepsin. Binding to pepsin was examined using fluorescence quenching of pepsin's intrinsic fluorescent properties. Scatchard analysis was used to estimate a binding constant of 0.717×10^4 at 298 K. Spectroscopy including ultraviolet-visible absorption, Fourier transform infrared fluorescence, and circular dichroism indicated that PFOA induces a conformation change in pepsin associated with decreased α -helical and β -sheet content. Molecular docking analysis suggested that PFOA interacts with 16 amino acid residues of pepsin. It is unclear whether PFOA-pepsin interactions impact absorption or distribution from the gut to other compartments in the body.

PFAS also binds 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 concentrations (IC_{50} s) for PFOA and PFOS were 9.0 ± 0.7 and 3.3 ± 0.1 µmol, respectively, suggesting that PFOA has a lower binding affinity than PFOS. PFOA was bound to the carrier protein in a 1:1 ratio, and the interaction was mediated by electrostatic interactions and hydrogen binding with the fatty acid binding site. Using size-exclusion column coelution and nontarget analysis to identify additional PFAS ligands from contaminated environmental sources, Yang et al. (2020, 6356370) also found that both polar and hydrophobic interactions are crucial for binding affinities to L-FABP for PFOA and PFOS.

A computational modeling approach that combined molecular docking and molecular dynamics simulation techniques was used to estimate the relative binding of affinity of PFOS for human and rat L-FABP (Cheng and Ng, 2018, 5024207). The authors found that predicted free energies

correlated well with binding affinities measured in 3 previous studies (Woodcroft et al., 2010, 2919284; Zhang et al., 2013, 5081488; Sheng et al., 2018, 4199441). Key residues contributing to free binding energies (ΔG_{bind}) for L-FABP include ARG 122, SER 124, SER 39, and ILE 52 (human) and ARG 122, TYR 55, and ILE 60 (rat).

D.2.2 Subcellular Distribution

Han et al. (2005, 5081570) examined the subcellular distribution of PFOA in the liver and kidney of male and female rats. Male and female Sprague-Dawley CrI:CD (SD)IGS BR rats were gavage-dosed with 25 mg/kg [^{14}C] PFOA and necropsied 2 hours after dosing. Blood was collected and the liver and kidneys were removed. Five subcellular fractions (nuclei and cell debris, lysosome and mitochondria, microsome, light microsome and ribosome, and membrane-free cytosol) were obtained by differential centrifugation. In the male liver, the highest proportion of total reactive residues (TRR) of PFOA was located in the nuclei and cell debris (40%), followed by membrane-free cytosol (26% TRR), lysosome and mitochondria (~14% TRR), microsome (~16% TRR), and light microsome and ribosome (~1% TRR). In the female liver, the highest proportion of TRR of PFOA was found in the membrane-free cytosol (48%), followed by nuclei and cell debris (~31% TRR), lysosome and mitochondria (~12% TRR), microsome (~8% TRR), and light microsome and ribosome (~1% TRR). Based on the results, the authors concluded that subcellular distribution of PFOA in the rat liver was sex-dependent because the proportion of PFOA in the liver cytosol of female rats was almost twice that of the male rats. They hypothesized that females might have a greater amount than males of an unknown liver cytosolic binding protein with an affinity for perfluorinated acids. They also hypothesized that the unknown protein or protein complex might normally aid in transport of fatty acids from the liver. In the kidney, the subcellular distribution did not show the sex difference seen with the liver; however, the protein-bound fraction for the males (42%) was about twice that for the females (17%).

Zhang et al. (2020, 6316915) examined the subcellular distribution of PFOA in human colorectal cancer cells (DLD-1), human lung epithelial cells (A549), and human normal liver cells (L-02). Cells were incubated with 100 or 300 μM PFOA for 48 hours and mitochondria, nucleus, and cytosol were isolated and examined for PFOA levels. Accumulation in these subcellular compartments corresponded to exposure levels with the highest amounts accumulating in cytosol followed by nuclei and mitochondria. Cytosolic accumulation was more than 100 times greater than accumulation in the other analyzed subcellular compartments. The PFOA concentration in cytosol was highest for liver cells and was comparable between colorectal cancer and lung epithelial cells. The patterns of accumulation (cytosol > nuclei > mitochondria) were also comparable.

D.2.3 Tissue Distribution

D.2.3.1 Human Studies

D.2.3.1.1 Distribution in Blood Fractions

Human blood is a major site of PFOA 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 PFOA concentration was 4.8 ng/mL in adults and 3.0 ng/mL in children (Kotlarz et al., 2020, 6833715). This value was similar to the estimate of 3.8 ng/mL predicted using a pharmacokinetic model based on drinking water containing 15 ng/L PFOA and using the average length of residence of 20 years for the participants.

PFOA accumulation in blood impacts distribution to various tissues and organs, but few studies have examined PFOA partitioning to human blood fractions. Forsthuber et al., (2020, 6311640) measured the distribution of PFOA 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 PFOA and that there was no affinity for lipoproteins. The concentration of PFOA in these fractions was below the LOD.

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 from C6 (mean 0.24) to C11 (0.87). The PFOA plasma:whole blood ratio in the Jin et al. (2016, 3859825) study was lower (1.2 ± 0.43) compared to the mean plasma:whole blood (2.0–2.1) (Ehresman et al., 2007, 1429928) and serum:whole blood (1.4–2.2) (Karmen et al., 2006, 2159543 and Hanssen et al., 2013, 3859848) ratios previously reported. In blood samples obtained from three highly exposed Canadian subjects, the highest levels of PFOA were measured in plasma (0.27 ng/mL) compared to red blood cells (RBCs, 0.13 ng/mL) and washed RBCs (0.12 ng/mL). The authors suggest that these values could be used as more accurate conversion factors to convert 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 et al., 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 PFOA concentrations in plasma, serum, and whole blood were 1.90, 1.60 and 0.93 ng/mL, respectively. Similar to other studies, PFOA 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 will not provide accurate estimates for PFOA.

In another study (De Toni et al., 2020, 6316907) in which blood from healthy low-exposed donors was exposed to PFOA, platelets were identified as the preferential site of PFOA accumulation. The concentrations observed among blood cell components were below the LOQ in erythrocytes, 6.2 ± 0.4 pg/ 10^6 cells in leukocytes, and 243.9 ± 122.6 pg/ 10^6 cells in platelets. The authors also incubated platelets with Merocyanine 540, a fluorescent dye that has been used as a marker of membrane fluidity. Fluorescence intensity increased in a dose-dependent manner up to, but not beyond, 400ng/mL. The authors suggest these findings support an association between PFOA accumulation and increased membrane fluidity.

D.2.3.1.2 Distribution in Tissues

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

Pirali et al. (2009, 757881) measured intrathyroidal PFOA levels (0.4–6.0 ng/g) in thyroid surgical patients and found no correlation between serum and thyroid PFOA concentrations. PFOA has been detected in breast milk samples (Tao et al. 2008, 1290895; Völkel et al. 2008, 3103448), cord blood samples (Apelberg et al. 2007, 1290833; Monroy et al. 2008, 2349575), and follicular fluid samples (Kang et al., 2020, 6356899) at concentrations above the LOQ. These studies indicate that PFOA is distributed within the body, including reproductive tissues.

PFOA concentrations above the LOQ were detected in 5 of 6 postmortem liver samples from males in Catalonia, Spain. In females, only 1 of 6 liver samples was above LOQ of 0.77 ng/g (Kärman et al. 2009). 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. PFOA was present in 45% of the samples but could be quantified in only 20% (median 1.9 ng/g). PFOA accumulated primarily in the bone (60.2 ng/g), lung (29.2 ng/g), liver (13.6 ng/g), and kidney (2.0 ng/g), with levels below LOD (2.4 ng/g) in the brain.

Two studies examined accumulation of PFOA in cerebrospinal fluid (CSF) and serum (Fujii et al., 2015, 2816710; Wang et al., 2018, 5080654). In both studies, PFOA levels in CSF were two orders of magnitude lower than in the serum. These results indicate that PFOA does not easily cross the adult BBB.

D.2.3.2 Animal Studies

Studies of tissue distributions are available for several species including non-human primates, rats, and mice. Experiments in non-human primates provide evidence of serum and liver accumulation of PFOA. While only a few studies exist, they document distribution with repeated measurements over long periods of time and include recovery time after exposure termination. Murine studies demonstrate that PFOA primarily distributes to serum, liver, lungs, and kidney; however, several of these studies also measure PFOA in different organs and tissues. These include the central nervous system, cardiovascular, gastrointestinal, renal, reproductive, endocrine, and musculoskeletal systems. Recent studies have also indicated that a moderate amount of PFOA enters bone and even crosses the barriers into the central nervous system. Adipose tissue was observed as a site that contained very little amounts of accumulation.

These data are characterized based on dosing (low, medium, and high), time exposed (acute vs. chronic), sex differences between males and females, as well as toxicokinetics, where data is applicable. Ranges of dose regimens indicate changes in deposition patterns as animals are exposed to increased concentrations of PFOA, indicating possible changes in excretion through bile and urine. Several studies corroborate to show that there are sex-specific deposition patterns, primarily that male animals accumulate more PFOA in serum and some tissues including liver. Overall, these studies provide a wide range of deposition data that when taken together can illustrate short- and long-term accumulation of PFOA.

D.2.3.2.1 Non-Human Primates

One of the few studies in cynomolgus monkey that measured distribution of PFOA was performed by Butenhoff et al. (2002, 1276161; 2004b, 3749227). The study followed four to six male monkeys that received PFOA (0, 3, 10, or 20 mg/kg) daily via oral capsule. Serum, urine, and fecal samples were collected at 2-week intervals and liver samples were collected at necropsy. Steady-state concentrations of PFOA in serum were 77 ± 39 , 86 ± 33 , and 158 ± 100 $\mu\text{g/ml}$ after 6 weeks and 81 ± 40 , 99 ± 50 , and 156 ± 103 $\mu\text{g/ml}$ after 6 months for the 3-, 10-, and 20-mg/kg dose groups, respectively (Butenhoff et al. 2002, 1276161; 2004b, 3749227). The mean serum concentration of PFOA in control monkeys was 0.134–0.203 $\mu\text{g/ml}$. Urine PFOA concentrations reached steady state after 4 weeks and were 53 ± 25 , 166 ± 83 , and 181 ± 100 $\mu\text{g/ml}$ in the 3, 10, and 20-mg/kg dose groups, respectively, for the duration of the study. Liver PFOA concentrations at necropsy in the 3-mg/kg and 10-mg/kg dose groups were similar and ranged from 6.29–21.9 $\mu\text{g/g}$, while concentrations in two monkeys exposed to 20 mg/kg were 16.0 and 83.3 $\mu\text{g/g}$. Liver PFOA concentrations in two monkeys dosed with 10 mg/kg/day at the end of a 13-week recovery period were 0.08 and 0.15 $\mu\text{g/g}$ (Butenhoff et al., 2004b, 3749227).

D.2.3.2.2 Rats

Numerous studies have been performed on PFOA distribution in rats. These studies range from acute (hours) to chronic (2 years) and include various levels of dosing. Previous studies have indicated that humans and rats have similar serum albumin binding, suggesting circulation of PFOA in the body would be similar {Harkness, 1983, 9641985}{Saladin, 2004, 9642161}.

In adult male Sprague-Dawley rats, animals were exposed by gavage to PFOA (20 mg/kg/day) for 1, 3, or 5 days (Martin et al., 2007, 758419). While serum data was only presented for 3-day exposure animals, it is clear that serum levels had a moderate accumulation of 245 ± 41 $\mu\text{g/mL}$. Additionally, liver concentrations were 92 ± 6 , 250 ± 32 , and 243 ± 23 $\mu\text{g/g}$ after 1, 3, and 5 daily doses, respectively. Liver accumulation appeared to reach its peak by day 3 and remained steady at this level through day 5. While limited serum levels were presented, data indicates that at day 3, serum and liver levels were in a 1:1 ratio.

Several studies indicate that the major target organs of PFOA accumulation are liver, kidneys, and lungs with a large concentration remaining in blood serum. In an earlier study of PFOA, Ylinen et al. (1990, 5085631) administered male and female Wistar rats doses of 3, 10, and 30 mg/kg/day PFOA via gavage for 28 days. At necropsy, serum, brain, liver, kidney, lung, spleen, ovary, testis, and adipose tissue were collected (Table D-4). At 3 mg/kg/day, male rats exhibited the highest concentration of PFOA in serum followed by, liver, kidneys and then lungs with notable accumulation in testis. Interestingly, measurements of PFOA from adipose tissue resulted in no detectable levels at any dose or timepoint. In higher doses of 10 and 30 mg/kg/day, male mice had a significant increase in PFOA present in kidneys. The levels of PFOA in male rat serum were generally lower in the 30 mg/kg/day dose group than in the 10 mg/kg/day dose group, presumably due to increased urinary elimination in the 30 mg/kg/day group. The tissue levels were similar for the 10 and 30 mg/kg/day doses. Interestingly, female rats exhibited only 5, 14, and 27% of PFOA in serum when compared to male concentrations at 3, 10, and 30 mg/kg/day doses, respectively. These low levels of absorption were also seen in solid tissue as liver and kidney measurements were ~10 and 30% of levels detected in males, respectively. In females, there was a dose-related increase in tissue levels and serum. Concentrations of PFOA in tissue at the low dose was highest in serum followed by liver, lungs, then spleen. At the higher

doses of 10 and 30 mg/kg/day, the highest PFOA levels were in serum and kidney, a pattern also observed in male mice.

Table D-4. Tissue Distribution of PFOA in Wistar Rats After Exposure via Gavage for 28 Days

Tissue ^a	Males			Females		
	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Serum (µg/mL)	48.60 ± 10.30	87.27 ± 20.09	51.65 ± 11.47	2.40 ^b	12.47 ± 4.07	13.92 ± 6.06
Liver (µg/g)	39.90 ± 7.25	51.71 ± 11.18	49.77 ± 10.76	1.81 ± 0.49	3.45 ± 1.36	6.64 ± 2.64
Kidney (µg/g)	1.55 ± 0.71	40.56 ± 14.94	39.81 ± 17.67	0.06 ± 0.02	7.36 ± 3.19	12.54 ± 8.24
Spleen (µg/g)	4.75 ± 1.66	7.59 ± 3.50	4.10 ± 1.57	0.15 ± 0.04	0.38 ± 0.17	1.59 ± 0.49
Lung (µg/g)	2.95 ± 0.54	22.58 ± 4.59	23.71 ± 5.42	0.24 ^b	0.22 ± 0.15	0.75 ± 0.26
Brain (µg/g)	0.398 ± 0.144	1.464 ± 0.211	0.710 ± 0.320	< LOQ ^c	0.029 ± 0.019	0.044 ± 0.018
Ovary (µg/g)	–	–	–	< LOQ	0.41 ± 0.27	1.16 ± 0.58
Testis (µg/g)	6.24 ± 2.04	9.35 ± 4.02	7.22 ± 3.17	–	–	–

LOQ = limit of quantification.

^aData are presented as mean ± standard deviation (n = 6).

^bData are presented as the mean (n = 3).

^cLOQ = 1 µg/mL.

In the Ylinen study (1990, 5085631), concentrations of PFOA in brains were an order of magnitude higher in males than females (Table D-4). Kawabata et al. (2017, 3858489) also measured PFOA in brains of male Wistar rats 9 days after administration of a single dose of 50 mg/kg. Serum PFOA was 33.3 µg/mL and liver concentrations were 58.7 µg/g. However, levels in brain were below LOQ. Although levels are low and detection is variable, these studies do support PFOA accumulation to low levels in brains of adult rats.

PFOA distribution followed a similar pattern in Sprague-Dawley rats administered a single ¹⁴C-PFOA dose via oral gavage to male (Table D-5) and female (Table D-6) rats (Kemper et al., 2003, 6302380). Tissues from male rats were collected at 10.5 hours (T_{max}) and 171 hours (T_{max/2}) (time to return to 50% maximum plasma concentration) after dosing. Tissues from female rats were collected at 1.25 hours (T_{max}) and 4 hours (T_{max/2}) after dosing. Liver, blood, skin, muscle, bone, gastrointestinal tract, and adipose were the primary tissues for distribution of ¹⁴C-PFOA. In males, the fraction of dose found in the liver increased between T_{max} and T_{max/2} but remained constant or decreased in other tissues. In females, the fraction of the dose present in all tissues remained constant or decreased between T_{max} and T_{max/2}. Liver:blood ratios for ¹⁴C-PFOA at T_{max} in males were approximately 1:1 but increased between T_{max} and T_{max/2}. In females, the liver:blood ratio was ~1.2:1 at the low dose but increased to ~1.5 at higher doses. In males, the blood distributions levels were tenfold or higher than kidney levels at T_{max} and declined slightly at T_{max/2}. In the female tissues at T_{max/2}, ~30% of the dosed PFOA retained was present in the liver, blood, kidney, muscle, and skin tissues in decreasing amounts. This study confirmed sex-specific differences in PFOA distribution and identified accumulation in reproductive tissues including testis and ovaries.

Table D-5. Distribution of PFOA in Male Sprague-Dawley Rats After a Single Oral Gavage Dose^a

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	% at T _{max}	% at T _{max/2}	% at T _{max}	% at T _{max/2}	% at T _{max}	% at T _{max/2}
Prostate	0.083 ± 0.039	0.030 ± 0.002	0.071 ± 0.045	0.057 ± 0.020	0.067 ± 0.018	0.028 ± 0.012
Skin ^b	14.772 ± 2.135	6.061 ± 0.274	15.565 ± 0.899	7.233 ± 0.430	13.836 ± 0.969	5.419 ± 0.237
Blood ^b	22.148 ± 0.692	8.232 ± 1.218	24.919 ± 1.942	11.140 ± 0.624	22.905 ± 1.177	7.904 ± 1.032
Brain	0.071 ± 0.018	0.022 ± 0.002	0.051 ± 0.021	0.023 ± 0.008	0.063 ± 0.007	0.019 ± 0.002
Fat ^b	2.281 ± 0.467	0.593 ± 0.136	2.815 ± 0.225	0.916 ± 0.205	2.153 ± 0.430	0.628 ± 0.110
Heart	0.451 ± 0.119	0.195 ± 0.024	0.443 ± 0.037	0.252 ± 0.030	0.461 ± 0.053	0.164 ± 0.032
Lungs	0.740 ± 0.147	0.341 ± 0.043	0.593 ± 0.376	0.344 ± 0.194	0.863 ± 0.103	0.303 ± 0.057
Spleen	0.086 ± 0.011	0.045 ± 0.006	0.096 ± 0.017	0.060 ± 0.007	0.106 ± 0.015	0.042 ± 0.005
Liver	21.708 ± 5.627	32.627 ± 3.601	18.750 ± 2.434	25.231 ± 1.289	17.528 ± 0.900	20.145 ± 3.098
Kidney	1.949 ± 0.402	1.140 ± 0.215	2.170 ± 0.354	1.212 ± 0.115	2.293 ± 0.286	1.003 ± 0.122
G.I. tract	2.930 ± 0.929	0.980 ± 0.300	2.508 ± 0.713	1.052 ± 0.202	2.784 ± 0.608	0.808 ± 0.189
G.I. contents	2.083 ± 0.625	0.239 ± 0.025	2.632 ± 0.934	0.270 ± 0.028	4.186 ± 1.349	0.210 ± 0.084
Thyroid	0.008 ± 0.005	0.004 ± 0.003	0.011 ± 0.006	0.004 ± 0.002	0.009 ± 0.002	0.005 ± 0.001
Thymus	0.085 ± 0.008	0.051 ± 0.018	0.085 ± 0.012	0.053 ± 0.003	0.120 ± 0.025	0.045 ± 0.010
Testes	0.755 ± 0.079	0.356 ± 0.037	0.693 ± 0.180	0.372 ± 0.062	0.623 ± 0.098	0.224 ± 0.031
Adrenals	0.019 ± 0.004	0.010 ± 0.001	0.022 ± 0.004	0.009 ± 0.001	0.026 ± 0.004	0.009 ± 0.003
Muscle ^b	12.025 ± 0.648	4.984 ± 0.745	13.565 ± 0.576	6.429 ± 0.648	12.855 ± 0.841	4.253 ± 0.358
Bone ^b	3.273 ± 0.538	1.120 ± 0.094	3.047 ± 0.544	1.375 ± 0.169	3.062 ± 0.438	0.906 ± 0.100
Total ^c	85.465 ± 6.426	57.026 ± 3.379	88.033 ± 1.420	56.031 ± 1.025	83.937 ± 3.680	42.112 ± 4.740

G.I. = gastrointestinal; T_{max} = time to reach maximum plasma concentration; T_{max/2} = time to return to 50% maximum plasma concentration.^aData are presented as mean percent of dose ± standard deviation recovered at T_{max} and T_{max/2} in tissues.^bPercent recovery scaled to whole animal assuming the following: skin=19%, whole blood=7.4%, fat=7%, muscle=40.4%, bone=7.3% of body weight.^cTotals are calculated from individual animal data.

Table D-6. Distribution of PFOA in Female Sprague-Dawley Rats After a Single Oral Gavage Dose^a

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	% at T _{max}	% at T _{max/2}	% at T _{max}	% at T _{max/2}	% at T _{max}	% at T _{max/2}
Skin ^b	0.434 ± 0.162	0.403 ± 0.096	0.624 ± 0.142	0.307 ± 0.121	0.380 ± 0.166	0.415 ± 0.175
Blood ^b	5.740 ± 1.507	4.438 ± 1.625	8.089 ± 2.080	5.411 ± 1.466	7.158 ± 2.232	6.407 ± 1.406
Brain	0.037 ± 0.009	0.047 ± 0.008	0.066 ± 0.019	0.045 ± 0.010	0.058 ± 0.008	0.058 ± 0.018
Fat ^b	0.134 ± 0.032	0.164 ± 0.079	0.220 ± 0.111	0.110 ± 0.069	0.147 ± 0.053	0.148 ± 0.065
Heart	0.198 ± 0.079	0.253 ± 0.055	0.388 ± 0.057	0.236 ± 0.051	0.317 ± 0.035	0.287 ± 0.069
Lungs	0.454 ± 0.148	0.546 ± 0.082	0.827 ± 0.102	0.570 ± 0.179	0.678 ± 0.067	0.775 ± 0.204
Spleen	0.063 ± 0.027	0.058 ± 0.006	0.101 ± 0.021	0.060 ± 0.012	0.091 ± 0.007	0.070 ± 0.002
Liver	7.060 ± 1.266	6.817 ± 1.537	11.190 ± 2.192	7.176 ± 0.982	10.538 ± 1.723	9.080 ± 0.895
Kidney	3.288 ± 0.948	2.769 ± 0.784	4.293 ± 0.771	2.685 ± 0.736	5.867 ± 0.946	4.749 ± 0.393
G.I. tract	10.699 ± 9.066	8.462 ± 6.519	7.142 ± 2.594	8.255 ± 8.967	6.923 ± 1.846	3.547 ± 1.306
G.I. contents	21.956 ± 13.48	3.891 ± 2.395	2.896 ± 2.305	5.601 ± 6.165	2.491 ± 1.548	1.121 ± 1.010
Thyroid	0.010 ± 0.003	0.016 ± 0.021	0.008 ± 0.002	0.006 ± 0.002	0.009 ± 0.003	0.007 ± 0.002
Thymus	0.052 ± 0.017	0.058 ± 0.024	0.105 ± 0.030	0.068 ± 0.021	0.091 ± 0.032	0.077 ± 0.020
Ovaries	0.047 ± 0.019	0.048 ± 0.006	0.071 ± 0.012	0.041 ± 0.012	0.071 ± 0.012	0.070 ± 0.012
Adrenals	0.014 ± 0.005	0.018 ± 0.004	0.026 ± 0.005	0.015 ± 0.004	0.031 ± 0.005	0.021 ± 0.001
Muscle ^b	0.170 ± 0.051	0.258 ± 0.089	0.325 ± 0.010	0.229 ± 0.031	0.441 ± 0.116	0.304 ± 0.099
Uterus	0.243 ± 0.091	0.374 ± 0.247	0.354 ± 0.046	0.247 ± 0.068	0.358 ± 0.124	0.365 ± 0.029
Bone ^b	0.101 ± 0.017	0.153 ± 0.052	0.174 ± 0.057	0.142 ± 0.078	0.157 ± 0.072	0.181 ± 0.090
Total ^c	50.698 ± 16.485	28.772 ± 10.976	36.897 ± 3.187	31.201 ± 12.63	35.803 ± 2.554	27.680 ± 2.569

G.I. = gastrointestinal; T_{max} = time to reach maximum plasma concentration; T_{max/2} = time to return to 50% maximum plasma concentration.^aData are presented as mean percent of dose ± standard deviation recovered at T_{max} and T_{max/2} in tissues.^bPercent recovery scaled to whole animal assuming the following: skin = 19%, whole blood = 7.4%, fat = 7%, muscle = 40.4%, bone = 7.3% of body weight.^cTotals are calculated from individual animal data.

Sex dependent dose distribution similar to results found in Ylinen et al. (1990, 5085631) have also been found in several other reports (Kemper et al., 2003, 6302380, Lau et al., 2006, 1276159). According to Kemper et al., T_{max} plasma concentration occurred in 1/10th the time and at much lower levels in females when compared to males. Lau et al. (2006, 1276159) dosed male and female Sprague-Dawley rats with 10 mg/kg for 20 days and necropsied them 24 hours after the last dose. Male rats had serum PFOA levels of 111 µg/mL compared to 0.69 µg/mL in female rats, a sex ratio that was in line with the Kemper et al. results.

Kemper et al. (2003, 6302380) observed levels of PFOA accumulation in the kidneys of females that were consistently elevated compared to males, indicating that excretion of PFOA may play a role. Furthermore, at $T_{max/2}$ there was ~1% of ¹⁴C-PFOA in the gastrointestinal tissues and contents in males compared to ~14% in females. Based on the timing of the measurements and the results, females appear to absorb and excrete PFOA more rapidly than males, however, the samples were collected at 1.25 and 4 hours in females and 10.5 and 171 hours in males, providing more time for absorption in the males (Kemper et al., 2003, 6302380). This study also confirmed accumulation in reproductive organs (testes) and detected PFOA accumulation in endocrine (thyroid, adrenals) and immune (thymus) tissues.

Two NTP studies exemplify sex-specific patterns of PFOA accumulation in blood and liver. PFOA levels were measured in the context of both a 28-day toxicity study (NTP, 2019, 5400977) and a two-year chronic toxicity study (NTP, 2020, 7330145). In the 28-day study (NTP, 2019, 5400977), male and female Sprague-Dawley rats were administered 0 to 10 mg/kg/day (males) or 0 to 100 mg/kg/day (females) of PFOA by oral gavage. Although females were administered a 10-fold higher dose of PFOA, males exhibited higher plasma concentrations than females across all dose groups. The plasma concentrations in males were 50.7 ± 2.2 and 148.6 ± 15.4 µg/mL at the lowest and highest dose groups respectively. In females, plasma concentrations were $0.4905 \pm 0.072.1$ and 23.444 ± 3.247 µg/mL at the lowest highest dose groups respectively. When normalized to dose administered (µM/mmol/kg), males had a 1,000-fold higher level than females at the lowest dose and a 63-fold higher level at the highest dose. Males exhibited a decreasing normalized plasma concentration with dose, whereas females exhibited an increasing normalized plasma concentration with dose. PFOA in liver was only measured in males, and the liver:plasma ratios were fairly consistent across dose groups, ranging from 0.87 to 1.17.

In the two-year study (NTP, 2020, 7330145), Sprague-Dawley rats were exposed to 0, 150, or 300 ppm PFOA during the perinatal periods. During the postweaning period, F₁ male rats were provided 0, 150, or 300 ppm and F₁ female rats were provided 0, 300, or 1,000 ppm PFOA via feed. Plasma and liver PFOA levels were measured at the 16-week interval. Plasma and liver PFOA concentrations in males were within 10% of each other regardless of whether animals were also dosed during the perinatal period. Plasma concentrations in females showed a similar pattern to the males (e.g., minor differences between perinatal exposures and liver patterns). Although exposures in females were 2–3 times higher than in males, PFOA plasma concentrations were much lower compared to males. For example, at the highest dose in rats exposed during both perinatal and postweaning periods, plasma concentrations were 223.4 ± 8.4 µg/mL in males compared to 70.2 ± 6.9 µg/mL in females. The liver:plasma ratios were again fairly consistent across dose groups, ranging from 0.73 to 0.88 in males and from 0.81 to 0.99 in females.

In a repeated inhalation exposure study, Hinderliter et al. (2006, 135732) exposed male and female rats to 0, 1, 10, or 25 mg/m³ aerosol concentrations of PFOA for 6 hours/day, 5 days/week for 3 weeks. Blood was collected immediately before and after the daily exposure period 3 days/week. The aerosols had mass median aerodynamic diameters of 1.3–1.9 µm with geometric standard deviations (GSDs) of 1.5–2.1. PFOA plasma concentrations were proportional to the inhalation exposure concentrations, and repeated exposures produced little plasma carryover in females, but significant day-to-day carryover in males. By 3 weeks, males reached steady-state plasma levels of 8, 21, and 36 µg/mL for the 1, 10, and 25 mg/m³ groups, respectively. In females, the postexposure plasma levels were 1, 2, and 4 µg/mL for the 1, 10, and 25 mg/m³ groups, respectively. When measured immediately before the next daily exposure, plasma levels had returned to baseline in females, demonstrating clearance within 24 hours of each daily dose.

D.2.3.2.3 Mice

Measurements of serum PFOA concentrations in mice have differed from results in rat studies. Lau and colleagues (2006, 1276159) dosed male and female CD-1 mice with 20 mg/kg/day of PFOA for 7 or 17 days and analyzed serum levels. After 7 days, male mice had serum PFOA levels of 181 µg/mL and females had levels of 178 µg/mL. After 17 days of treatment, male mice had serum PFOA levels of 199 µg/mL and females had levels of 171 µg/mL (Lau et al., 2006, 1276159). Additionally, in a separate experiment performed by Lou et al., female CD-1 mice were dosed with 20 mg/kg/day for 17 days (Lou et al., 2009, 2919359). Serum samples were collected 24 hours after the final dose and analyzed for PFOA. The mean serum concentration was 130 ± 23 mg/L, which is comparable to the reported value of 171 µg/mL reported above by Lau et al. (2006, 1276159). These data suggest that the sex difference observed by Lau et al. (2006, 1276159) in rats was not seen in the mice under the conditions of this study.

Lou et al. (2009, 2919359) measured pharmacokinetics of PFOA in mice administered single doses of 1 and 10 mg/kg to groups of male and female CD-1 mice. Plasma, liver, and kidney tissues were collected at multiple early time points (4, 8, 12, and 24 hours) as well as a dozen time points between 3 and 80 days. In female mice, peak serum concentrations were measured at 10 and 100 mg/L and declined to 2 mg/L and <0.2 mg/L after 80 days for the 1 and 10 mg/kg/day doses, respectively. Peak serum concentrations were slightly lower in the males at ~8 and 80 mg/L, but final serum concentrations were higher in the males at ~0.5 and 8 mg/L, respectively. Liver and kidney concentrations also were higher in males than in females for each of the two doses. These data suggest a longer half-life in males than in females. Additionally, this group dosed 60 mg/kg to female mice and measured serum levels over the course of 28 days. Based on their findings, these mice were able to clear a higher dose of PFOA much more quickly than animals who had received a 1 or 10 mg/kg dose (Lou et al., 2009, 2919359). The 60 mg/kg dose animals were able to return to a 0.4 mg/L serum concentration in about 28 days while the 10 mg/kg and 1 mg/kg groups took 61 days and 70 days to reach 1 mg/L, respectively.

Several studies of short-term distribution of PFOA in mice have been published that vary between 4 hours and 28 days and demonstrate the range of PFOA tissue distribution. One of the earliest of these time points was performed by Burkemper et al., (2017, 3858622) who used a radioisotope injection (¹⁸F-PFOA) and measured deposition in 14 different tissues as well as serum 4 hours later. Despite the observation that radiolabel was associated with ~29% of serum

protein, the majority of signal was found in the bone (femur), liver, and lungs. The next highest levels of radioisotope detection were in the heart, spleen, large intestines, and then kidneys. These findings were consistent with recent work by Bogdanska et al. (2020, 6315801). Using a ¹⁴C-PFOA radioisotope, authors measured low (0.06 mg/kg/day) and high dose (22 mg/kg/day) PFOA delivered via feed to C57Bl/6 mice and collected measurements at 1, 3 and 5 days postexposure (Table D-7). Similar to previous finding of the Burkemper paper, liver accumulation was consistently 4-5 times greater than what was found in serum at all doses and time points. Lung deposition was also found to be at elevated levels and was measured at nearly half serum concentrations at all doses and time points. In a study by Li et al. (2017, 4238518) conducted in BALB/c mice after a 28-day exposure, PFOA concentrations in both liver and serum increased with PFOA dose in mice, with PFOA concentrations being generally higher in the liver than the serum.

Table D-7. Distribution of PFOA in Male C57BL/6 Mice Following Exposure to 14C-PFOA for 1, 3, or 5 days in Feed^a

Tissue	0.06 mg/kg/day			22 mg/kg/day		
	Dose Duration			Dose Duration		
	1 Day	3 Days	5 Days	1 Day	3 Days	5 Days
Blood	0.328	1.222	1.645	90	183	192
Liver	1.59	5.229	7.507	281	671	756
Lung	0.179	0.606	0.873	40	96	110
Kidney	0.16	0.556	0.783	42	91	104
Pancreas	0.087	0.258	0.344	22	51	61
Thyroid gland	0.082	0.294	0.421	24	48	57
Skin	0.096	0.337	0.501	25	47	52
Stomach	0.125	0.259	0.345	14	45	48
Thymus	0.089	0.197	0.237	16	34	47
Inguinal fat pad	0.064	0.209	0.273	15	37	40
Whole bone	0.105	0.282	0.452	20	30	40
Small intestine	0.057	0.174	0.269	10	37	36
Large intestine	0.05	0.166	0.204	10	32	32
Testis	0.054	0.156	0.235	12	28	29
Epididymal fat	0.053	0.152	0.153	12	23	24
Muscle	0.032	0.116	0.169	9	19	20
Brain	0.008	0.029	0.024	2	3	4
Spleen	0.022	< LOD	< LOD	< LOD	5	1
Heart	< LOD	< LOD	< LOD	14	15	< LOD

LOD = limit of detection.

^aData are presented as mean (nmol/g).

Interestingly, while Burkemper et al., (2017, 3858622) measured equal levels of kidney and large intestine depositions at very early time points (4 hours), Bogdanska et al. (2020, 6315801)

registered a far greater amount of PFOA in the kidneys at the slightly later time points of 1, 3, and 5 days. This may indicate a change in excretion methods over the course of exposure and/or reflect differential distribution or detection of ^{18}F -PFOA relative to ^{14}C -PFOA. Burkemper et al. also measured a large uptake of ^{18}F -PFOA in mouse femurs at 4 hours, while Bogdanska et al. found moderately low levels at later time points. This difference could be due to rapid fluorine intake of the bone by potential ^{18}F radioisotope artifacts.

Bogdanska et al. (2020, 6315801) also observed accumulation of PFOA in testes of C57BL/6 mice at levels similar to those observed in epididymal fat and in intestines. In BALB/c mice exposed to PFOA (0.31 to 20 mg/kg/d) for 28 days, PFOA levels in the testes increased with increasing dose (Zhang 2014a, 2850230). Further evidence of distribution to reproductive tissues in male mice comes from the finding that PFOA accumulated in the epididymis of BALB/c mice in a dose-dependent manner (Lu et al., 2016, 3981459).

Accumulation in both small intestine and the colon was observed in CD-1 mice administered between 1 and 20 mg/kg/day for 10 days (Rashid et al., 2020, 6833711). Higher levels of PFOA were measured in the small intestine relative to colon. The mean concentration of PFOA in small intestine detected was 1.0, 2.3, 4.4, and 6.5 $\mu\text{g/g}$ in the 1, 5, 10, and 20 mg/kg/day groups, respectively. Dose-dependent accumulation was also seen in the colon, where mean concentrations ranged from 211.12 to 1,834.27 ng/g in colon tissue.

Fujii et al. (2015, 2816710) performed IV injections of 0.313 $\mu\text{mol/kg}$ of PFOA on male and female animals and collected serum and organ samples after 24 hours. Distribution was calculated as percentage of total recovered dose from serum and organs. The majority of administered PFOA was retained in the serum and liver of mice and less than 2% of administered dose was found in kidney and adipose tissue. While a relatively small amount of PFOA was measured in the brain (0.1%), it is noteworthy that PFOA can cross the BBB in healthy animals. Similar findings were observed in both the Burkemper et al., (2017, 3858622) and Bogdanska et al. (2020, 6315801) studies. Levels in female mouse livers were ~30% of the levels measured in male samples. A larger portion of PFOA was not recovered from serum, organ, and excretions of female mice, indicating that there may be further distribution in organs that were not examined in this study. Fujii and colleagues (2015, 2816710) examined distribution based on chain length. They observed that perfluoroalkyl carboxylic acids (PFCAs) with shorter chain length (C6 and C7) were excreted rapidly through urine, while longer chains ($\geq\text{C8}$) accumulated in the liver. Moreover, PFCA with longer chain lengths were found to exhibit increasing affinity for serum and liver fatty acid binding proteins. The authors suggest that lipophilicity driven by chain length may account for the distribution patterns of PFCA, which is consistent with the high levels of PFOA accumulation in serum and liver. These large sequestration volumes of PFOA observed in the liver seem to be attributable to the liver's large binding capacity in mice.

Studies that examined PFOA distribution for longer time periods also reveal that the liver is a primary site of PFOA accumulation. Adult BALB/c mice exposed to PFOA (0.4, 2, and 10 mg/kg/day) via oral gavage for 28 days exhibited dose dependent increase in both serum and liver (Guo et al., 2019, 5080372). At every dose tested, liver:serum ratios appeared to stay near 2:1. Additionally, it was found that the liver consistently absorbed 10% of the total PFOA each animal was exposed to. In a study with the same 28-day exposure and similar low dose (1.25 mg/kg/day via oral gavage), Zheng and colleagues found that PFOA distributed in the liver and serum in an ~2.5:1 ratio (Zheng et al., 2017, 4238507). These findings further corroborate the

previous radioisotope studies that PFOA accumulates primarily within the liver and secondarily in serum.

One potential method of removal of PFOA from liver is through activation of PPAR α . In human and rodent hepatocytes, PPAR α activation induces expression of genes involved in lipid metabolism and cholesterol homeostasis. PFOS and PFOA structurally resemble fatty acids and are well-established ligands of PPAR α in the rat and mouse liver. As PPAR α agonists, PFOS and PFOA can induce the β -oxidation of fatty acids, induce fatty acid transport across the mitochondrial membrane, decrease hepatic very low-density lipoprotein-triglyceride and apolipoprotein B (apoB) production, and promote lipolysis of triglyceride-rich plasma lipoproteins (Fragki et al., 2021, 8442211). In an experiment using male wild-type 129S4/SvImJ mice and PPAR α -null 129S4/SvJae-Pparatm1Gonz/J mice, animals were orally administered 0, 12.5, 25, and 50 μ mol/kg/day PFOA (~0, 5.4, 10.8, and 21.6 mg/kg/day PFOA, respectively) for four weeks (Minata et al., 2010, 1937251). Blood, liver, and bile were collected for determination of PFOA concentration at the end of 4 weeks (Table D-8). The PFOA concentration in whole blood and the liver were similar between wild-type and PPAR α -null mice and increased in proportion to dose. In bile, PFOA concentration in wild-type mice increased by a factor of 13.8 from 12.5 to 25 μ mol/kg and by a factor of 2.8 from 25 to 50 μ mol/kg; however, in bile of PPAR α -null mice, PFOA concentration increased by a factor of only 3.2 from 12.5 to 25 μ mol/kg and by a factor of 6.1 from 25 to 50 μ mol/kg. The liver can transport PFOA from hepatocytes to bile ducts that is mediated at least partly by PPAR α . The lower PFOA levels in bile of PPAR α null mice suggest a role for PPAR α in PFOA clearance in the liver (Minata et al., 2010, 1937251).

Table D-8. PFOA Concentrations in Wild-type and PPAR α -null Male Mice Exposed to PFOA by Gavage for Four Weeks^a

Dose (μ mol/kg)	Whole Blood		Bile		Liver	
	Wild-type	PPAR α -null	Wild-type	PPAR α -null	Wild-type	PPAR α -null
0	ND	ND	ND	ND	ND	ND
12.5	20.6 \pm 2.4 ^a	19.3 \pm 2.2	56.8 \pm 26.9	19.6 \pm 2.2	181.2 \pm 6.3	172.3 \pm 8.9
25	46.9 \pm 3.2	36.4 \pm 2.7	784 \pm 137.6	62.9 \pm 16.7	198.8 \pm 15.4	218.3 \pm 14.5
50	64.2 \pm 6.5	71.2 \pm 8.0	2174 \pm 322.4	383 \pm 109.9	211.6 \pm 13.3	239.7 \pm 25.0

Wild-type = 129S4/SvImJ mice; PPAR α -null = peroxisome proliferator-activated receptor alpha-null 129S4/SvJae-Pparatm1Gonz/J mice; ND = not detected.

^aData are presented as mean \pm standard deviation (μ g/mL).

D.2.3.3 Tissue Transporters

As described earlier, protein transporters from a number of families play a role in the tissue uptake of orally ingested PFOA. The transporters are located at the interface between serum and a variety of tissues (e.g., liver, kidneys, lungs, heart, brain, testes, ovaries, placenta, and uterus) {Klaassen, 2010, 9641804}. The liver is an important uptake site for PFOA. OATPs and MRPs, at least one OAT, and the sodium-taurocholate cotransporting polypeptide (NTCP)—a hepatic bile uptake transporter—have been identified at the boundary of the liver at the portal blood and/or the canalicular membranes within the liver {Kim, 2003, 9641809} {Kusuhara, 2009, 9641810} {Zaïr, 2007, 9641805}.

Transporters responsible for PFOA transport across the placenta are not well understood. Kumm 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 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, reduced folate carrier 1 (*RFC-1*), equilibrative nucleoside transporter (*ENT1*), folate receptor alpha (*FRα*), heme carrier protein 1, serotonin transporter (*SERT*), p-glycoprotein (*MDR1*), multi-drug resistance-associated protein 2 (*MRP2*), and breast cancer resistance protein (*BCRP*). *MDR1* expression levels were significantly associated with TTEs of branched PFOS and iso-PFOS, (3+4+5)m-PFOS, but not linear PFOS or PFOA. *MRP2* expression was associated with total PFOS, linear PFOS, branched PFOS, and iso-PFOS, (3+4+5)m-PFOS, but not PFOA. *BCRP* expression levels did not significantly change with PFOA or PFOS. Interestingly, the pattern of expression of *MDR1*, *MRP2* and *BCRP* were only observed in full-term placentas. Preterm placentas showed significant expression levels of *ENT1*, *FRα*, and *SERT* and were associated with 1m-PFOS and iso-PFOS. Thus, the expression of transporters and TTEs appear to differ between preterm and full-term placentas. Authors noted that the three transporters that were significantly associated with PFOS (*MDR1*, *MRP2*, and *BCRP*) are also ATP-binding cassette (ABC) transporters, which play a protective role for the placenta tissue and the fetus by effluxing xenobiotics across the placental barrier thereby reducing exposure to PFOS. It is unclear why there were no correlations with PFOA although this may be related to the fact that gene expression associations with TTE were not confirmed using protein expression data of the candidate genes.

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

D.2.4 Distribution during Reproduction and Development

The availability of distribution data from pregnant females plus animal pups and neonates is a strength of the PFOA pharmacokinetic database because it helps to identify those tissues receiving the highest concentration of PFOA 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.2.4.1 Human Studies

T. Zhang et al. (2013, 3859792) recruited 32 pregnant females (21–39 years) from Tianjin, China, for a study to examine the distribution of PFOA between maternal blood, cord blood, the placenta, and amniotic fluid. Samples were collected at time of delivery (35–37 weeks). The study yielded 31 maternal whole blood samples, 30 cord blood samples, 29 amniotic fluid samples, and 29 placentas. PFOA was found in all fluids/tissues sampled. Maternal blood contained variable levels of 10 PFAS: 8 acids and 2 sulfonates. 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. PFOA was transferred to the amniotic fluid to a greater extent than PFOS based on their relative proportions in the maternal blood and cord blood. Compared with mean PFOA blood levels in the pregnant females, mean levels of PFOA in the cord blood, placenta, and amniotic fluid were 47%, 59%, and 1.3%, respectively, of those in the mother's blood. The correlation coefficients between the maternal PFOA blood levels and placenta, cord blood, and amniotic fluid levels (0.7–0.9) were statistically significant ($p < 0.001$).

D.2.4.1.1 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 R_{PM} . Chen et al. (2017a, 3859806) examined distribution of PFAS chemicals and their isomers 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. PFOA isomers examined included nPFOA (linear PFOA), iso-PFOA, 5m-PFOA, 4m-PFOA, tb-PFOA, and 3m-PFOA; however, all were below the level of detection in maternal blood and placentas except linear PFOA and 3m-PFOA. Linear PFOA contributed approximately 74% of total placenta PFOA, and 73% of maternal serum PFOA. Branched PFOA (3m-PFOA) contributed approximately 10% of the total PFOA in placenta and 3% of total PFOA in maternal serum (Table D-9). Notably, the increased proportion of linear isomers was also observed in other PFAS chemicals including PFOS and PFHxS. Similar findings have been reported in Cai et al. (2020, 6318671) and Li et al. (2020, 6505874).

Despite the reduced proportion of branched PFOA within each biological compartment, the proportion of maternal branched PFOA that accumulated in the placenta was significantly higher than the proportion of linear PFOA. The ratio of placental:maternal concentrations (R_{PM}) for 3m-PFOA was greater than that for linear PFOA (Table D-9), suggesting that 3m-PFOA is transferred more efficiently than linear PFOA.

T. Zhang et al. (2013a, 3859792) recruited 32 female subjects (mean age of 30.9 years) from a hospital in Tianjin, China, who reported full-term pregnancies (average gestation period of 40.3 weeks). The authors reported an average of 1.58 ng/g of PFOA in the placenta and 3.35 ng/mL in maternal serum (Table D-9). The R_{PM} for total PFOA was approximately 0.47, which is higher than the proportion of total PFOA reported by Chen et al. (2013). For PFOA levels in maternal serum, Zhang et al. (2013a, 3859792) reported significantly higher levels which may have contributed to the increased PFOA accumulation. The fact that participants in the T. Zhang et al. (2013a, 3859792) study were further along in gestation than participants in the Chen et al. (2017, 3859806) study may have contributed to their higher maternal PFOA levels.

Table D-9. PFOA Concentrations in Human Placenta, Maternal Blood, and Transplacental Transfer Ratios (RPM)

Study	Gestational Age (weeks) ^a	Isomer	Placenta (ng/g)	Maternal Serum (ng/mL)	Placenta: Maternal Serum RPM ^b
Chen et al., 2017, 3859806	38.9 ± 1.6	Total PFOA	0.484 ± 0.202	1.560 ± 0.611	0.326
		Linear PFOA	0.359	1.150	0.328
		3m-PFOA	0.049	0.060	0.460
Zhang et al., 2013, 3859792	1.58 ± 0.54	Total PFOA	1.58 ± 0.543	3.35 ± 1.03	0.471

RPM = ratio of cord serum to maternal serum; T1 = first trimester; T2 = second trimester; T3 = third trimester.

^a Data are presented as mean ± standard deviation.

^b RPM values were reported by authors as the ratio of the concentration in placenta to the concentration in maternal serum.

Mamsen et al. (2019, 5080595) demonstrated that factors such as gestational age can affect PFOA concentrations in maternal serum and placentas. Using a linear graph of normalized percentage accumulation as a function of gestational age, the authors found that, for male and female infant placentas, there was a steady increase in PFOA accumulation during gestation days 50 to 300, with male placentas showing higher levels of than female placentas. Authors estimated a placenta PFOA accumulation rate of 0.11% per day during gestation.

In summary, the findings from these studies highlight four important points: 1) Linear PFOA is more is detected at higher frequency and at higher levels in maternal serum than branched isomers likely due to different binding affinities in plasma; 2) branched and linear PFOA crosses the placental barrier and are distributed in different proportions within the placenta; 3) branched PFOA is more efficiently transferred into the placenta than linear PFOA; and 4) PFOA concentrations within the placenta increase during gestation from the first to third trimester.

Several studies have investigated distribution from mother to fetus through analysis of detected PFAS chemicals in cord blood. Kato et al. (2014, 2851230) collected blood samples from 71 mothers and their infants in a prospective birth cohort in the Cincinnati, Ohio, metropolitan area. They quantified PFAS in maternal blood at 16 weeks of gestation and, at the time of delivery, evaluated the correlation between PFAS levels in maternal serum and matched cord blood. Maternal serum PFOA levels at 16 weeks of gestation and at time of delivery were 4.8 µg/L and 3.3 µg/L, respectively. Authors reported a positive correlation between maternal serum PFOA concentration at 16 weeks of gestation and cord serum (correlation coefficient = 0.94). Similarly,

the correlation between maternal serum at the time of delivery and cord serum was also positive (correlation coefficient = 0.88).

Porpora et al. (2013, 2150057) quantified PFOA 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 PFOA concentrations were 2.9 ng/g and 1.6 ng/g, respectively. A strong positive correlation was observed between maternal and cord serum concentrations ($r = 0.70$, $p < 0.001$). These values suggest a cord to maternal serum ratio of 0.55.

Wang et al. (2019, 5083694) measured the levels of 10 PFAS chemicals in paired maternal and umbilical cord serum from a prospective birth cohort ($n = 369$) in Shandong, China. The average maternal and gestational ages of the participants were 28.4 years and 39.4 weeks, respectively. PFOA was detected in all maternal and umbilical cord serum samples at a geometric mean of 39.27 ng/mL (range of 1.16–602.79 ng/mL) in maternal serum and 31.83 ng/mL (range 1.52–291.56 ng/mL) in cord serum. Of the 10 PFAS chemicals measured, PFOA showed the highest concentration in both maternal and cord serum ($r = 0.908$). Authors did not explain why PFOA levels were high. Comparing the studies in Table D-10, geographic location could be a factor in population exposure to a particular PFAS chemical. In the case of Shandong, China, PFOA production may be a reason for the high PFOA levels in serum samples. Based on these studies, cord blood PFOA levels is a biomarker for in utero exposure and provides further evidence that PFOA readily accumulates in cord blood during gestation.

Study participants from various geographical locations, whether it be Ohio, USA (Kato et al., 2014, 2851230), Rome, Italy (Porpora et al., 2013, 2150057), Spain (Manzano-Salgado et al., 2015, 3448674), France (Cariou et al., 2015, 3859840), Farose island, Denmark (Eryasa et al., 2019, 5412430), Tianjin China (Zhang et al., 2013a, 3859792), or Shandong, China (Wang et al., 2019, 5083694), show consistently higher levels of PFOA in maternal serum versus cord serum regardless of the gestational age. Moreover, for studies with participants of similar gestational ages, the PFOA concentrations in both maternal and cord serum varied substantially across studies that were reflected in RCM ratios from 0.57 to 1.33 (Table D-10). Factors such as exposure sources, parity, and other maternal demographics can potentially account for these variations. For example, nulliparous mothers generally have significantly higher serum PFOA than parous women (Kato et al., 2014, 2851230). Conversely, younger women tend to have lower serum PFOA than older women (Kato et al., 2014, 2851230). Therefore, studies with high percentages of young, multiparous women may report lower levels of PFOA in maternal and cord blood.

To understand the role of the placenta in facilitating the transport of PFAS compounds to the fetal compartment during gestation, it is important to highlight the transplacental transfer efficiency (TTE) and the factors that can potentially modulate *in utero* transport of PFAS. TTE is a measure of a compound's ability to cross the placenta barrier and is often reported as the ratio of cord blood to maternal blood concentrations (RCM). A summary of recent studies examining RCM is presented in Table D-10. The percentages of maternal PFOA that accumulate in cord blood ranged from 57 to 133% and did not strictly correlate to maternal serum values. This variability suggests that TTE may differ across populations likely due to maternal characteristics or differing levels of exposure. For example, Manzano-Salgado et al. (2015, 3448674)

demonstrated that the percentage of maternal PFOA that accumulates in cord blood tends to increase with maternal age.

T. Zhang et al (2013a, 3859792) calculated the RCM of 11 PFAS compounds in matched maternal-cord blood from a population of 32 mothers in Tianjin, China, who delivered their infants at full term. Authors noted an interesting trend where the highest RCM was reported for PFHpA (C7) and a descending trend of RCM was observed with increasing chain length from PFHpA (C7) to PFDA (C10). There was then an increasing trend in RCM with increasing chain length from PFDA (C10) to PFDoDA (C12), creating a “U” shaped curve where the RCM decreases with increasing chain length until a certain threshold is reached and then the RCM increases. The authors suggest that this non-linear relationship may be due to differential binding affinities to maternal serum proteins and that high-affinity PFAS-serum protein interactions may result in PFAS not being able to cross the placental barrier as efficiently through passive diffusion. In line with most previous reports (Zhang et al., 2013, 3859792; Beesoon et al., 2011, 2050293; Hanssen et al., 2010, 2919297; Lee et al., 2013, 3859850), but not all (Gützkow et al., 2012, 1290978; Kim et al., 2011, 1424975), Wang et al. (2019, 5083694) reported that short-chain PFASs were transferred to cord serum at higher efficiencies than longer-chain PFASs.

Branching also impacts TTE (Zhao et al., 2017, 5085130) with branched isomers transferring more efficiently than their linear isomers. The authors observed a U-shaped trend of TTEs with increasing carbon chain lengths as well as the position of the branching point. TTEs of branched PFOA isomers (iso-, 5m-, and 4m-PFOA) were 0.71, 0.94, and 2.00, respectively compared to a TTE of 0.56 for linear isomer (n-PFOA). Thus, higher efficiencies were observed as the branching point moved closer to the carboxyl moiety of PFOA, which may be due to lower affinities of branched PFOA isomers for HSA allowing for more efficient transfer to the fetus.

The efficiency of the placenta to modulate the transfer of xenobiotic varies across gestation. To determine whether RCMs of PFAS in preterm placentas differed from full-term placentas, Li et al (2020, 6505874) assessed the RCMs of 32 PFAS chemicals in preterm and full-term deliveries in the Maoming Birth Cohort in South China. The concentration of PFOA in maternal blood from preterm subjects (mean = 1.2 ng/mL) did not differ significantly from blood levels in full-term subjects (mean = 1.34 ng/mL). However, the concentration of PFOA in preterm cord blood (0.70 ng/mL) was significantly lower than full-term cord blood (1.25 ng/mL, $p < 0.001$). Interestingly, the proportion of maternal PFOA in cord blood was 33% higher in full-term pregnancies than in preterm pregnancies. Authors attributed the differences in RCM between preterm and full-term deliveries to several factors, such as the difference in gestational age between the two groups. Full-term deliveries have longer gestation periods which means longer exposure duration. Second, the ability of the placenta to reduce toxin transfer reduces in the later stages of pregnancy, making it easier for PFAS to diffuse into fetal circulation. Third, most preterm pregnancies have impaired uteroplacental circulation, potentially reducing the amount of PFAS entering fetal circulation. Finally, gene expression of RCM transporters varies during the different stages of gestation, consequently affecting placenta barrier efficiency.

Table D-10. PFOA 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	PFOA Measurement	Cord Serum (ng/mL) ^c	Maternal Serum (ng/mL) ^c	Cord: Maternal Serum Ratios (RCM) ^d
Chen et al., 2017, 3859806	Wuhan, China	32	38.9 ± 1.6	total PFOA	1.237±0.577	1.56 ± 0.611	0.808
				n-PFOA	0.947	1.15	0.842
				Iso-PFOA	0.067	0.053	1.267
				3m-PFOA	0.08	0.06	0.587
Note: Authors measured n, iso, 5m, 4m, and 3m-PFOA. However, only n, iso, and 3m isomers had PFOA levels above the LOD.							
Cariou et al., 2015, 3859840	Toulouse, France	89	NR	total PFOA	0.919	1.22	0.78
Note: Concentrations represent mean values from 100 pairs. Semi-quantified values below LOD were taken into account for mean calculation.							
Cai et al., 2020, 6318671	Maoming birth cohort, China	424	39.3 ± 1.1	total PFOA	0.85 ± 0.52	1.21 ± 1.01	0.80
Note: Ratios were calculated from matched maternal and infant pairs for which all cord blood samples were > limit LOD. PFOA was detected 98.28% of samples PFOA.							
Wang et al., 2019, 5083694	Shandong, China	369	39.4 ± 1.3	total PFOA	31.83	39.27	0.83
Note: PFOA detected in 100% of maternal and cord samples.							
Li et al., 2020, 6505874	Maoming Birth Cohort, China (Pre-term births)	86	33.8 ± 3.0	total PFOA	0.7	1.2	0.57
	Maoming Birth Cohort, China (Full term births)	187	39.5 ± 1.1	total PFOA	1.25	1.34	0.85
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 were included in generating mean concentration values.							
Li et al, 2020, 6506038	Beijing, China	86	39.0± 1.2	total PFOA	4.98	3.63	1.33
Note: PFOA detection rate was 84.62% in maternal serum and 83.76% in cord serum. For PFOA, 86 of 117 matched cord and maternal serum samples were used to generate RCM.							
Eryasa et al., 2019, 5412430	Faroe Birth Cohort, Denmark (cohort 3)	100	39.9 ± 1.3	total PFOA	1.97 (1.42-2.76)	2.33 (1.79-3.29)	0.82
	Faroe Birth Cohort, Denmark (cohort 5)	51	39.7 ± 1.1	total PFOA	0.81 (0.56-1.26)	1.03(0.75-1.41)	0.77

Study	Country, Cohort	Number of Maternal-Infant Pairs ^a	Mean Gestational Age (weeks) ^b	PFOA Measurement	Cord Serum (ng/mL) ^c	Maternal Serum (ng/mL) ^c	Cord: Maternal Serum Ratios (RCM) ^d
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).							
Pan et al., 2017, 3981900	Wuhan, China	100	39.4 ± 1.3	total PFOA	1.42	2.19	0.65
Note: Maternal blood collected in third trimester (38.4 ± 1.6 weeks). PFOA was detected in 100% of maternal and cord samples.							
Zhao et al., 2017, 5085130	People’s Hospital of Hong’an County, China	63	39.3 ± 0.82	n-PFOA	0.551	0.966	0.59
		49	39.3 ± 0.82	iso-PFOA	0.01	0.014	0.81
		36	39.3 ± 0.82	5m-PFOA	0.003	0.003	1.7
		7	39.3 ± 0.82	4m-PFOA	0.001	0.001	2
		63	39.3 ± 0.82	total-PFOA	0.565	0.984	0.59
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 PFOA	1.1	1.8	0.61
		20	NR	n-PFOA	NR	NR	0.62
		20	NR	Iso-PFOA	NR	NR	0.84
		4	NR	5m-PFOA	NR	NR	0.86
		19	NR	4m-PFOA	NR	NR	0.64
		18	NR	3m-PFOA	NR	NR	0.76
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).							
Fei et al., 2007, 1005775	Danish National Birth Cohort, maternal blood obtained in first trimester	50	40.06 ± 1.57	total PFOA	3.7 ± 4.7	5.6 ± 2.5	0.55
	Danish National Birth Cohort, maternal blood obtained in second trimester	50	40.06 ± 1.57	total PFOA	3.7 ± 4.7	4.5 ± 1.9	0.68
Note: Authors did not specify if matched maternal and cord blood samples were used to derive ratios.							

Study	Country, Cohort	Number of Maternal-Infant Pairs ^a	Mean Gestational Age (weeks) ^b	PFOA Measurement	Cord Serum (ng/mL) ^c	Maternal Serum (ng/mL) ^c	Cord: Maternal Serum Ratios (RCM) ^d
Hanssen et al., 2010, 2919297	Johannesburg, South Africa	71 maternal serum, 58 cord blood	NR	total PFOA	1.3	1.3	0.71
Note: Maternal and cord blood samples taken at time of delivery.							
Fromme et al., 2010, 1290877	Munich, Germany	38 maternal and 33 cord serum	NR	total PFOA	1.4	1.9	1.02
Note: Maternal and cord blood samples taken at time of delivery.							
Kim et al., 2011, 1424975	Seoul and Gumi, South Korea	44 mothers, 43 infants	39±1.6	total PFOA	1.15 (0.95 - 1.86)	1.46 (1.15 - 1.91)	0.98
Note: Median serum concentrations reported. Values in parentheses are 25-75% IQRs.							
Needham et al., 2011, 1312781	Faroe Islands	12	NR	total PFOA	3.1	4.2	0.72
Note: Serum concentrations reported as median values, RCMs reported as arithmetic means.							
Liu et al., 2011, 2919240	Jinhu, China	50 (all)	NR	total PFOA	1.5	1.655	0.91
		26 (males infants)	NR	total PFOA	NR	NR	0.87
		24 (female infants)	NR	total PFOA	NR	NR	0.95
Note: Maternal samples collected in the first weeks after delivery.							
Midasch et al., 2007, 1290901	NR	11	NR	total PFOA	3.4	2.6	1.26
Note: Serum concentrations reported as median values, RCMs reported as arithmetic means.							
Verner et al., 2015, 3299692	NA	NA	NA	NA	NA	NA	0.78
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 average of studies reported in Aylward et al., 2014, 2920555.							

NR = not reported, LOD = Level of Detection, NA= Not Applicable, IQR = Interquartile Range

^aNumber represents number of matched pairs used for RCM calculation unless otherwise noted in comments.^bGestational age reported as mean ± SD, represents gestational age at the time of cord blood sampling (delivery) and may not be the same as age at the time of maternal blood sampling.^cConcentrations 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.^dData are presented as a ratio of cord serum to maternal serum concentrations unless otherwise noted in comments.

D.2.4.1.2 Partitioning to Amniotic Fluid

T. Zhang et al (2013a, 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 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-11 presents means or medians and ranges of measured and estimated PFOA concentrations in maternal blood from recent studies (2013 to present) that also measured fetal indicators of exposure (cord blood, placenta, and amniotic fluid). These studies demonstrate the variability of PFOA accumulation in these tissues across geographic regions. Maternal serum values ranged from 0.02 ng/mL in Rome, Italy (Porpora 2013, 2150057) to 602.79 ng/mL in Shandong, China (Wang et al., 2019, 5083694). These same studies also showed the greatest range of PFOA in cord blood (0.17–291.56 ng/mL). Fewer studies measured PFOA in placentas and amniotic fluid. Placenta values ranged from <LOQ at hospitals in Skelby ad Randers, Denmark (Mamsen et al., 2017, 3858487) to 3.57 ng/g in Tianjin, China (Zhang 2013a, 3859792). The same two studies provided ranges detected in amniotic fluid (<LOQ to 0.145 ng/mL), which were lower than those observed in placentas. The very wide concentration ranges observed across these geographic locations and matrices highlight the challenges of comparing the portioning of PFOA 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 (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 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 rate 3-times higher than PFOS. The coefficients of variation of the maximal transfer rate across subjects were estimated at 75% for PFOA and 55% for PFOS. Variation was also observed in the ranking of PFOA and PFOS when comparing exposure levels to fetal indicators of exposure. Maternal daily intake estimates were then used as inputs to the PBPK model to simulate the fetal exposure in several target organs over the whole pregnancy. The PFOA and PFOS fetal plasma concentrations are quite similar at the end of pregnancy for the whole cohort. This similarity was also predicted for the brain, but not in the kidneys and liver. When examined at the individual level, the ranking of PFOA and PFOS exposure exhibited a wide range of variability. Interestingly, the model estimated that approximately one-third of the population has levels of one compound always higher than levels of the other compound, whereas the remaining

two-thirds exhibited differing 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-11. PFOA Concentrations in Human Maternal Blood, Cord Blood, Placenta and Amniotic Fluid Across Studies

Study (Study Location)	Maternal Blood	Cord Blood	Placenta	Amniotic Fluid
Porpora et al, 2013, 2150057 (Italy)	Maternal serum Mean: 2.9 ng/g Median: 2.4 ng/g Range: 0.20–9.1 ng/g	Cord serum Mean: 1.6 ng/g Median: 1.6 ng/g Range: 0.17–5.0 ng/g	NR	NR
Zhang et al, 2014, 2850251 (Tianjin, China)	NR	NR	Mean: 1.58 ng/g Median: 1.41 ng/g	Mean: 0.044 ng/mL Median: 0.043 ng/mL
Yang et al., 2016, 3858535 (Jiangsu, China)	Maternal serum Mean: 1.64 ng/mL SD: 1.11 ng/mL Median: 1.24 ng/mL Range: 0.34–5.30 ng/mL	Cord serum Mean: 1.45 ng/mL SD: 1.14 ng/mL Median: 1.03 ng/mL Range: 0.16–6.77 ng/mL	NR	NR
Manzano-Salgado et al., 2015, 3448674 (Sabadell and Valencia, Spain)	Maternal plasma Median: 2.85 ng/mL Range: 0.78–11.93 ng/mL IQR: 1.87–6.00 ng/mL Maternal serum Median: 2.97 ng/mL Range: 0.86–14.54 ng/mL IQR: 2.26–4.85 ng/mL	Cord serum Median: 1.90 ng/mL Range: 0.60–10.56 ng/mL IQR: 1.45–4.70 ng/mL	NR	NR
Zhang et al., 2013, 3859792 (Tianjin, China)	Mean: 3.35 ng/mL RSD: 1.03 Range: 1.17–8.94 ng/mL	1.95 ng/mL RSD: 0.71 Range: 0.70–4.31 ng/mL	Mean: 1.58 ng/g RSD: 0.54 Range: 0.45–3.57 ng/g	Mean: 0.044 ng/mL RSD: 0.021 Range: < LOQ - 0.145 ng/mL
Cariou et al., 2015, 3859840 (Toulouse, France)	Maternal serum Mean: 1.22 ng/mL Median: 1.045 ng/mL Range: 0.309–7.31 ng/mL	Cord serum Mean: 0.919 ng/mL Median: 0.860 ng/mL Range: 0.311–7.06 ng/mL	NR	NR
Pan et al., 2017, 3981900 (Wuhan, China) ^{a,c}	Maternal Serum T1 Mean: 3.15 ng/mL Median: 3.24 ng/mL IQR: 2.44–3.88 ng/mL T2 serum Mean: 2.52 ng/mL Median: 2.50 ng/mL IQR: 2.05–3.13 ng/mL	Cord serum Mean: 1.42 ng/mL Median: 1.41 ng/mL IQR: 1.14–1.84 ng/mL	NR NR	NR NR

Study (Study Location)	Maternal Blood	Cord Blood	Placenta	Amniotic Fluid
	T3 serum Mean: 2.19 ng/mL Median: 2.16 ng/mL IQR: 1.81–2.73 ng/mL		NR	NR
Caserta et al., 2018, 4728855 (Rome, Italy)	Mean: 1.05 ng/mL SD: 0.35 ng/mL Range: 0.45–1.9 ng/mL	Mean: 0.98 ng/mL SD: 0.54 ng/mL Range: 0.30–2.50 ng/mL	NR	NR
Wang et al., 2019, 5083694 (Shandong, China)	Maternal serum GM: 39.27 ng/mL Median: 42.83 ng/mL Range: 1.16–602.79 ng/mL	Cord serum GM: 31.83 ng/mL Median: 34.67 ng/mL Range: 1.52–291.56 ng/mL	NR	NR
Zhao et al., 2017b (Hong'an, China)	Maternal blood Mean: 0.984 ng/mL Median: 0.987 ng/mL Range: 0.274–2.72 ng/mL	Cord blood Mean: 0.585 ng/mL Median: 0.535 ng/mL Range: 0.126 – 1.44 ng/mL	NR	NR
Brochot et al., 2019, 5381552 (INMA prospective birth cohort, Spain) ^{a,d}	Group 1 mean (plasma): 3.26 ± 1.87 [0.39–11.93] ng/mL Group 2 mean (plasma): 2.78 ± 2.18 [0.20–31.64] ng/mL	Mean: 2.54 ± 1.64 [0.86–10.56] ng/mL	NR	NR
Gao et al., 2019, 5387135 (Beijing, China)	Mean: 2.85 ng/mL Median: 2.21 ng/mL Range: < LOD–25.4 ng/mL	Mean: 2.29 ng/mL Median: 1.88 ng/mL Range: 0.03–10.2 ng/mL	NR	NR
Eryasa et al., 2019, 5412430 (Faroes Birth Cohorts, Denmark) ^b (Cohort 3)	GM serum: 2.33 ng/mL SD: 0.12 ng/mL IQR: 1.79–3.29 ng/mL	Cord serum Mean: 1.97 ng/mL SD: 0.10 ng/mL IQR: 1.42–2.76 ng/mL Whole cord blood Mean: 1.08 ng/mL SD: 0.05 ng/mL IQR: 0.8–1.45 ng/mL	NR	NR
Eryasa et al., 2019, 5412430 (Faroes Birth Cohorts, Denmark) ^b (Cohort 5)	Mean: 1.03 ng/mL SD: 0.08 ng/mL IQR: 0.75–1.41 ng/mL	Cord serum Mean: 0.81 ng/mL SD: 0.07 ng/mL IQR: 0.56–1.26 ng/mL Whole cord blood Mean: 0.41 ng/mL SD: 0.03 ng/mL IQR: 0.29–0.59 ng/mL	NR	NR
Cai et al., 2020, 6318671 (Maoming Birth Cohort, China)	Maternal serum Mean: 1.21 ng/mL SD: 1.01 ng/mL Median: 0.99 ng/mL IQR: 0.74–1.37/mL	Cord serum Mean: 0.85 ng/mL SD: 0.52 ng/mL Median: 0.75 ng/mL IQR: 0.52–1.09 ng/mL	NR	NR

Study (Study Location)	Maternal Blood	Cord Blood	Placenta	Amniotic Fluid
Li et al., 2020, 6505874 (Maoming Birth Cohort, China)	Preterm delivery Mean serum: 1.20 ng/mL Median: 1.00 ng/mL IQR: 0.69–1.47	Preterm delivery Mean: 0.70 ng/mL Median: 0.57 ng/mL IQR: 0.43–0.91	NR	NR
	Full-term delivery Mean: 1.34 Median: 1.13 ng/mL IQR 0.72–1.74	Full-term delivery Mean: 1.25 ng/mL Median: 0.99 ng/mL IQR 0.64–1.49		
Li et al, 2020, 6506038 (Beijing, China)	Mean serum: 3.63 ng/mL (95% CI 3.26, 4.49) Median: 3.20 ng/mL	Mean: 4.98 ng/mL (95% CI 4.41, 7.38) Median: 3.80 ng/mL	NR	NR
Mamsen et al, 2017, 3858487 (Hospitals in Skelby and Randers, Denmark)	Mean: 2.1 ng/g, Range: 0.6–8.0 ng/g	NR	Mean: 0.23 ng/g, Range: 0.04–0.45 ng/g	NR
Mamsen et al., 2019, 5080595 (Denmark) ^a	T1 serum Mean: 2.04 ng/mL SD: 1.63 ng/mL Median: 1.51 ng/mL Range: 0.55–7.95 ng/mL	NR	Mean: 0.28 ng/g SD: 0.09 ng/g Median: 0.27 ng/g Range: 0.15–0.45 ng/g	NR
	T2 serum Mean: 1.62 ng/mL SD: 0.71 ng/mL Median: 1.58 ng/mL Range: 0.72–3.78 ng/mL	NR	Mean: 0.39 ng/g SD: 0.26 ng/g Median: 0.26 ng/g Range: 0.19–0.99 ng/g	NR
	T3 serum Mean: 1.62 ng/mL SD: 0.85 ng/mL Median: 1.36 ng/mL Range: 0.62–4.62 ng/mL	NR	Mean: 0.43 ng/g SD: 0.16 ng/g Median: 0.36 ng/g Range: 0.21–0.82 ng/g	NR
Hanssen et al., 2013, 3859848 (Norilsk, Russia) ^e	Plasma Median: 1.61 ng/mL Mean: 1.50 ng/mL Range: 0.63–2.48 ng/mL	Cord plasma Median: 1.00 ng/mL Mean: 1.26 ng/mL Range: 0.36–2.32 ng/mL	NR	NR
	Whole blood Median: 0.89 ng/mL Mean: 0.89 ng/mL Range: 0.33–1.40 ng/mL	Cord whole blood Median: 0.49 ng/mL Mean: 0.58 ng/mL Range: 0.15–1.12 ng/mL	NR	NR
Kato et al, 2014, 2851230 (Ohio, USA) ^f	Maternal Serum at 16 weeks Median: 4.80 µg/L	Cord serum at delivery Median: 3.10 µg/L		
	Maternal serum at delivery Median: 3.30 µg/L			

NR = Not reported; SD = Standard deviations; GM= Geometric mean; LOD = limit of detection; LOQ = limit of quantification; IQR = Interquartile range; T1= first trimester; T2 = Second trimester; T3 = Third trimester.

^aFor studies that quantified PFOA at different trimesters, first trimester (T1), second trimester (T2) and third trimester (T3).

^bEryasa et al., 2019 sampled participants from two birth cohorts: Cohort 3 (100 Singleton births from 1999 to 2001), and Cohort 5 (50 singleton birth from 2008 to 2005). Both cohorts had the same source of exposure and are similar in maternal characteristics.

^cPan et al., 2017 measured PFOA in maternal serum at first, second and third trimester and measured cord blood only at the time of full-term delivery.

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

^eHanssen et al., 2013 measured PFOA in whole blood and plasma from mothers and their infants at the time of delivery.

^fKato et al., 2014 measured PFOA in 71 matched maternal and cord serum pairs. Maternal serum samples were collected at 16 weeks of gestation and at the time of delivery.

D.2.4.1.3 Distribution in Fetal Tissues

Mamsen et al. (2017, 3858487) measured the concentrations of 5 PFAS chemicals in human fetuses, placentas, and maternal plasma from a cohort of 39 pregnant women in Denmark, who legally terminated their pregnancies before gestational week 12 for reasons other than fetal abnormality. The samples collected included 24 maternal blood, 34 placenta, and 108 fetal organs. The participants were healthy women ages 18–46 years with an average BMI of 22.7. About 51% of the mothers smoked during pregnancy at an average of 10 cigarettes per day or were exposed to secondhand cigarette smoke for an average of 1.8 hours per day. PFOA was detected in placenta, fetal liver, extremities, heart, intestines, lungs, connective tissues, spinal cord, and ribs at different concentrations. Notably, PFOA levels were highest in the placenta and lung. Mean concentrations of PFOA in maternal serum, placenta, and fetal organs were reported as 1.9 (0.6–4.1), 0.2 (0.0–0.4), and 0.1 (0–0.3) ng/g, respectively. 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 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 PFOA decreased from maternal serum to fetal tissues as follows: maternal serum > placenta > fetal tissues. The relative concentration of PFOA in the placenta was 11% of the concentrations found in maternal plasma and were further reduced to 7% in fetal tissues. In general, a positive trend was observed between fetal tissue-maternal serum ratio and gestational age. Although the gestational age reported in this study is short (37–68 days post conception), the results suggest that PFOA is retained in several fetal organs and may potentially continue to accumulate across gestation.

To determine whether PFOA 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. Fetal tissue:maternal serum ratios of PFASs were calculated by dividing the fetal tissue concentration by the maternal serum concentration. In general, fetal tissue:maternal ratios of PFOA in fetal tissue increased from first trimester to third trimester except for the liver and heart which showed the highest tissue:maternal serum ratios in the

second trimester compared with the third trimester. The fetal tissue:maternal serum ratio of PFOA was highest in adipose tissue during the second trimester than in any other tissue across gestation.

Interestingly, PFOA concentration in the liver was also highest in the second trimester compared with the first and third trimesters. Authors attributed this phenomenon to the unique architecture of the fetal liver during early gestation when 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, 9642023). This reduction results in increased flow of cord blood through the liver, thus increasing levels of PFOA and PFOS during the second trimester. Furthermore, Mamsen et al. (2019, 5080595) observed that PFOA and PFOS levels were lowest in the CNS than any of the tissues examined, suggesting that the CNS has less PFAS exposure and may be protected by the 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.2.4.1.4 Partitioning to Infants

Four studies shown in Table D-12 analyzed PFOA levels in maternal serum and levels in breast milk and/or infant blood. Maternal and infant serum PFOA levels were an order of magnitude higher in subjects in the United States exposed to contaminated drinking water (Mondal et al., 2014, 2850916) compared to subjects analyzed in France, Denmark (Faroe Islands), or Sweden (Cariou et al., 2015, 3859840; Mogensen et al., 2015, 3859839; Gyllenhammar et al., 2018, 4778766). In the Mondal study, geometric mean (GM) maternal serum PFOA concentrations were lower in breastfeeding mothers (18.32 ng/mL) versus non-breastfeeding mothers (19.26 ng/mL). Conversely, breastfed infants had higher GM serum PFOA (48.55 g/mL) than infants who were never breastfed (21.74 ng/mL).

Cariou et al. (2015, 3859840) reported that PFOA levels in breastmilk were approximately 30-fold lower relative to maternal serum and the ratio between breastmilk and maternal serum PFOA was 0.038 ± 0.013 ($n = 10$). 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 inter-individual 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 PFOA, although it did reach significance for PFHxS. Only PFOA exhibited a significant correlation between the total duration of breastfeeding and serum PFOA levels after adjustment ($0.87 [0.80-0.94]$, $p = 0.0007$).

Mogensen et al. (2015, 3859839) relied on maternal PFOA 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-12, the increases in infant blood PFOA 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 PFOA concentrations in 2–4-month-old infants and maternal PFOA 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. Of note, the authors observed that variation in maternal PFOA concentrations explained 53% of the infant concentration variation, whereas only 13% of the variation in infant PFUnDA was explained by maternal variation. Also, the PFOA infant:maternal serum ratio was higher than ratios for other PFAAs [2.8 (0.43–5.7)].

Table D-12. Summary of Studies Evaluating PFOA concentrations in Maternal Serum, Breast Milk, and Infant Serum

Study	Subjects	Maternal Blood	Breastmilk	Infant Blood
Mondal et al., 2014, 2850916	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 mean: 18.69 ng/mL 95% CI: 17.13, 20.28 Breastfed GM: 18.32 ng/mL 95% CI: 16.36, 20.50 Not breastfed GM: 19.26ng/mL 95% CI: 16.80, 22.08	NR	Infant serum Breastfed & not breastfed mean: 36.14 ng/mL 95% CI: 24.87, 52.52 Breastfed GM: 48.55g/mL 95% CI: 31.17, 75.61 Not breastfed GM: 21.74ng/mL 95% CI: 11.21, 42.17

Study	Subjects	Maternal Blood	Breastmilk	Infant Blood
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.	Mean: 1.22 ng/mL Median: 1.045 ng/mL Range: 0.309–7.31 ng/mL	Mean: 0.041 ng/mL Median: < LOQ LOQ = 0.050 ng/mL Range: < LOD–0.308 ng/mL	NR
Mogensen et al., 2016, 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. A piece-wise linear model was used to estimate the age dependence of the PFOA Concentration.	NR	NR	Median at birth: 2.0 ng/mL (IQR 1.7,2.7) Median at 11 months: 8.2 ng/mL (IQR 6.1, 10.9) Median at 18 months: 6.1 ng/mL (IQR 5.1, 10) Median at 60 months: 3.8 ng/mL (IQR 3.1, 4.9)
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: 2.8 ng/g SD: 0.96 ng/g Median: 2.7 ng/g Range: 1.2–6.7 ng/g	NR	Infant serum Mean: 7.7 ng/g SD: 3.7 ng/g Median: 7.2 ng/g Range: 1.3–20 ng/g

GM = geometric mean; CI = confidence interval; SD = standard deviation; IQR = interquartile range; LOD = limit of detection; LOQ = limit of quantification; 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.; Sjobin, 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 PFOA levels with duration of breastfeeding (Table D-13). Maternal serum concentrations decreased with each month of breastfeeding (–3%; 95% CI: –5%, –2%) with the greatest decrease observed after 12 months of breastfeeding (–41%). Correspondingly, the infant PFOA serum concentrations increased by 6% (95% CI: 1%, 10%) with each month of breastfeeding, lower than the estimate of 30% per month in Swedish infants found by Gyllenhammar et al. (2018, 4778766). Increases were modest in the first 6 months (13%) but increased to 141% after 12 months of breastfeeding. Using mixed linear model regression (Table D-14), Mogensen et al. (2015, 3859839) calculated

that, during months with exclusive breastfeeding, significant increases in the PFOA concentrations in infant serum were estimated (27.8% and 31.2% per month at 18 and 60 months, respectively). These levels were higher than the continuous (per month) 6% estimated increases in the Mondal study, respectively. Increases were less striking for months with partial breastfeeding and small or none for months without breastfeeding. Altogether, these findings support breastfeeding as the primary source of infant PFOA accumulation and that distribution to the infant correlates with the length of breastfeeding.

Table D-13. Percent Change in PFOA Ratios in Maternal Serum to Breast Milk and Breast Milk to Infant Serum by Infant Age in Humans

PFOA (Mondal, 2014)	Maternal Serum: Breast Milk		Breastmilk: Infant Serum	
Infant Age	Percent Change	95% CI	Percent Change	95% CI
≤ 6 months	–5%	(–18, 8)	13%	(–46, 139)
7-12 months	–29%	(–41, –13)	82%	(–23, –334)
> 12 months	–41%	(–57, –17)	141%	(4, 460)
Continuous (per month)	–3%	(–5, –2)	6%	(1, 10)

CI = confidence interval.

Table D-14. Percent Change in PFOA Serum Concentration by Exclusive, Mixed or No Breastfeeding Per Month in Humans

Variable	Mixed Model up to 18 Months			Mixed Model up to 60 Months		
	Percent Change	95% CI	p-value	Percent Change	95% CI	p-value
Exclusive	27.8	(23.6, 32.1)	< 0.0001	31.2	(28.0, 34.5)	< 0.0001
Partial	3.9	(0.5, 7.3)	0.0252	0.1	(–1.6, 1.9)	0.8951
None	0.7	(–1.1, 2.5)	0.4528	–1.3	(–1.5, –1.0)	< 0.0001

CI = confidence interval.

The contributions of placental transfer, breastfeeding, and ingestion of PFAS-contaminated drinking water to early life PFOA levels in children were analyzed (Gyllenhammar et al. (2019, 5919402). This study measured PFOA 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 PFOA for these exposure modes. PFOA concentrations increased 10% per unit (ng/g serum) of increase in the maternal serum level at delivery. The association was strongest in 4-year-old children. Duration of breastfeeding only correlated with 4-year-old children but not older children in the MLR model (partial R² = 0.05 for children in this age group). PFOA increased 1.2% 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 PFOA contamination is an important source of exposure for children.

D.2.4.2 Animal Studies

D.2.4.2.1 Rats

PFOA levels during gestation and lactation were studied by Hinderliter et al. (2005, 1332671) and Mylchreest et al. (2003, 9642031). Time-mated female Sprague-Dawley rats were dosed with 0, 3, 10, or 30 mg/kg/day of PFOA during days 4–10, 4–15, and 4–21 of gestation, or from GD4 to LD21. Maternal blood samples were collected at 2 hours \pm 30 minutes (mins) post-dose on a daily basis. Plasma, milk, amniotic fluid extract, and tissue homogenate (placenta, embryo, and fetus) supernatants were analyzed for PFOA concentrations by HPLC/MS. Maternal PFOA plasma levels during gestation and lactation are presented in Table D-15. Maternal plasma levels at 2 hours post-dosing (approximately the time of peak blood levels following a gavage dose) were fairly similar during the course of the study with mean levels of 11.2, 26.8, and 66.6 $\mu\text{g/mL}$ in the 3, 10, and 30 mg/kg/day groups, respectively; PFOA levels in the control group were below the LOQ (0.05 $\mu\text{g/mL}$).

Table D-15. Maternal Plasma PFOA Levels in Sprague-Dawley Rats During Gestation and Lactation^a

Exposure Period	Sample Time	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD4–GD 10	GD 10 plasma	8.53 \pm 1.06	23.32 \pm 2.15	70.49 \pm 8.94
GD4–GD 15	GD 15 plasma	15.92 \pm 12.96	29.40 \pm 14.19	79.55 \pm 3.11
GD4–GD 21	GD 21 plasma	14.04 \pm 2.27	34.20 \pm 6.68	76.36 \pm 14.76
GD4–LD 3	LD 3 plasma	11.01 \pm 2.11	22.47 \pm 2.74	54.39 \pm 17.86
GD4–LD 7	LD 7 plasma	10.09 \pm 2.90	25.83 \pm 2.07	66.91 \pm 11.82
GD4–LD 14	LD 14 plasma	9.69 \pm 0.92	23.79 \pm 2.81	54.65 \pm 11.63
GD4–LD 21	LD 21 plasma	9.04 \pm 1.01	28.84 \pm 5.15	64.13 \pm 1.45
NA	Average plasma	11.19 \pm 2.76	26.84 \pm 4.21	66.64 \pm 9.80

GD = gestation day; LD = lactation day; NA = not applicable.

^aData are presented as mean \pm standard deviation ($\mu\text{g/mL}$).

PFOA levels in the placenta, amniotic fluid, and embryo/fetus are presented in Table D-16. The levels of PFOA in the placenta on GD21 were approximately twice the levels observed on GD15, and the levels of PFOA in the amniotic fluid were approximately four times higher on GD21 than on GD15. The concentration of PFOA in the embryo/fetus was highest in the GD 10 embryo and lowest in the GD15 embryo; PFOA levels in the GD21 fetus were intermediate.

Fetal and pup PFOA plasma levels during gestation and lactation are presented in Table D-17, and PFOA levels in maternal milk during lactation are provided in Table D-18. The concentrations of PFOA in the plasma of the GD21 fetus (5.88, 14.48, and 33.11 $\mu\text{g/mL}$, respectively, in the 3-, 10-, and 30-mg/kg/day groups) were approximately half the levels observed in the maternal plasma (Table D-15). Pup plasma levels decreased between birth and LD 7 (Table D-17) and were, thereafter, similar to the levels observed in the milk (Table D-18). The pups were not separated by sex. The concentrations of PFOA in maternal milk also were fairly similar throughout lactation (means of 1.1, 2.8, and 6.2 $\mu\text{g/mL}$ in the 3, 10, and 30

mg/kg/day groups, respectively) and were approximately one-tenth of the PFOA levels in the maternal plasma.

Table D-16. Placenta, Amniotic Fluid, and Embryo/Fetus PFOA Concentrations in Sprague-Dawley Rats^a

Exposure Period	Tissue	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD4–GD10	GD10—embryo	1.40 ± 0.30	3.33 ± 0.81	12.49 ± 3.50
GD4–GD15	GD15—placenta	2.22 ± 1.79	5.10 ± 1.70	13.22 ± 1.03
	—amniotic fluid	0.60 ± 0.69	0.70 ± 0.15	1.70 ± 0.91
	—embryo	0.24 ± 0.19	0.53 ± 0.18	1.24 ± 0.22
GD4–GD21	GD21—placenta	3.55 ± 0.57	9.37 ± 1.76	24.37 ± 4.13
	—amniotic fluid	1.50 ± 0.32	3.76 ± 0.81	8.13 ± 0.86
	—fetus	1.27 ± 0.26	2.61 ± 0.37	8.77 ± 2.36

GD = gestation day.

^aData are presented as mean ± standard deviation (µg/mL). Samples were pooled by litter and were collected 2 hours post-dosing.

Table D-17. Fetus/Pup PFOA Concentration in Sprague-Dawley Rats During Gestation and Lactation^a

Exposure Period	Tissue	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD4–GD21	GD21—fetal plasma	5.88 ± 0.69	14.48 ± 1.51	33.11 ± 4.64
GD4–LD3	LD3—pup plasma	2.89 ± 0.70	5.94 ± 1.44	11.96 ± 1.66
GD4–LD7	LD7—pup plasma	0.65 ± 0.20	2.77 ± 0.58	4.92 ± 1.28
GD4–LD14	LD14—pup plasma	0.77 ± 0.10	2.22 ± 0.38	4.91 ± 1.12
GD4–LD21	LD21—pup plasma	1.28 ± 0.72	3.25 ± 0.52	7.36 ± 2.17

GD = gestation day; LD = lactation day.

^aData are presented as mean ± standard deviation (µg/mL). Samples were pooled by litter and were collected 2 hours post-dosing.

Table D-18. Maternal Milk PFOA Concentration in Sprague-Dawley Rat During Lactation^a

Exposure Period	Sample Time	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD4–LD3	LD3—milk	1.07 ± 0.26	2.03 ± 0.33	4.97 ± 1.20
GD4–LD7	LD7—milk	0.94 ± 0.22	2.74 ± 0.91	5.76 ± 1.26
GD4–LD14	LD14—milk	1.15 ± 0.06	3.45 ± 1.18	6.45 ± 1.38
GD4–LD21	LD21—milk	1.13 ± 0.08	3.07 ± 0.51	7.48 ± 1.63
NA	Average milk	1.07 ± 0.09	2.82 ± 0.60	6.16 ± 1.06

GD = gestation day; LD = lactation day; NA = not applicable.

^aData are presented as mean ± standard deviation (µg/mL). Samples were from 5 dams/group/time point and were collected 2 hours post-dosing.

PFOA accumulation in young rats is impacted by both sex and age. Han (2003, 3749132) administered groups of 4–8-week-old Sprague-Dawley rats (10 per sex per age) a single dose of 10 mg/kg/day PFOA by oral gavage. Blood samples were collected 24 hours after dosing and the plasma concentration of PFOA was measured by high-performance liquid chromatography mass spectrometry (HPLC/MS). In the 5- and 6-week-old female rats, the plasma PFOA concentrations were about twofold lower than in the 4-week-old rats (Table D-19). However, in the 5-week-old males, the concentration of plasma PFOA was about fivefold higher than in the 4-week-old group, suggesting a developmental change in excretion rate. PFOA plasma concentrations were 35–65-fold higher in males than in females at every age except at 4 weeks. Thus, it appears that maturation of the transport features responsible for the sex difference in elimination occurs between the ages of 4 and 5 weeks in the rat.

Table D-19. Plasma PFOA Concentrations in Postweaning Sprague-Dawley Rats^a

Age (weeks)	Males	Females
4	7.32 ± 1.01	2.68 ± 0.64
5	39.24 ± 3.89	1.13 ± 0.46
6	43.19 ± 3.79	1.18 ± 0.52
7	37.12 ± 4.07	0.57 ± 0.29
8	38.55 ± 5.44	0.81 ± 0.27

^aData are presented as mean ± standard deviation (µg/ml).

Hinderliter et al. (2006, 3749132) continued the investigation of the relationship between age and plasma PFOA in male and female Sprague-Dawley rats. Immature rats at 3, 4, and 5 weeks of age were administered PFOA via oral gavage at a single dose of 10 or 30 mg/kg. Rats were not fasted prior to dosing. Two hours after dosing, five rats per sex per age group and dose group were sacrificed and blood samples were collected. The remaining five rats per sex per age and dose group were placed in metabolism cages for 24-hour urine collection. These rats were sacrificed at 24 hours and blood samples were collected.

In the male rats, plasma PFOA concentrations for either the 10- or 30-mg/kg dosage groups did not differ significantly by sample time (at 2 and 24 hours) or by animal age (3, 4, and 5 weeks), except at 2 hours for the 5-week-old group ($p < 0.01$), which showed the lowest PFOA level (Table D-20). PFOA plasma concentrations following a 30-mg/kg dose were 2–3 times higher than those following a 10-mg/kg dose. These data do not demonstrate a difference between the 5-week-old rats and the younger 3- and 4-week-old groups at 24 hours after dosing, and thus do not support the observations from the Han study (2003, 3749132).

Table D-20. Plasma PFOA Concentrations in Male Sprague-Dawley Rats at 2 and 24 hours after Oral Gavage

Age (weeks)	Dose (mg/kg)	Plasma PFOA (µg/mL)			
		2 Hours Post-Dose		24 Hours Post-Dose	
		Mean	SD	Mean	SD
3	10	41.87	4.01	34.22	7.89

Age (weeks)	Dose (mg/kg)	Plasma PFOA (µg/mL)			
		2 Hours Post-Dose		24 Hours Post-Dose	
		Mean	SD	Mean	SD
4	10	39.92	4.45	42.94	5.33
5	10	26.32*	6.89	40.60	3.69
3	30	120.65	12.78	74.16	18.23
4	30	117.40	18.10	100.81	13.18
5	30	65.66*	15.53	113.86	23.36

SD = standard deviation.

*Statistically significantly different by sample time and animal age ($p < 0.01$).

In the female rats, plasma PFOA concentrations were significantly lower in the 5-week-old group than in the 3- or 4-week-old groups at the 24-hour time period for both doses and for the 30-mg/kg dose group at 2 hours (Table D-21). Plasma PFOA concentrations following a 30-mg/kg dose were approximately one and one half to four times higher than those observed following a 10-mg/kg dose.

At 24 hours post-dose, plasma PFOA levels in the female rats were significantly lower than the plasma PFOA levels in male rats, especially at 5 weeks of age. The data for the 5-week-old female rats compared to the 3- and 4-week-old groups at 24 hours are consistent with the Han (2003, 3749132) data in that they demonstrate a decline in plasma levels compared to their earlier measurements. Thus, the developmental change is one that appears to be unique to the female rat.

Table D-21. Plasma PFOA Concentrations in Female Sprague-Dawley Rats at 2 and 24 hours after Oral Gavage

Age (weeks)	Dose (mg/kg)	Plasma PFOA (µg/ml)			
		2 Hours Post-Dose		24 Hours Post-Dose	
		Mean	SD	Mean	SD
3	10	37.87	5.77	13.55 ^b	3.83
4	10	29.88	12.15	18.98 ^b	7.01
5	10	33.23	7.41	1.36 ^{a, b}	0.87
3	30	84.86	10.51	51.43 ^b	13.61
4	30	80.67	14.10	28.01 ^b	9.90
5	30	56.90 ^a	29.66	3.42 ^{a, b}	1.95

SD = standard deviation.

^aStatistically significantly different from the 3- and 4-week values ($p < 0.01$).

^bStatistically significantly different from 2-hour values ($p < 0.01$).

The data demonstrate that both dose and sex influence plasma levels. Post-dosing clearance (CL) is slow for both doses at 2 and 24 hours in males and females at PNW3 and 4. At 5 weeks, however, the plasma levels after 24 hours are greater than those at 2 hours in males. In females, for the high dose at 2 hours, plasma levels are similar to those in males, while at 24 hours they

are only 3% of the value for males. This suggests that uptake from the intestines is similar while the rate of excretion at 5 weeks and beyond is considerably greater for female rats than males. They are comparable for PNW3 and 4.

In a supplemental study to determine the effect of fasting (Hinderliter et al., 2006, 3749132), 4-week-old rats, 4 rats per sex, were administered 10 mg/kg PFOA via oral gavage. Animals (two per sex) were fasted overnight for 12 hours before dosing with PFOA. All the rats were sacrificed at 24 hours post dosing and blood was collected for analysis of PFOA in plasma. Plasma PFOA concentrations in male rats were 64.95 and 30.00 µg/ml for the fasted and nonfasted animals, respectively. Plasma PFOA concentrations in the female rats were 68.16 and 26.54 µg/ml for the fasted and nonfasted animals, respectively. Given the consistency in the 4-week-old rat plasma PFOA concentrations, the authors concluded that age-dependent changes in female PFOA elimination are observable between 3 and 5 weeks of age. PFOA uptake was greater in the fasted animals than the fed animals, suggesting competition for uptake in the presence of food components that share common transporters and/or decreased contact of PFOA with the intestinal epithelium in the presence of dietary materials. This is consistent with the finding that dietary fat may negatively impact absorption (Li 2015, 2851033).

An oral two-generation reproductive toxicity study of PFOA in rats was conducted (Butenhoff et al., 2004, 1291063). Five groups of rats (30 sex/group) were administered PFOA by gavage at doses of 0, 1, 3, 10, or 30 mg/kg/day. At scheduled sacrifice, after completion of the cohabitation period in F₀ male rats and on lactation day (LD) 22 in F₀ female rats, blood samples were collected. Serum analysis for the F₀ generation males showed that PFOA was present in all samples tested, including low levels in controls (0.0344 ± 0.0148 µg/mL). Levels of PFOA were similar in the two male dose groups (51.1 ± 9.30 and 45.3 ± 12.6 µg/mL, respectively, for 10- and 30-mg/kg/day dose groups). In the F₀ female controls, serum PFOA was below LOQ (0.00528 µg/mL). Levels of PFOA found in female sera were lower than in males but increased between the two dose groups; treated females had an average concentration of 0.37 ± 0.0805 and 1.02 ± 0.425 µg/ml, respectively, for the 10- and 30-mg/kg/day dose groups.

D.2.4.2.2 Mice

Fenton et al. (2009, 194799) orally dosed pregnant CD-1 mice (n = 25/group) with 0, 0.1, 1, or 5 mg PFOA/kg on GD17. On GD18, five dams/group were sacrificed and trunk blood, urine, amniotic fluid, and the fourth and fifth mammary glands were collected. One fetus/dam was euthanized and retained for whole-pup analysis. The remaining dams were allowed to litter and samples (excluding amniotic fluid) also were collected on PND1, 4, 8, and 18. At each time-point, a single pup was euthanized and retained for whole-pup analysis. Blood from the remaining pups was collected and pooled. Milk was collected from dams on PND2, 8, 11, and 18 following a 2-hour separation of the pups from the dam.

PFOA levels in mice during gestation and lactation in selected fluids and tissues are summarized in Table D-22. The concentrations of PFOA in dam serum were approximately twice that detected in amniotic fluid. Compared to the amniotic fluid, concentrations of PFOA in the fetuses were increased by 2.3-, 3.1-, and 2.7-fold at 0.1, 1, and 5 mg/kg, respectively. The highest concentration of PFOA was detected in the serum of nursing dams. In the dams, the concentrations of PFOA in serum exhibited a U-shaped response curve; the lowest serum concentrations were observed at the time of peak lactation. Dam mammary tissue and milk

PFOA concentrations showed a U-shaped response that mirrored that found in dam serum. The concentrations of PFOA in pup serum were significantly higher than PFOA concentrations in dam serum and appeared to decrease as the time for weaning approached. When pup PFOA concentrations were calculated with consideration for pup body weight gain, PFOA body burden increased through the peak of lactation and began to decrease by PND18, showing an inverse U-shaped response curve.

Table D-22. Select Fluids and Tissues PFOA Concentrations in CD-1 Mice During Gestation and Lactation

Tissue	Day	Dose		
		0.1 mg/kg	1 mg/kg	5 mg/kg
Dam Serum ^a	GD18	143 ± 19	1697 ± 203	7897 ± 663
	PND1	217.5 ± 35	1957.0 ± 84	9845.6 ± 1478
	PND4	110.0 ± 12	1269.4 ± 235	6776.6 ± 561
	PND8	46.7 ± 21	360.8 ± 98	1961.8 ± 414
	PND18	123.3 ± 41	1035.2 ± 305	5156.5 ± 1201
Amniotic Fluid ^a	GD18	99.0 ± 28	865.3 ± 191	3203.8 ± 492
Dam Urine ^a	GD18	21.9 ± 8.6	104.9 ± 69.7	666.7 ± 169
	PND1	7.7 ± 1.7	116.8 ± 64	492.3 ± 119
	PND4	8.4 ± 6.4	53.5 ± 15	401.5 ± 117
	PND8	0.8 ± 0.22	11.6 ± 6.2	40.1 ± 17
	PND18	1.8 ± 1.1	18.7 ± 8.6	91.7 ± 49
Mammary Gland ^b	GD18	18.9 ± 1.9	307.2 ± 30.4	1429 ± 186
	PND1	27.4 ± 6.8	343.8 ± 53	1933.5 ± 194
	PND4	9.6 ± 8.4	239.2 ± 53	1461.8 ± 267
	PND8	2.4 ± 3.8	71.7 ± 22	411.8 ± 78
	PND18	17.1 ± 10	239.9 ± 76	1372.8 ± 240
Milk ^a	PND2	32.5 ± 12	716.7 ± 145	1236.6 ± 1370
	PND8	11.6 ± 8.1	77.4 ± 19	245.1 ± 26
	PND11	5.4 ± 1.0	42.3 ± 9.1	282.5 ± 162
	PND18	43.5 ± 19	251.8 ± 147	909.8 ± 308
Whole Pup ^b	GD18	136.3 ± 15	1665.8 ± 213	6256.5 ± 751
	PND1	150.9 ± 21	1606.9 ± 288	7134.5 ± 1097
	PND4	91.8 ± 8.9	1183.2 ± 187	5071.4 ± 267
	PND8	60.9 ± 16	729.0 ± 92	3118.5 ± 424
	PND18	17.5 ± 11	251.9 ± 112	1391.5 ± 118
Pup Serum ^a	PND1	324.7 ± 36	3926.8 ± 480	16,286.4 ± 1372
	PND4	267.6 ± 47	3020.8 ± 223	11,925.2 ± 1077
	PND8	260.2 ± 56	2548.2 ± 245	9215.8 ± 594
	PND18	111.8 ± 30	1124.8 ± 236	5894.3 ± 743

GD = gestation day; PND = postnatal day.

^aData are presented as mean ± standard deviation (ng/mL)

^bData are presented as mean ± standard deviation (ng/g)

Macon et al. (2011, 1276151) gavaged CD-1 mice with 0, 0.3, 1.0, or 3.0 mg PFOA/kg from GD1 to GD17 or with 0, 0.01, 0.1, or 1.0 mg PFOA/kg from GD10 to GD17. As shown in Table D-23, at the lowest dose, PFOA concentrations in the serum peaked at or before PND7, but peaked around PND14 for the two higher doses. Calculated blood burdens, which take into account the increasing blood volumes and body weights for females, showed an inverted U-shaped curve peaking at PND14 for all doses. In the liver, PFOA concentrations decreased over time with the highest concentration observed at PND7. Lower concentrations of PFOA were detected in the brain of the offspring on PND7 and PND14. As shown in Table D-24, after exposure to low doses of PFOA from GD10 to GD17, serum PFOA concentration in the female offspring declined from PND1 through the end of the experiment. Calculated blood burden showed a gradual increase from PND1 to PND14, followed by a decline through PND21.

Table D-23. Serum, Liver, and Brain PFOA Concentration in Female CD-1 Mouse Pups After GD10-17 Exposure^a

Tissue	Day	Dose		
		0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
Serum ^a	PND7	4980 ± 218	11026 ± 915	20700 ± 3900
	PND14	4535 ± 920	16950 ± 3606	26525 ± 2446
	PND21	1194 ± 394	377 ± 607	8343 ± 1078
	PND28	630 ± 162	1247 ± 208	4883 ± 1378
	PND42	377 ± 81	663 ± 185	2058 ± 348
	PND63	55 ± 17	176 ± 85	–
	PND84	16 ± 5	71 ± 8	125
	PND84	16 ± 5	71 ± 8	125
Liver ^b	PND7	2078 ± 90	8134 ± 740	16700 ± 749
	PND14	972 ± 124	4152 ± 483	10290 ± 1028
	PND21	1188 ± 182	1939 ± 637	2339 ± 1241
	PND28	678 ± 130	2007 ± 560	7124 ± 1081
	PND42	342 ± 87	617 ± 145	1145 ± 274
	PND63	118 ± 22	320 ± 113	417 ± 160
	PND84	43 ± 12	55 ± 12	235 ± 79
	PND84	43 ± 12	55 ± 12	235 ± 79
Brain ^b	PND7	150 ± 26	479 ± 41	1594 ± 162
	PND14	65 ± 12	241 ± 20	650 ± 44
	PND21	< LOQ ^c	31 ± 5	133 ± 23
	PND28	< LOQ	< LOQ	62 ± 93
	PND42	< LOQ	< LOQ	< LOQ
	PND63	< LOQ	< LOQ	< LOQ
	PND84	< LOQ	< LOQ	< LOQ
	PND84	< LOQ	< LOQ	< LOQ

GD = gestation day; PND = postnatal day; LOQ = limit of quantification; – = not measured.

^aData are presented as mean ± standard deviation (ng/mL)

^bData are presented as mean ± standard deviation (ng/g)

^cLOQ: serum full gestation = 10-20 ng/g; liver = 35 ng/g; brain = 35 ng/g; late gestation serum = 5 ng/mL

Table D-24. Serum PFOA Concentrations in Female CD-1 Mouse Pups After GD10-17 Exposure

Tissue	Day	Dose		
		0.01 mg/kg	0.1 mg/kg	1.0 mg/kg
Serum ^a	PND1	284.5 ± 21.0	2303.5 ± 114.4	16305.5 ± 873.5
	PND4	184.1 ± 12.1	–	–
	PND7	150.7 ± 20.9	1277.8 ± 122.6	11880.3 ± 1447.6
	PND14	80.2 ± 13.9	645.4 ± 114.2	6083.7 ± 662.6
	PND21	16.5 ± 2.1	131.7 ± 24.5	2025.1 ± 281.9
Blood Burden (calculated) ^b	PND1	15.2 ± 1.7	114.3 ± 5.4	926.0 ± 47.6
	PND4	20.6 ± 0.1	–	–
	PND7	27.3 ± 3.8	221.7 ± 24.9	1965.9 ± 256.7
	PND14	27.0 ± 4.6	218.5 ± 39.8	2033.6 ± 293.5
	PND21	7.9 ± 1.0	66.4 ± 12.8	984.7 ± 142.8

PND = postnatal day.

^aData are presented as mean ± standard deviation (ng/mL).

^bBlood burden determined by (body weight x (58.5/1000) x serum x 0.55).

White et al. (2011, 1276150) measured serum PFOA concentrations in three generations of CD-1 mice (Table D-25). Pregnant mice (F₀, n = 10–12 dams/group) were gavage-dosed with 0, 1, or 5 mg PFOA/kg from GD1 to GD17. A separate group of pregnant mice (n = 7–10 dams/group) were gavage-dosed with either 0 or 1 mg PFOA/kg from GD1 to GD17 and received drinking water containing 5 parts per billion (ppb) PFOA beginning on GD7 and continuing until the end of the study for their offspring—except during breeding and early gestation—to simulate a chronic low-dose exposure. Increases in serum PFOA concentrations were observed in the control + 5 ppb PFOA groups of the F₁ and F₂ generations and in the 1 mg/kg + 5 ppb PFOA group of the F₂ generation. Decreases were observed for the remaining groups.

Table D-25. Serum PFOA Concentration in CD-1 Mice Over Three Generations^a

Generation/ Day	Dose			
	0 mg/kg + 5 ppb	1 mg/kg	1 mg/kg + 5 ppb	5 mg/kg
Dams at Weaning				
F ₀ / PND22	74.8 ± 11.3	6658.0 ± 650.5	4772.0 ± 282.4	26980.0 ± 1288.2
F ₁ /~PND91	86.9 ± 14.5	9.3 ± 2.6	173.3 ± 36.4	18.7 ± 5.2
Offspring				
F ₁ /PND22	21.3 ± 2.1	2443.8 ± 256.4	2743.8 ± 129.7	10045 ± 1125.6
F ₁ /PND42	48.9 ± 4.7	609.5 ± 72.2	558.0 ± 55.8	1581.0 ± 245.1
F ₁ /PND63	66.2 ± 4.1	210.7 ± 21.9	187.0 ± 24.1	760.3 ± 188.3
F ₂ /PND22	26.6 ± 2.4	4.6 ± 1.2	28.5 ± 3.7	7.8 ± 1.9
F ₂ /PND42	57.4 ± 2.9	0.4 ± 0.0	72.8 ± 5.8	0.4 ± 0.0

Generation/ Day	Dose			
	0 mg/kg + 5 ppb	1 mg/kg	1 mg/kg + 5 ppb	5 mg/kg
F ₂ /PND63	68.5 ± 9.4	1.1 ± 0.5	69.2 ± 4.3	1.2 ± 0.5

F₀ = parent generation; F₁ = offspring generation 1; F₂ = offspring generation 2; PND = postnatal day.

Data are presented as mean ± standard deviation (ng/mL)

To examine the effect of PFOA on the embryo-placenta unit, Blake et al. (2020, 6305864) exposed CD-1 mice to PFOA at 0, 0.1, or 5 mg/kg-day from embryonic day (E) 1.5 to 11.5 or 17.5 via oral gavage. PFOA levels in the maternal serum, amniotic fluid, and whole embryo are presented in Table D-26. The mean concentration of PFOA in whole embryo is approximately 7 times higher on E 17.5 than E 11.5 for both the 1- and 5-mg/kg/day dose groups. At E 11.5, the levels of PFOA in maternal serum is approximately 5.5 times the levels observed in the amniotic fluid for the 1-mg/kg/day group and 13 times the levels observed in the 5-mg/kg/day group. Dosimetry for amniotic fluid was not reported for the mice examined at E 17.5.

Table D-26. Maternal Serum, Amniotic Fluid, and Whole Embryo PFOA Concentrations in CD-1 Mice Exposed During Gestation Day 1.5-17.5

Biological Matrix	Gestational Age	Dose	
		1 mg/kg/day	5 mg/kg/day
Maternal serum ^a	E 11.5	25.4 ± 3.7	117.3 ± 20.6
	E 17.5	18.7 ± 3.2	95.1 ± 14.1
Amniotic fluid ^a	E 11.5	4.6 ± 2.8	8.8 ± 2.7
	E 17.5	NR	NR
Whole embryo ^b	E 11.5	0.80 ± 0.10	2.34 ± 0.27
	E 17.5	5.78 ± 0.71	16.4 ± 1.75

E = embryonic day; SD = standard deviation; NR = not reported.

^aData are presented as mean ± standard deviation (ng/mL).

^bData are presented as mean ± standard deviation (ng/g).

Transfer of PFASs via lactation does not appear to correlate with lipophilicity (Fujii et al., 2020, 6512379). Lactating FVB/NJcl mice were given a single IV dose of PFOA and other PFCAs chemicals with chain lengths from C8 to C13 on PND8–PND13. Maternal blood and milk were collected from the dam 24 h after administration. The milk/plasma (M/P) concentration ratio for PFOA was 0.32. Ratios exhibited a U-shaped curve with increasing chain length: 0.30 for C9, 0.17 for C10, 0.21 for C11, 0.32 for C12, and 0.49 for C13. While the M/P concentration ratio did not correlate to lipophilicity of PFCAs, the estimated relative daily intake increased with chain length: 4.16 for C8, 8.98 for C9, 9.35 for C10, 9.51 for C11, 10.20 for C12, and 10.49 for C13. These findings suggest that the amount transferred from mothers to pup during lactation may also relate to chain length-dependent clearance.

D.2.5 Volume of Distribution Data

D.2.5.1 Human Studies

Several researchers have attempted to characterize PFOA exposure and intake in humans through PK modeling {Lorber and Egeghy, 2011, 2914150; Thompson, 2010, 2919278}. As an integral

part of model validation, the parameter for V_d of PFOA within the body was calibrated from the available data. In the models discussed in Section 3.2, V_d was defined as the total amount of PFOA in the body divided by the blood or serum concentration.

Two groups of researchers defined a V_d of 170 mL/kg body weight for humans for use in a simple, single compartment, first-order PK model (Lorber and Egeghy, 2011, 2914150; Thompson, 2010, 2919278). The models developed by these groups were designed to estimate intakes of PFOA by young children and adults (Lorber and Egeghy, 2011, 2914150) 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 the NHANES and assumes that most PFOA intake is from contaminated drinking water. Thus, in using the models to derive an intake from contaminated water, the V_d was calibrated so that model prediction of elevated blood levels of PFOA matched those seen in residents.

The assignment of V_d values used in several modelling studies is shown in Table D-27. The value of 170 mL/Kg is frequently used when considering both males and females. Mondal et al. (2014, 2850916) assigned a value 198 mL/kg for breastfeeding females. Shin et al. (2013, 5082426) assigned values by sex (181 mL/kg for males and 198 mL/kg for females). Gomis et al. (2017, 3981280) used a higher V_d of 200 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-27. Summary of PFOA Volume of Distribution Values Assigned in Human Studies

Study	Population	Sex	Compartment	V _d	AUC or Mean/Median Concentration Measured in Compartment	Steady State Considerations
Mondal et al., 2014, 2850916	Adult, breastfeeding	Females	Maternal serum	198 mL/kg	GM Breastfeeding :18.32 ng/mL (95% CI: 16.36, 20.50) GM Non-breastfeeding: 19.26 (16.80, 22.08)	NR
Zhang et al., 2015	Adult	Males and females	Whole blood	170 mL/kg	Mean: 2.71; GM: 2.47	Steady state assumed
	Adult, pregnant	Females	Whole blood	170mL/kg	Mean: 3.36; GM: 3.09	Steady state not assumed due to variable PFAS levels during pregnancy
Worley et al., 2017, 3859800	> 12 years	Males and females	Blood (2016)	170 mL/kg bodyweight	Mean: 11.7 µg/L (95 CI: 8.7–14.6)	NR
	> 12 years	Males and females	Blood (2010)	170 mL/kg bodyweight	Mean: 16.3 (95 CI: 13.2–19.6)	NR
Fu et al., 2016, 3859819	Adult, occupational	Males and females	Serum	170 mL/kg	Mean: 1052 ng/mL Median: 427 ng/mL	NR
Zhang et al., 2013, 3859849	Adults	Males and females	Serum and whole blood	170 mL/kg	Mean: 3.1 ng/mL	NR
Shin et al., 2013, 5082426	Adult, nonoccupational	Males	Serum	181 mL/kg	Median predicted: 13.7ppb; observed 23.5ppb (updated values in Erratum)	NR
	Adult, nonoccupational	Females	Serum	198 mL/kg	Median predicted: 13.7ppb; observed 23.5ppb (updated values in Erratum)	NR
Gomis et al., 2017, 3981280	Human and animals	Males and females	Serum	200 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 in the past decade.

V_d = volume of distribution; AUC = area under curve; GM = geometric mean; NR = not reported; CI = confidence interval.

D.2.5.2 Animal Studies

In Fujii et al. (2015, 2816710), PFOA distribution in male and female FVB/NJcl mice (8–10 weeks of age) administered by IV (0.31 $\mu\text{mol/kg}$) or gavage (3.13 $\mu\text{mol/kg}$) was determined using a two-compartment model. Serum PFOA concentrations varied linearly by dose regardless of route. The V_d after IV injection was calculated as dose/ $C(0)$. As shown in Table D-28, the V_d of PFOA was low in mice after IV injection and exhibited no differences between sexes. The low serum V_d was consistent with the high percentage (32.3%) of administered dose calculated for serum. The measured percentage of administered dose was higher in the liver (47.4%) although V_d for this compartment was not calculated.

In this study, the authors examined perfluoroalkyl carboxylic acids (PFCAs) with chain lengths between 6 and 14 and observed that V_d increased as a function of chain length in both males and females. The authors suggested that this may be linked to the lipophilicity of PFCAs and their increasing affinity for serum and liver fatty acid binding proteins. For PFOA, V_d corresponded to the volume of extracellular water. Interestingly, V_d values corresponded to different compartments based on chain length, specifically the total volume of blood for C7 and the volume body water for C11 and C12).

Table D-28. PFOA Volume of Distribution in Serum of FVB/NJcl Mice

Route	Dose ($\mu\text{mol/kg}$)	Sex	V_d l kg^{-1} a	AUC $\mu\text{mol l}^{-1}$ hour (0 to 24 hours)a
IV	0.313	Male	0.18 ± 0.04	42.2 ± 9.9
IV	0.313	Female	0.15 ± 0.04	49.5 ± 11.9
Oral ^b	3.13	Male	NR	348 ± 76
Oral ^b	3.13	Female	NR	495 ± 64

V_d = volume of distribution; AUC = area under curve; NR = not reported.

^a V_d and AUC reported as means \pm standard deviation.

^bSteady state achieved 8 days after initial dose (oral).

Two recent studies (Kim et al., 2016, 3749289; Dzierlenga et al., 2019, 5916078) measured toxicokinetic parameters in rats, including V_d . 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 (Table D-29). Similar to the Fujii et al. (2015, 2816710) study in mice, V_d values were similar in males and females. While organ specific V_d values were not determined, the liver and kidney exhibited partition coefficients greater than 1 in males (2.31 ± 0.38 for liver and 1.18 ± 0.47 for kidney). While the partition coefficients in females for the kidney (1.23 ± 0.39) were similar to males, they were significantly lower in the livers of females (0.81 ± 0.36) compared with males. Partition coefficients were similar in males and females for the heart, lung, and spleen. Although V_d values were not significantly different between males and females, the differential partition coefficients in liver and kidney may relate to the higher V_d values calculated for females compared to males.

Dzierlenga et al. (2019, 5916078) calculated the apparent volume of central (V_1) and peripheral (V_2) distribution in rats. In this study, the plasma concentration-time profiles were best described using one-compartment models in males and a two-compartment model in females. As detailed in Table D-29, males and females were administered different doses that were higher than those used in the Kim et al. (2016) study. Females were administered 40–320 mg/kg compared to 6–

48mg/kg in males. Several observations were apparent for V_d in males. V_d values were substantially lower in the peripheral compartment compared to the central compartment, and V_{ds} were substantially lower in the peripheral compartment after IV administration relative to oral administration. V_{ds} were similar after oral dosing at 6 and 12 mg/kg (159 ± 12 and 154 ± 11 mL/kg, respectively) and only increased at the highest dose of 48 mg/kg (202 ± 18 mL/kg). In contrast to males after IV dosing, female V_d values were similar in central and peripheral compartments (108 ± 24 and 98.7 ± 39.8 mL/kg, respectively) although the dose in females of 40 mg/kg was substantially higher than the 6 mg/kg dose in males.

In females, both peripheral and central V_{ds} were calculated after oral dosing at all doses. Peripheral V_d values were dramatically lower than central V_d values at all doses by the oral route (Table D-29). These trends are consistent with the observations that peak tissue levels were reached readily in both males and females. However, while tissue levels in males were steady over the course of several days, tissue levels in females dropped quickly (in the span of hours), which likely reflects the shorter half-life in females.

In a third study (Iwabuchi et al., 2017, 3859701), PFOA 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 blood, serum, and several tissues. The whole blood V_d (0.42 kg tissue volume/kg BW) was almost threefold higher than the serum V_d . Organ V_d values were highest in the brain (9.0 kg tissue volume/kg BW) and spleen (2.3 kg tissue volume/kg BW). V_{ds} were 1–2 orders of magnitude lower in the heart, kidney, and liver (0.91, 0.27, and 0.083 kg tissue volume/kg BW, respectively). An interesting observation from this analysis is that, for PFOA, 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.

A single study examined V_d in primates. Butenhoff et al. (2004b, 3749227) calculated a V_d from noncompartmental PK analysis of data from cynomolgus monkeys. Three males and three females were administered a single IV dose of 10 mg/kg, and serum PFOA concentrations were measured in samples collected up to 123 days postdosing. The V_d of PFOA at steady state (V_{dss}) was similar for both sexes at 181 ± 12 mL/kg for males and 198 ± 69 mL/kg for females.

Table D-29. Summary of PFOA Volume of Distribution Calculations in Rats

Study	Method of V_d Calculation	Route	Dose	Strain	Age	Sex	V_d	Compartment	Concentration Measured in Compartment ^a	C_{max}
Kim et al., 2016, 3749289	Dose \times AUMC/(AUC0- ∞) ²	Oral	1 mg/kg	Sprague-Dawley	8-12 weeks	Males	106.4 \pm 8.90 mL/kg	Blood plasma	AUC: 24.81 \pm 1.41 μ g day/mL	7.55 \pm 0.51 μ g/mL
						Females	153.83 \pm 9.19 mL/kg	Blood plasma	AUC: 1.39 \pm 0.06 μ g day/mL	5.41 \pm 0.38 μ g/mL
		IV	1 mg/kg	Sprague-Dawley	8-12 weeks	Males	112.12 \pm 29.41 mL/kg	Blood plasma	AUC: 21.10 \pm 1.51 μ g day/mL	8.92 \pm 2.34 μ g/mL
						Females	171.37 \pm 11.19 mL/kg	Blood plasma	AUC: 1.63 \pm 0.09 μ g day/mL	5.84 \pm 0.38 μ g/mL
Dzierlenga et al., 2019, 5916078	Standard equations (Gabrielsson, 2000, 9642135)	Oral	6 mg/kg	Sprague-Dawley	8 weeks	Males	159 \pm 12 mL/kg	Peripheral	AUC: 39.37 \pm 2.42 mM h	0.089 \pm 0.007 mM
			12 mg/kg	Sprague-Dawley	8 weeks	Males	154 \pm 11 mL/kg	Peripheral	AUC: 69.79 \pm 3.86 mM h	0.185 \pm 0.013 mM
			48 mg/kg	Sprague-Dawley	8 weeks	Males	202 \pm 18 mL/kg	Peripheral	AUC: 178.4 \pm 12.1 mM h	0.560 \pm 0.048 mM
			40 mg/kg	Sprague-Dawley	8 weeks	Females	73.6 \pm 20.6 mL/kg	Central	AUC: 5.217 \pm 0.507 mM h	0.580 \pm 0.060 mM
							5.55 \pm 1.62 mL/kg	Peripheral	AUC: 5.217 \pm 0.507 mM h	0.580 \pm 0.060 mM
			80 mg/kg	Sprague-Dawley	8 weeks	Females	130 \pm 24 mL/kg	Central	AUC: 8.066 \pm 0.869 mM h	0.961 \pm 0.118 mM
							19.9 \pm 12.9 mL/kg	Peripheral	AUC: 8.066 \pm 0.869 mM h	0.961 \pm 0.118 mM
			320 mg/kg	Sprague-Dawley	8 weeks	Females	272 \pm 1990 mL/kg	Central	AUC: 57.00 \pm 7.97 mM h	2.06 \pm 0.61 mM

Study	Method of V_d Calculation	Route	Dose	Strain	Age	Sex	V_d	Compartment	Concentration Measured in Compartment ^a	C_{max}
		IV	6 mg/kg	Sprague-Dawley	8 weeks	Males	69.9 ± 1849.1 mL/kg	Peripheral	AUC: 57.00 ± 7.97 mM h	2.06 ± 0.61 mM
							114 ± 5 mL/kg	Central	AUC: 28.0 ± 1.69 mM h	0.127 ± 0.006 mM
							39.2 ± 14.5 mL/kg	Peripheral	AUC: 28.0 ± 1.69 mM h	0.127 ± 0.006 mM
			40 mg/kg	Sprague-Dawley	8 weeks	Females	108 ± 24 mL/kg	Central	AUC: 2.87 ± 0.31 mM h	0.893 ± 0.196 mM
							98.7 ± 39.8	Peripheral	AUC: 2.87 ± 0.31 mM h	0.893 ± 0.196 mM
Iwabuchi et al., 2017, 3859701	Dose / $k_e \times$ plasma concentration (AUC)	Oral	100 µg/kg, single dose	Wistar	7–9 weeks	Males	9.0 kg tissue volume/kg BW	Brain	160 µg/kg tissue volume - day	8.77 µg/kg
							0.91 kg tissue volume/kg BW	Heart	1500 µg/kg tissue volume - day	108 µg/kg
							0.083 kg tissue volume/kg BW	Liver	35000 µg/kg tissue volume - day	1270 µg/kg
							2.3 kg tissue volume/kg BW	Spleen	630 µg/kg tissue volume - day	49.2 µg/kg
							0.27 kg tissue volume/kg BW	Kidney	6600 µg/kg tissue volume - day	624 µg/kg

Study	Method of V_d Calculation	Route	Dose	Strain	Age	Sex	V_d	Compartment	Concentration Measured in Compartment ^a	C_{max}
							0.42 kg tissue volume/kg BW	Whole blood	4300 µg/kg tissue volume - day	265 µg/kg
							0.15 kg tissue volume/kg BW	Serum	9200 µg/kg tissue volume - day	759 µg/kg

V_d = volume of distribution; AUC = area under curve; NR = not reported; AUMC = area under first moment curve; BW = body weight; C_{max} = maximum plasma concentration; k_e = elimination rate constant.

^aPresented as AUC or Mean/Median.

D.3 Metabolism

PFOA does not appear to be metabolized in mammals. In a recent study, Gannon et al. (2016, 3810188) investigated the metabolism of PFOA in vivo and in vitro using rodent models. Specifically, male and female mice (Crl:CD1(ICR)) and rats (Sprague-Dawley) were exposed to a single oral dose of PFOA at 3 mg/kg and 30 mg/kg, respectively. Urine samples collected from both rodent species were analyzed by high-performance liquid chromatography. The authors subsequently screened for metabolites using the control-comparison tool, IntelliExtract™. Only the anionic form of PFOA was detected. There was almost complete recovery of the dose in the urine, confirming that PFOA is not metabolized. In addition, normal and heat-inactivated rat hepatocytes (5×10^6 cells/mL) were exposed to 50 μ M of PFOA in a 3-mL suspension. No differences in clearance rate were found and no metabolites were detected.

D.4 Excretion

D.4.1 Urinary and Fecal Excretion

D.4.1.1 Human Studies

The majority of human studies predominantly consider PFOA excretion after oral exposure, either implicitly or explicitly. The urinary excretion of PFOA in humans is impacted by the isomeric composition of the mixture present in blood and the sex/age of the individual. 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. Fewer studies have examined excretion through the fecal route. Animal studies suggest that sex and competing PFAS compounds influence fecal excretion.

Several major studies highlight the urinary excretion of PFOA in humans. T. Zhang et al. (2014, 2851103) derived estimates for PFOA'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 detected PFOA concentration in first-draw morning urine samples by the predicted urinary volume (1600 mL/day for males and 1200 mL/day for females). PFOA was detected in the blood samples for all participants but for only 76% of the urine samples from the general population and 30% for the pregnant females. Total daily PFOA intake was modeled for the general population and used to estimate a daily urinary excretion rate of 25%, but was higher in males than in females (31% and 19%, respectively). In contrast to the estimates relating to PFOS, there was little difference in urine:blood ratio between nonpregnant females age 21-50 and those age 51-61, although the urine:blood ratio was found to be lower for pregnant females than nonpregnant females (0.0011 and 0.0029, respectively), suggesting the placenta and cord blood as possible elimination pathways. There was a direct correlation between the PFOA concentrations in blood and creatinine adjusted urine ($r = 0.348$ $p = 0.013$) for the general population but not for the pregnant females. When limited to the eight females who had detectable levels in both blood and urine, there was a significant correlation ($r = 0.724$, $p = 0.042$).

Zhang et al. (2013, 3859849) calculated median renal clearance rates of 0.16 mL/kg/day in young women and 0.19 mL/kg/day in men and older women for total PFOA. In a later study, Fu et al. (2016, 3859819) determined the renal clearance half-lives of PFOA 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. The participants were subdivided based on their work assignment. Serum PFOS and PFHxS were highest in workers of the sulfonation department and the serum PFOA levels were highest in workers from the electrochemical fluorination department.

Serum PFOA concentrations were in the ranges of 2.52-32,000 ng/mL (median 424 ng/mL). The average concentrations of serum PFOA was significantly higher in males ($1,215 \pm 2,936$) ng/mL than in females (659 ± 743) ng/mL. The median urine concentration for all workers was 4.3 ng/mL (range (LOD – 53.6 ng/mL). The correlation coefficient of PFOA concentrations in paired serum and urine samples of 0.64 was found to be highly statistically significant, ($p < 0.01$), suggesting that urine concentrations could serve as effective bioindicators for PFOA 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 0.067mL/day/kg) followed by PFOS (GM0.010 mL/day/kg). The high efficiency of PFOA renal clearance was reflected in the relative abundance of PFOA from 12% in the serum samples to 42% in the urine samples. Sex did impact daily renal clearance values, which were significantly lower in males compared to females ($p < 0.01$).

A single case report study demonstrated fecal excretion of PFOA in humans. Fecal PFOA was measured in an exposed man before and after treatment with bile sequestering agents (Genuis et al., 2010, 2583643). Before treatment, his urine and stool levels of PFOA levels were 3.72 ng/mL and below detectable limits (0.5 ng/g), respectively. After treatment with cholestyramine, PFOA measurements in stool increased to 0.96 ng/g in the first weeks after treatment and to 1.19 ng/g several months later after subsequent treatments with saponins.

Urinary clearance of PFCAs in humans was observed to decrease with increasing alkyl chain lengths, while biliary clearances increased (Fujii et al., 2015, 2816710). In these studies, paired bile-serum and urine-serum were obtained from the archived samples in the Kyoto University Human Specimen Bank. Bile samples were taken by nasobiliary drainage, percutaneous transhepatic biliary drainage or percutaneous transhepatic gallbladder drainage for 24 hours. Blood samples were taken from the same patients on the same day. Blood and urine were also collected from healthy volunteers. Human data were analyzed from paired (bile-serum) archived samples from patients undergoing nasobiliary drainage, percutaneous transhepatic biliary drainage, or percutaneous transhepatic gallbladder drainage for 24 hours. Urine-serum pairs were collected from healthy donors. Urinary and biliary clearance was determined by dividing the cumulative urine or bile excretion in a 24-h period with the serum concentration. Fecal clearance was calculated using the estimated biliary resorption rate.

The authors estimated that human total clearances were 0.096 mL/kg/day and were 50-100 times smaller than those estimated in mice after oral gavage dosing. In humans, PFOA clearance rates via urinary, biliary, and fecal routes were estimated to be 0.044, 2.62, and 0.052 mL/kg/day, respectively. The reabsorption rate of bile excreting C8 was estimated to be 0.98 (derived by assigning a V_d of 200 mL/kg, a serum half-life of 3.8 years, and the presumption that that C8 could only be excreted into the urine and feces via the bile).

Interestingly, perfluoroalkyl carboxylic acids (PFCAs) with chain lengths of C6 and C7 were rapidly excreted into urine, whereas PFOA and PFCAs with longer chain lengths were deposited mainly in the liver. Thus, chain length for PFCAs may be a major determinant of bioaccumulation as well as excretion rate and route. These authors also conducted a toxicokinetics analysis in mice (discussed in the next section). The ascertained that human urinary clearances for PFCAs were more than 200 times smaller than those in mice. Fecal clearances in humans were also an order of magnitude lower than those estimated in mice after oral gavage and IV dosing (ranging from 1.1 to 4.3 mL/day/kg) also differed by one order of magnitude, indicating the other membrane transporters in the liver may also be involved.

Although no data were identified on urine or fecal excretion of PFOA following inhalation exposures in humans, the Hinderliter study (2006, 135732) provides evidence of clearance following single and repeated inhalation exposures in Sprague-Dawley rats. Plasma PFOA concentrations following a single exposure to 1, 10, or 25 mg/m³ PFOA declined 1 hour after exposure in females and 6 hours after exposure in males. In females, the elimination of PFOA was rapid at all exposure levels and, by 12 hours after exposure, their plasma levels had dropped below the analytical LOQ (0.1 µg/mL). In males, the plasma elimination was much slower and, at 24 hours after exposure, the plasma concentrations were approximately 90% of the peak concentrations at all exposure levels. In the repeated exposure study, male and female rats were exposed to the same concentrations for 6 hours/day, 5 days/week for 3 weeks. Steady-state plasma levels were reached in males by 3 weeks, but plasma PFOA levels in females returned to baseline within 24 hours of each dose.

No data were identified on excretion following dermal exposures. Minimal fecal excretion is anticipated for the dermal route of exposure although the biliary pathway can be a route for excretion of material absorbed through the skin, distributed to the liver, and discharged to the gastrointestinal tract.

D.4.1.2 Animal Studies

Butenhoff et al. (2004, 3749227) studied the fate of PFOA in cynomolgus monkeys in a 6-month oral exposure study. Groups of four to six male monkeys each were administered PFOA daily via oral capsule at DRs of 0, 3, 10, and 30/20 mg/kg for 6 months, with urine and fecal samples collected at 2-week intervals. All dosed groups reached steady-state urine PFOA levels after four weeks, which were 53 ± 25 , 166 ± 83 , and 181 ± 100 µg/mL, respectively. Two monkeys exposed to 10 mg/kg and three monkeys exposed to 20 mg/kg were monitored for 21 weeks (recovery period) following dosing. Within two weeks of recovery, urine PFOA concentrations were <1% of the value measured during treatment and decreased slowly thereafter. Lower amounts were excreted in feces. These results are consistent with both renal and biliary excretion in male monkeys.

There have been a number of studies of excretion in rats because of the sex differences noted in serum levels. Hinderliter et al. (2006, 3749132) investigated the relationship between age and urine PFOA concentrations in male and female Sprague-Dawley rats. Immature rats 3, 4, or 5 weeks of age were administered PFOA via oral gavage as a single dose of 10 or 30 mg/kg, and urine was collected for 24 hours.

Urine PFOA concentrations differed significantly ($p < 0.01$) with age, dose, and sex. For all doses and ages, urinary excretion of PFOA was substantially higher in females than in males, and this difference increased with age, as female excretion increased and male excretion decreased. In both sexes, urine PFOA was higher (2.5 to 6.5 times) at the 30-mg/kg dose as compared to the 10-mg/kg dose (Table D-30).

Table D-30. Urine PFOA Concentrations in Male and Female Sprague-Dawley Rats, 24-Hours After Oral Gavage^a

Age (weeks)	Dose (mg/kg)	Urine PFOA			
		Male		Female	
		Mean	SD	Mean	SD
3	10	9.57	4.86	21.17	8.95
4	10	4.53	2.45	23.26	15.27
5	10	4.03	2.36	49.77	24.64
3	30	51.76	28.86	94.89	26.26
4	30	28.70	18.84	104.12	28.97
5	30	15.65	6.24	123.16	51.56

SD = standard deviation.

Data are presented as mean \pm standard deviation ($\mu\text{g/mL}$)

Kim and colleagues (2016, 3749289) extended the study of male and female Sprague Dawley rats to evaluate fecal excretion. They also compared oral and intravenous administration of PFOA, giving a single 1 mg/mL dose by either pathway. Urine and feces were measured daily for 12 days in males and females after dosing. Like previous studies, the highest concentrations were found in urine under all conditions. In males, the levels detected in urine and feces were very similar from both oral and intravenous exposure. By the oral route, 26.42 ± 2.64 ug was detected in urine vs 23.60 ± 9.45 ug in feces. Levels were even more similar in male rats dosed intravenously (22.47 ± 1.94 ug in urine vs 21.13 ± 12.31 ug in feces). In contrast, females excreted much higher levels in urine compared to males and compared to feces. After oral administration, urine and fecal levels were 124.95 ± 6.38 ug and 24.60 ± 4.18 ug, respectively. The values measured after intravenous administration were similar to those observed after oral dosing (131.87 ± 6.82 ug in urine vs 18.04 ± 1.35 ug in feces). The differences between males and females in amounts detected in urine and feces translated to significant differences in the estimated half-life values (1.64 and 1.83 days in males versus 0.15 and 0.19 days in females by the oral and intravenous routes).

Other studies comparing urinary and fecal excretion following PFOA administration by gavage among male Sprague-Dawley rats have found much higher excretion rates from urine than from feces (Benskin et al., 2009, 1617974; Cui et al., 2010, 2919335). Benskin et al. (2009, 1617974)

gave single doses of 0.5 mg PFOA/kg to each rat and monitored for 38 days, while Cui et al. (2010, 2919335) gave 0, 5, or 20 mg/kg/day over 28 days and monitored for the duration. Among the single-dose rats, 91–95% of the daily excreted PFOA was eliminated in the urine after the initial 24 hours. On day 3, the mean PFOA concentration in urine and feces were 265 ng/g and 28 ng/g. The half-life for elimination from plasma in male rats was 13.4 days (Benskin et al., 2009, 1617974).

Among the repeated dose rats, a sharp increase in urinary and fecal excretion expressed as percent of dose/day was observed during week 1 in rats of both dose groups. The excretion rate leveled off at about 50% for the low-dose animals for the remainder of the 28 days. In the case of the high-dose animals, the urinary excretion remained level at about 80% for the second and third weeks and then increased sharply to about 140% at 28 days. The fecal excretion rates followed an upward trend throughout the 28 days with the terminal percent/day about 25% for the low-dose group and 40% for the high-dose group.

Studies on male and female CD rats have similar findings to those done in Sprague-Dawley rats; namely, that females excreted PFOA more efficiently than males, excretion rates increased with higher dosages, and both sexes excreted more PFOA by urine than by feces. Hundley et al. (2006, 3749054) examined excretion of PFOA in one male and one female CD rat, giving each a single dose of 10 mg/kg ¹⁴C-PFOA and collecting urine and feces at 12-24 hour intervals for five days post-dose (Table D-31). Kemper gave either single or repeat doses ranging from 1-25 mg/kg (Table D-32) and collected urine and feces for 7 or 28 days for females and males, respectively. Hundley et al. found that the female rat had excreted almost all dosed ¹⁴C-PFOA within 48 hours, with urinary excretion accounting for about 2.65 times the amount of fecal excretion. In the male rat, PFOA was excreted from urine at a similar rate relative to fecal excretion, but much slower overall; only about 19% had been excreted after 48 hours, and only 34% after 120 hours. Kemper (2003, 6302380) found that after 28 days, singly dosed male rats excreted 47-68% of the initial dose; interestingly, while the females consistently excreted more of the PFOA than males, none of the dose groups were found to eliminate 100% of the ¹⁴C-PFOA after 7 days.

Table D-31. Cumulative Percent ¹⁴C-PFOA Excreted in Urine and Feces by Male and Female CD Rats^a

Rat	Hours After Dosing					
	12	24	48	72	96	120
Male	0.6	8.7	19.2	23.4	30.2	34.3
Female	52.5	96.4	99.8	100.0	100.0	100.0

¹⁴C -PFOA = ¹⁴C-Radiocarbon perfluorooctanoic acid.

^aData is presented in % total dose administered.

Table D-32. Percentage of Dose Excreted in Urine and Feces of Male and Female Sprague-Dawley Rats exposed to ¹⁴C-PFOA via Oral Gavage

Dose and Regimen	Sex	Urine ^a	Feces ^b
Single Dose 1 mg/kg	Male	43.238 ± 3.015	14.055 ± 4.003

Dose and Regimen	Sex	Urine ^a	Feces ^b
Single Dose 5 mg/kg	Female	75.872 ± 4.066	2.169 ± 2.923
	Male	62.201 ± 3.656	5.568 ± 1.779
Single Dose 25 mg/kg	Female	77.867 ± 6.034	5.886 ± 5.387
	Male	53.265 ± 8.490	12.490 ± 4.153
Repeated Dose 1 mg/kg/day	Female	84.381 ± 12.023	1.868 ± 2.546
	Male	52.430 ± 7.959	19.841 ± 6.620
	Female	68.537 ± 16.631	12.384 ± 15.775

¹⁴C -PFOA = ¹⁴C-Radiocarbon perfluorooctanoic acid; SD = standard deviation.

^aData are presented as mean ± standard deviation (µg/ml)

^bData are presented as mean ± standard deviation (µg/g)

Dose is an important variable that impacts excretion. Rigden et al. (2015, 7907801) exposed groups of five male Sprague-Dawley rats to doses of 0, 10, 33, and 100 mg/kg/day for 3 days and maintained them for 3 additional days; overnight urine was collected and body weight was measured daily. Of greatest interest relative to the limitations on renal resorption, is the dose-related increase in urine PFOA concentration and urine PFOA concentration per mg creatinine for the 33- and 100-mg/kg/day groups compared to the 10-mg/kg/day group. The peak in PFOA excretion normalized to creatinine occurred on day 3 after the cessation of dosing. The concentration at 33 mg/kg/day was 500 times greater than that at 10 mg/kg/day. At the 100-mg/kg/day dose, the peak concentration was about 3,200 times greater than for the low dose. The low-dose excretion was only slightly greater than the controls. The urine results support the renal resorption hypothesis concept and suggest that there is a threshold limit on resorption that, once exceeded, dramatically increases PFOA loss in urine. As a consequence, half-life for continuous low-dose exposures will be longer than for single or short-term high-dose exposures.

Another study (Gao et al., 2014, 2851191) also compared concentrations in urine and feces of male and female Wistar rats. A mixture of PFOA/PFNA/PFOS were administered to the 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 PFAS 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. These translated to intake values for PFOA, PFNA and PFOS of 0.15 and 0.12 mg/kg bw, 1.52 and 1.22 mg/kg bw, and 13.6 and 17.7 mg/kg bw for female and male rats, respectively. At the high dose of 5 mg/L, there were higher levels of PFOA in urine and feces of males and females. However, and in contrast to that observed by others, there were far higher levels of PFOS in feces compared to urine for both males and females. 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 PFNAs.

Hundley et al. (2006, 3749054) examined excretion of PFOA in CD mice, BIO-15.16 hamsters, and New Zealand White rabbits. One male and one female of each species was given a single dose of 10-mg/kg ¹⁴C-PFOA and housed in metabolism cages. Urine and feces were collected at

12-24 hour intervals for five days post-dose. Additional samples were collected from rabbits at 144 and 168 hours post-dose.

Over 120 hours, both mice excreted similar amounts of PFOA, although the male mouse excreted a greater proportion in feces (3.4% ¹⁴C-PFOA in urine and 8.3% ¹⁴C-PFOA in feces), and the female mouse excreted more via urine (6.7% ¹⁴C-PFOA in urine and 5.7% ¹⁴C-PFOA in feces). The male hamster excreted far more than the female, although both excreted more via urine than by feces; the male excreted 90.3% and 8.2% ¹⁴C-PFOA in urine and feces, respectively, and the female hamster excreted 45.3% and 9.3% ¹⁴C-PFOA. Over 168 hours, both rabbits excreted most of the original dose, and both predominantly excreted via urine (76.8% and 4.2% ¹⁴C-PFOA from the male, and 87.9% and 4.6% ¹⁴C-PFOA from the female in urine and feces, respectively). The cumulative percentages of ¹⁴C-PFOA excreted are shown in Table D-33.

Table D-33. Cumulative Percent ¹⁴C-PFOA Excreted in Urine and Feces in Mouse, Hamster, and Rabbit^a

Species	Sex	Hours After Dosing						
		12	24	48	72	96	120	168
Mouse	Male	0.4	4.1	6.7	8.6	9.1	10.8	–
	Female	0.2	4.1	6.5	8.4	9.0	11.0	–
Hamster	Male	67.3	84.5	96.1	97.4	98.2	98.4	–
	Female	11.3	24.6	36.4	43.9	50.1	54.0	–
Rabbit	Male	77.8	80.2	80.4	80.4	80.4	80.4	80.4
	Female	86.7	90.5	92.0	92.2	92.7	92.9	93.0

¹⁴C -PFOA = ¹⁴C-Radiocarbon perfluorooctanoic acid.

^aData is presented in % of total dose administered

Fujii and colleagues (2015, 2816710) compared elimination in humans and mice exposed to using a two-compartment model. Toxicokinetics and clearance was investigated in FVB/NJcl mice exposed by oral gavage and intravenous administration of perfluoroalkyl carboxylic acids (PFCAs) with carbon chain lengths between C6 and C10. At 24 hours after exposure, urine and feces were collected in metabolic cages. In mice, the short-chained PFCAs (C6 and C7) were rapidly eliminated in the urine, whereas long-chain PFCAs (C8 to C14) accumulated in the liver and were excreted slowly in feces. For PFOA administered IV, urinary clearance was higher in males (13.1 ml/day/kg) compared to females (9.8 ml/day/kg). PFOA administered by oral gavage was also higher in males (9.2 ml/day/kg) compared females (6.6 ml/day/kg), but clearance was significantly lower than rates measured after IV administration.

Fecal clearance of PFOA after IV administration was higher in females (2.0 mL//day/kg) compared to males (1.1 mL/day/kg). After gavage administration, the opposite was observed with higher rates observed in males (4.0 mL//day/kg) compared to females (2.4 mL/kg/day). The feces clearance after 24 hours of gavage administration represents PFOA contained in the bile and unabsorbed PFCAs that passed through the gut, and this likely accounts for the higher fecal

clearance after gavage dosing. The actual fecal clearances of PFCAs were represented by the fecal clearances of IV-administrated PFCAs. In contrast to urinary clearance, fecal clearance rates were still lower than urinary clearance rates by both dosing routes.

Interestingly, these authors also estimated urinary and fecal clearance rates in humans, which were 1-2 orders of magnitude lower than rates estimated in mice. This study illustrates chain length, sex, and species have dramatic impacts on the rate and route of PFOA excretion.

Studies in animals provide evidence that urine is typically the primary route of excretion but that sex impacts excretion by both routes, and these sex differences appear to be species-specific. Limited evidence supports excretion through the fecal route in animals and humans and through hair in animals. Most studies indicate excretion by the fecal route is substantially lower than that observed by the urinary route. Excretion through the fecal route appears to be more efficient in males compared to females and in rodents compared to humans. Also, exposures to mixtures of PFNAs may also alter the relative amounts of PFOA excreted through the fecal route, quite possibly due to differential lipophilicity and cellular uptake as well as differential affinities for transporters associated with chain length and branching. Nevertheless, a comprehensive set of principles governing resorption by renal, hepatic and enteric routes and how these impact excretion and retention of PFOA has not been established in either humans or animals.

D.4.2 Physiological and Mechanistic Factors Impacting Excretion

D.4.2.1 Renal Resorption

Several studies have been conducted to elucidate the cause of the sex difference in the elimination of PFOA by rats. Many of the studies have focused on the role of transporters in the kidney tubules, especially the OATs located in the proximal portion of the descending tubule. OATs are found in other tissues as well and were discussed earlier for their role in absorption and distribution. In the kidney, they are responsible for delivery of organic anions (including a large number of medications) from the serum into the kidney tubule for excretion, as well as reabsorption of anions from the glomerular filtrate. The transporters are particularly important in excretion of PFOA because it binds to surfaces of serum proteins (particularly albumin), which makes much of it unavailable for removal during glomerular filtration. Other transporter families believed to be involved in renal excretion are the OATPs and the MRPs. However, they have not been evaluated as extensively as the OATs for their role in renal excretion.

OATs are located on both the basolateral (serum interface) and apical surfaces of the brush boarder of the proximal tubule inner surface. At the basolateral surface, the OATs transport the perfluorooctanoate anion from the serum to the tubular cells {Anzai, 2006, 9642039}{Cheng, 2008, 758807}{Klaassen, 2010, 9641804}{Klaassen, 2008, 9642044}{Nakagawa, 2007, 2919370}{Nakagawa,2009, 2919342}. OAT1, 2, and 3 are located on the basolateral membrane surface. OAT4 and OAT5 are located on the apical surface of the tubular cells, where they reabsorb the PFOA anions from the glomerular filtrate. Figure D-1 diagrams the flow of organic anions such as the PFOA anion from serum to the glomerular filtrate for excretion and resorption of organic acids from the glomerular filtrate with transport back to serum. OATs can function for uptake into the cell across both the basolateral and apical surfaces.

Several MRP transporters also appear to function in the kidney and move organic anions in and out of cells at both the basolateral surface (e.g., MRP2/4) and the apical surface (e.g., MRP1) as well as one or more OATPs on each surface {Cheng, 2009, 4116789}{Klaassen, 2010, 9641804}{Klaassen, 2008, 9642044}{Kusuhara, 2009, 9641810}{Launay-Vacher, 2006, 9641802}{Yang, 2009, 2919328}. Bidirectional movement of PFOA across both the basolateral and apical surfaces is driven by concentration gradients and/or active transport. Far more data exist on PFOA and OATs in the kidneys than on OATPs and MRPs. Abbreviations for individual transporters on the basolateral and apical surfaces differ across publications. The accepted convention is to use uppercase letters to refer to human transporters and lowercase letters to refer to animal transporters. For this report, the data are not reported by species but by transporter family and the uppercase letters are used.

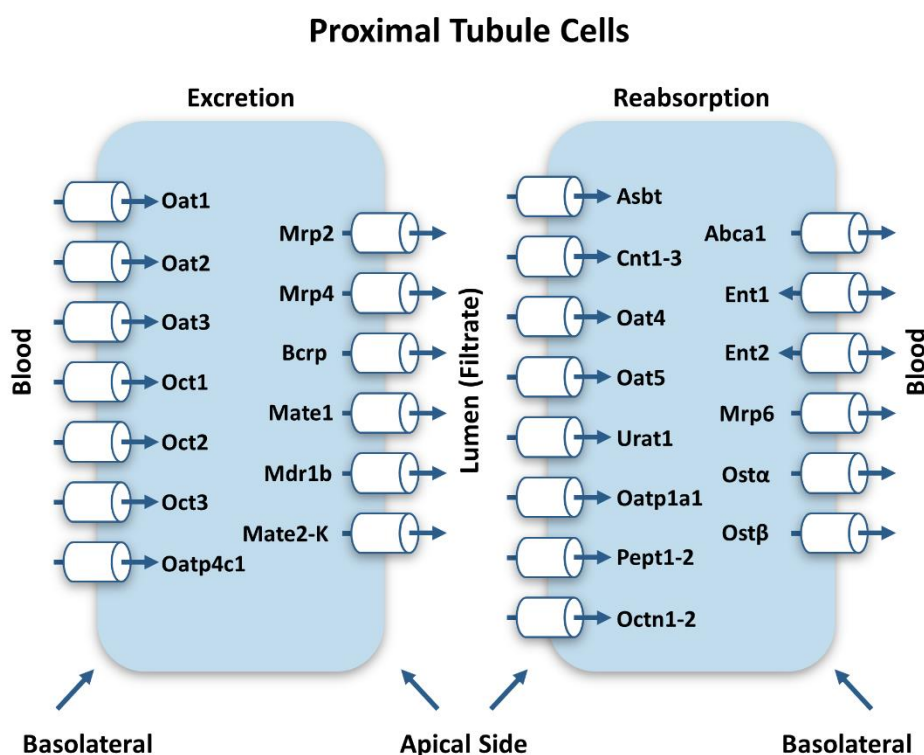


Figure D-1. Localization of Transport Proteins

Adapted from Klaassen and Aleksunes 2010.

Knowledge about specific OAT, OATP, and MRP transporters in the kidneys is rapidly evolving. A low membrane density or blockage of basolateral OATs will decrease PFOA excretion while low membrane densities or blockage of apical OATs will increase excretion because they decrease resorption of anions from the glomerular filtrate.

The earliest studies of the impact of sex on PFOA urinary excretion were conducted on male and female Holtzman rats by Hanhijarvi et al. (1982, 5085525) using probenecid, an inhibitor of renal excretion of organic acids that has since been found to specifically inhibit OAT1-6 and OAT8. The female rats that had not received the probenecid excreted 76% of the administered

dose of PFOA over a 7-hour period, while males excreted only 7.8% of the administered dose over the same period. The authors concluded that the female rat possesses an active secretory mechanism that rapidly eliminates PFOA from the body that male rats do not possess.

Kudo et al. (2002, 2990271) examined the role of sex hormones and OATs on the CL_R of PFOA. Gonadectomy alone caused an increase in CL_R of PFOA in both male and female rats (14-fold and twofold, respectively). Treatment with testosterone reduced the PFOA CL_R in castrated males and intact females. Conversely, treatment with estradiol increased the CL_R of PFOA in intact male rats, but reduced that of ovariectomized female rats back to normal values.

Early studies from Kudo et al. (2002, 2990271) and Cheng et al. (2006, 6551310) found that intact males were found to express less OAT2, more OATP1a1, and more OATP3a1 than their female counterparts. Castration was found to increase OAT2 and decrease OATP1a1. Ovariectomy increased OAT3 in female rats but did not affect OATP1a1, which was already virtually absent from intact female mice. Treatment with estradiol increased OAT2 in intact male rats, while 17- β estradiol decreased OATP1a1 in both castrated and ovariectomized mice but did not affect OATP3a1. Finally, treatment with testosterone increased OAT2 in castrated rats, while 5 α -dihydroxy-testosterone increased both OATP1a1 and OATP3a1 in castrated and ovariectomized mice. Multiple regression analysis of the data suggested that OAT2 and OAT3 are responsible for urinary elimination of PFOA in the rat; however, the possibility of a resorption process mediated by OATP1 was mentioned as a possible factor in male rat retention of PFOA. OAT2 and OAT3 are located on the basolateral cell surface. OATP1 is located on the apical surface of the renal tubule cells (Kudo et al., 2002, 2990271).

Based on Hinderliter et al. (2006, 3749132), a developmental change in renal transport occurs in rats between 3 and 5 weeks of age that allows for expedited excretion of PFOA in females and an inverse development in males. This was evidenced by changes in measured PFOA in plasma and urine, such that maturing females experienced decreased plasma PFOA and increased urine PFOA, while the opposite was seen in males. Taken together with previous information, the change in female rats seems to involve excretion-promoting OATs (Kudo et al., 2002, 2990271) while the change in males seems to involve excretion-reducing OATPs {Cheng, 2006, 6551310}.

Numerous in vitro studies using human embryonic kidney cells (HEK 293) and Chinese hamster ovary (CHO), time- and concentration-dependent studies as well as competition studies with known transporters have been utilized to evaluate the role of various transporters in the renal excretion of PFOA. For example, Yang et al. (2010, 2919288) examined cellular uptake of PFOA by OATP1A2 in CHO and HEK293 cells transfected with OATP1A2 plasmid DNA or vector DNA (control). PFOA uptake in OATP1A2-transfected HEK293 cells was no different than uptake in control cells. Uptake of estrone-3-sulfate (E3S), a known substrate of OATP1A2, was inhibited ~30% in the presence of 100 μ M PFOA (C8). Inhibition varied by PFAS's of different chain lengths (~62% by C9, ~70% by C10, ~42% by C11, and ~18% by C12). E3S uptake was not inhibited by C4–C7.

Other studies observed Michaelis-Menten kinetics in transporter-transfected cells compared to passive diffusion in control (vector only) cells, and several transporters have been identified as having PFOA renal transport activity, including OAT1, OAT3, OAT4, OATP1a1, and URAT1 (Nakagawa et al., 2007, 2919370; Nakagawa et al., 2009, 2919342; Yang et al., 2009, 2919328;

Yang et al., 2010, 2919288). Limited data suggest possible roles for OAT2 and OAT1PA2 in uptake of PFOA.

Yang et al. (2009, 2919328) investigated the role of OAT polypeptide 1a1 (OATP1a1) in PFOA uptake. In time-dependent uptake experiments using transfected CHO cells, uptake of PFOA by OATP1a1-transfected cells increased proportionally to time during the first 2 mins of incubation. Vector-transfected cells had a significant level of uptake of PFOA attributed to nonspecific passive diffusion. In the concentration-dependent uptake experiments, while saturation levels were not reached in OATP1a1-transfected cells, active PFOA uptake could be derived from the difference between the uptake of the OATP1a1 cells and the passive diffusion of the vector-transfected cells. Based on the results of the uptake and additional inhibition experiments, the authors suggested that passive diffusion could be an important route of PFOA distribution and that renal reabsorption in the male rat could be mediated by OATP1a1

In vitro studies were supported by in vivo analysis of OATPs gene and protein expression in rat kidneys (Yang et al., 2009, 2919328). OAT polypeptide 1a1 (OATP1a1), located on the apical side of proximal tubule cells and could be the mechanism for renal reabsorption of PFOA in rats. The level of mRNA of OATP1a1 in male rat kidney is 5–20-fold higher than in female rat kidney, OATP1a1 protein expression is higher in male rat kidneys, and it is regulated by sex hormones. One of its known substrates is estrone-3-sulfate (E3S). A substantial presence of OATP1a1 in male rats would favor resorption of PFOA in the glomerular filtrate and reduce excretion.

Limited evidence exists for a role of OAT and OATP1A2 in PFOA uptake. In transformed HEK 293 cells transfected with OAT 2, prostaglandin F2 α uptake by OAT2 was inhibited moderately by PFOA, 75–85% of control at 10 μ mol PFOA, and 65% of control at 100 μ mol PFOA (Nakagawa et al., 2007, 2919370). However, in the same study, the authors observed that HEK 293 cells or S2 (cells derived from proximal tubule) transfected with OAT failed to take up radiolabeled μ mol [14 C]PFOA. Similarly, Yang et al. (2010, 2919288) observed that PFOA uptake in OATP1A2-transfected HEK293 cells was no different than uptake in control cells though they did observe inhibition of E3S uptake. At 100 μ mol, E3S uptake was inhibited ~30% by PFOA (C8), ~62% by C9, ~70% by C10, ~42% by C11, and ~18% by C12. E3S uptake was not inhibited by C4–C7.

The kinetic response of the OAT1, OAT3, and OATP1a1 transporters to increasing concentrations of selected perfluorinated carboxylates also was evaluated by Weaver et al. (2010, 2010072). The change in transport velocity (ng/mg protein/min) with increasing concentrations of the perfluorinated carboxylate exhibited a Michaelis-Menten-type response. The kinetic data were analyzed to determine the K_m and V_{max} , and the results are summarized in Table D-34.

Table D-34. Kinetic Parameters of Perfluorinated Carboxylate Transport by OAT1, OAT3, and OATP1a1

Transporter	PFAS	K_m (μ mol)	V_{max} (nmol/mg protein/min)
OAT1	C7	50.5 \pm 13.9	2.2 \pm 0.2
	C8	43.2 \pm 15.5	2.6 \pm 0.3
OAT3	C8	65.7 \pm 12.1	3.8 \pm 0.5

Transporter	PFAS	K _m (μmol)	V _{max} (nmol/mg protein/min)
OATP1a1	C9	174.5 ± 32.4	8.7 ± 0.7
	C8	126.4 ± 23.9	9.3 ± 1.4
	C9	20.5 ± 6.8	3.6 ± 0.5
	C10	28.5 ± 5.6	3.8 ± 0.3

OAT = Organic Anion Transporter; PFAS = Per- and polyfluoroalkyl substances; K_m = Michaelis constant; V_{max} = maximum rate of transport

The Michaelis-Menten kinetic data (K_m and V_{max} [maximum initial rate of an enzyme catalyzed reaction]) indicate that there are substantial differences in the affinity of the perfluorinated carboxylate with 8 and 9 carbon chains for OAT3, with the C8 acid favored over the C9 acid. OAT3 is an export transporter located on the basolateral side of the tubular cells; thus, when present in a mixture consisting of comparable concentrations of both, renal tubular excretion of the C8 acid would tend to decrease excretion of the C9 acid. For OATP1a1, a resorption transporter located on the apical side of the renal tubular cells, the C9 and C10 acid have a greater affinity for the transport protein than the C8 acid. The kinetic data suggest that the net impact of these relationships would be to favor excretion of the C8 acid over the C9 acid and possibly the C10 acid when all three fluorocarbons are present in the exposure matrix at approximately equal concentrations. There were minimal kinetic differences between transport of the C7 and C8 acids by OAT1, an export transporter on the basolateral surface of the renal tubular cells.

Sakolish and colleagues developed a 3D microphysiological in vitro model using RPECs designated as a “kidney tubule chip” of the human proximal tubule (Sakolish et al., 2020, 6320196). The kidney tubule chip results for reabsorption were combined with a physiologically-based “parallel tube model” (Janku et al., 1993, 8630776) that was used to model overall renal clearance kinetics in humans in vivo. When compared to reported in vivo renal clearance (in vivo data were obtained from Reece et al. (1985, 9642054)) the kidney tubule chip combined with a physiologically-based kinetic model qualitatively and quantitatively recapitulated in vivo kinetics in the kidney.

PFOA, used as the positive control in this study, exhibited a low but measurable amount of re-absorption. The ratio of renal clearance using the combined chip and PBPK model for PFOA was estimated to be 0.40 μM at the low dose (0.01 μM) and 0.32 μM at the higher dose (1.0 μM). In contrast this ratio for creatinine (used as a negative control for resorption) was 0.54 mM and 1.17 mM for doses of 0.1 and 1.0 mM, respectively. The authors suggest the lower than expected levels of PFOA resorption may be due to one of the following factors: (1) the high degree of protein binding of PFOA in vivo actually is the primary driver of slow renal clearance as long as the unbound fraction is ≤0.01, with reabsorption contributing to a lesser degree; (2) the lack of a vascular channel in the tissue chip limits resorption (e.g., tubular secretion is not accounted for); and (3) basal OAT4 expression in the RPTECs used in the PFOA experiments was relatively low based on immunohistochemistry observations (Sakolish et al., 2020, 6320196).

When considered together, the studies of the transporters suggest that female rats are efficient in transporting PFOA across the basolateral and apical membranes of the proximal kidney tubules into the glomerular filtrate, but male rats are not. Males have a higher rate of resorption than

females for the smaller amount they can transport into the glomerular filtrate via OATP1a1 in the apical membrane.

Much work remains to be done to explain the sex differences between male and female rats and to determine whether it is relevant to humans. Similarities are possible in that the long half-life in humans resembles the male rat. The broad range of half-lives in human epidemiology studies suggests a variability in the unbound fraction of PFOA in serum or in human transport capabilities resulting from genetic variations in structures and consequently in function. Genetic variations in human OATs and OATPs are described in a review by Zair et al. (2008, 9641805).

D.4.2.2 Enterohepatic Resorption

In animals, the impact of PFOA on several membrane transporter systems linked to biliary transport was studied by Maher et al. (2008, 2919367) as part of a more detailed study of perfluorodecanoic acid (PFDA). A dose of 80 mg/kg by intraperitoneal (i.p.) injection (propylene glycol: water vehicle) was found to significantly increase ($p < 0.05$) the expression of MRP3 and MRP4 in the livers of C57BL/6 mice 2 days after treatment. MRP3 and MRP4 are believed to protect the liver from accumulation of bile acids, bilirubin, and potentially toxic exogenous substances by promoting their excretion in bile. There were significant increases in serum bilirubin and bile acids after PFDA exposure, signifying increased export. Conversely, Western Blot analysis and messenger RNA (mRNA) measurements showed significant decreases ($p < 0.05$) in the protein levels for OATP1a1, OATP1a4, and OATP1b2 following exposure to 40 mg PFOA/kg (Cheng, 2008, 758807). There was no significant impact on sodium-taurocholate cotransporting polypeptide (NTCP) protein or the serum levels of bile acids. The OATPs are transporters responsible for the uptake of bile acids and other hydrophobic substances such as steroid conjugates, ecosinoids, and thyroid hormones into the liver.

These studies, all by the same laboratory, were carried out at high, single-dose exposures, which limit their value in extrapolating to low- and repeat-dose scenarios. The results suggest a decrease in the uptake of favored substrates into the liver and an increase in removal of favored substrates from the liver via bile. Upregulation of MRP3 and MRP4, coupled with decreased OATp levels, could be beneficial due to increased biliary excretion of bile acids, bilirubin, and conjugated metabolites of toxic chemicals, including PFOA. Based on the results with the more extensive evaluation of PFDA including mouse strains null for several receptors (PPAR α , CAR, PXR, and FXR), the authors concluded that the changes in receptor proteins were primarily linked to activation of PPAR α .

Gastrointestinal elimination of PFOA was reported in a case history of a single human male with high serum levels of perfluorinated chemicals that was treated with a bile acid sequestrant (cholestyramine [CSM]) (Genuis et al. 2010, 2583643). Before treatment, PFOA was detected in urine (3.72 ng/mL) but not in stool (LOD = 0.5 ng/g) or sweat samples. After treatment with CSM for 1 week, his serum PFOA concentration lowered from 5.9 ng/g serum to 4.1 ng/g serum and stool PFOA levels increased to 0.96 ng/g. This observation suggests that PFOA is excreted in bile and that enterohepatic resorption via intestinal transporters limits the loss of PFOA via feces.

Zhao et al. (2017, 3856461) demonstrated that PFOA was a substrate for human OATP1B1, OATP1B3, and OATP2B1 transporters expressed in liver using in vitro studies of Chinese

hamster ovary (CHO) and human embryonic kidney (HEK-293) cells transfected with transporter cDNA, as well as CHO Flp-In cells expressing human OATP2B, and compared with wild-type control cells transfected with vector only. Under these conditions, the three OATPs expressed in human hepatocytes can transport the longer chain PFOA (C8) and perfluorononanoate (C9), but not the shorter chain perfluoroheptanoate (C7). The authors suggest that these results may relate to the longer serum elimination half-lives of these 2 PFCAs.

In summary, relatively few studies have investigated resorption through enterohepatic routes. The transporters involved in PFOA resorption through these routes may include MRP3 and MRP4 as well as OATP1A1, OATP1A4, OATP1B1, OATP1B2, OAT2B1, and OAT1B3. Preliminary evidence suggests enterohepatic resorption could limit elimination of PFOA by the fecal route, including the recent observation that PFOA binds to NTCP, an uptake transporter in the gut (Ruggiero, 2021, 9641806). The extent to which this pathway operates in vivo and whether enterohepatic resorption plays a substantial role in the retention of PFOA in humans and animals is still unknown.

D.4.3 Maternal Elimination Through Lactation and Fetal Partitioning

PFOA 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 clearly eliminate PFOA through routes not available to males.

The total daily elimination of PFOA in pregnant females was estimated to be 11.4 ng/day, lower than the 30.1 ng/day estimated for PFOS (Zhang and Qin, 2014, 2850251). The distribution of PFOA from maternal serum to the fetus and infants is discussed in detail above (Section D.2.4). A study by T. Zhang et al. (2013, 3859792) exemplifies the routes and amounts of PFOA 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 PFOA was 3.35 ng/mL. The mean levels in the cord blood, placenta, and amniotic fluid were 58%, 47%, and 1.3%, respectively, of those in the mother's blood. Thus, pregnant females may eliminate PFOA through cord blood, placenta, and amniotic fluids. Blood loss during childbirth could be another source of excretion.

The elimination of PFOA in pregnant women corresponds to an increase in concentrations in the placenta. Mamsen et al. (2019, 5080595) observed an increase in PFOA accumulation from gestational age 50 to 300 days, with male placentas showing higher levels of than female placentas. The authors estimated a placenta PFOA accumulation rate of 0.11% increase per day during gestation.

Mamsen and colleagues measured placental samples and fetal tissues in relation to maternal plasma levels of 5 PFASs in 39 Danish women who underwent legal termination of pregnancy before gestational week 12 (Mamsen et al., 2017, 3858487). All PFASs were transferred from mother to fetus albeit 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 PFOA were lower than maternal blood. The average concentration of PFOA was 0.17 ng/g in fetal tissues compared to 0.23 ng/g in placenta and 2.1

ng/g in maternal plasma. The increasing fetal PFOA level with fetal age finding suggest that the rate of elimination of PFAS from mother to fetus may increase through the gestational period.

The same group (Mamsen et al., 2019, 5080595) measured PFOA 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. PFOA was above LOQ in 100% of maternal serum samples, in 82% of placenta samples and 70% of fetal organs. In general, the concentrations of PFOA 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 5.6% higher ratio in male fetuses than in female fetuses ($p < 0.05$). These studies support the placenta and fetus as important routes of PFOA elimination in pregnant women and suggests that the magnitude of elimination may be influenced by the sex of the fetus.

Underscoring the importance of pregnancy as a life-stage when excretion is altered, Zhang et al (2015, 2851103) observed that the partitioning ratio of PFOA concentrations between urine and whole blood in pregnant women (0.0011) was significantly lower ($p=0.017$) than the ratios found in non-pregnant women (0.0028) 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 (2008, 1290895) 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. Elimination through breast was more recently measured in 293 samples collected from 127 mothers in the Children's Health and Environmental Chemicals in Korea (CHECK) Cohort (Lee et al. 2017, 3983576). Results were stratified by age, parity, body mass, delivery method, and infant sex. The median PFOA concentrations in breast milk across all samples was 38.5 ng/L (range of 25.1–61.5 ng/L) and the median concentration for all PFAS chemicals measured was 151 ng/L (range of 105–212 ng/L). Only PFOS concentrations were higher than PFOA with a median concentration of 47.4 ng/L (36.4–63.8 ng/L).

In this study, pooled breast milk samples were measured to follow the time course of PFOA in breast milk after birth. Concentrations in breast milk measured 30 days after birth were significantly higher (ANOVA, $p < 0.05$) than those measured prior to 7 days after birth. These findings are contrast with results of other studies. Thomsen et al. (2010, 759807) reported that breast milk levels of PFOA and PFOS decreased by 7% and 3.1%, respectively, during the first month after birth. PFOA levels significantly decreased in breast milk over a 4-month lactation period (Kang et al., 2016, 3859603). Demographic factors, maternal diets, sample sizes, the lactational periods measured may account for these discrepancies.

Lower PFOA levels in the breast milk of multiparous women provides further evidence for pregnancy and lactation as elimination pathways. Lee and colleagues observed that primiparous mothers showed higher levels of PFOA in breast milk with a median concentration of 46.0ng/L compared to 33.4 ng/L for mothers giving birth to more than 1 child ($p < 0.05$). In another study, multivariable models estimated that parous women had 40% lower PFOS (95% CI: –56 to –17%) and 40% lower PFOA (95% CI: –54 to –23%) concentrations compared with nulliparous

women (Jusko et al., 2016, 3981718). These authors also measured concentrations in colostrum. The geometric mean concentration in was 35.3 ng/L for PFOS and 32.8 ng/L for PFOA.

PFOA 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 et al., 2015, 3859840). Mean PFOA concentrations were 1.22, 0.9191 and 0.041 ng/mL in maternal serum, cord serum and breast milk respectively. The observed ratios of cord and maternal serum for PFOA was 0.78 in this study. However, the ratio between breast milk and maternal serum was 0.038 ± 0.013 suggesting a low transfer from maternal blood to breast milk relative to maternal blood to cord blood.

Studies in animals support elimination through pregnancy and lactation observed in humans. Fujii and colleagues (2020, 6512379) used the milk/plasma (M/P) concentration ratio as a measure of chemical transferability in FVB/NJcl mice. On PND8 to PND13, dams (n = 12) were given a single administration of PFOA by tail vein injection (3.13 $\mu\text{mol/kg}$). To facilitate milking, dams were administered 4.0 U/kg oxytocin and milk was collected from all dams by aspirating with pulsations using a novel apparatus. After milking, maternal blood was collected to obtain plasma. Maternal plasma PFOA concentrations were significantly higher than milk (13.78 vs 4.38 $\mu\text{mol/L}$, $P < 0.05$) and the M/P ratios was 0.32. The M/P ratios were similar for C8, C9, C12, and C13, arguing against a direct relationship with lipophilicity. Potential roles for binding proteins in breast milk or transporters in breast tissue have not been investigated.

In summary, partitioning to the placenta, amniotic fluid, 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

Menstruation may be an important factor in the sex-specific differences observed in PFOA elimination. Zhang et al., (2013, 3859849) estimated a menstrual serum clearance rate 0.029 mL/day/kg. The link between menstruation and PFOA elimination is based on several observations. First, older females have longer PFOA elimination half-lives than young females and males (Zhang et al., 2013, 3859849). 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.

Second, several studies examined the association between increased serum concentrations of PFOA and PFOS and early menopause (Knox et al., 2011, 1402395; Taylor et al., 2014, 2850915). However, a re-analysis of this data (Ruark et al., 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. 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 model 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 PFOA in menstrual blood were not identified. However, for

PFOA 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) examined the possibility that hair could be a potential route of PFAS elimination. They exposed adult Wistar male and female Wistar rats to 0, 0.05, 0.5, 5 mg/L of PFOA, PFNA, and PFOS via drinking water for 90 days. The hair samples were cleaned, sonicated, dried, and alkaline digested to extract PFAAs. PFOA, PFNA, and PFOS were detected in all the hair samples of treated groups. A dose-dependent increase in hair PFOA concentration was observed in all exposed animals. The mean hair concentrations of PFOA ranged from 3.31 to 444 ng/g. suggesting that hair may be a potential route for PFOA elimination. Interestingly, the hair PFOA concentrations for all treatment doses were significantly higher in males than in females. The sexual dimorphic difference in hair concentrations may be attributed to the sex differences observed in PFOA elimination rate and the transfer from serum to hair.

Gao also measured the composition of the mixture excreted in urine, feces and hair after administration of 0.5 or 0.05mg/mL. As summarized in Table D-35, at the lower dose of 0.05mg/mL, PFOA was not detected 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-35. Estimated Percentage of the Sum of PFOS, PFNA, and PFOA in Excreta and Serum of Male and Female Wistar Rats^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 sulfonate; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFAA = perfluoroalkyl acids ND = not detected.

^aData is presented in % total PFAAs administered. Animals exposed to 0.05 mg/mL

Excretion of PFOA through sweat was measured in one study (Genuis et al., 2013, 2149530). Sweat samples were collected during sauna or exercise from 20 human adult subjects. While another chemical class was readily detected in sweat (polychlorinated biphenyls [PCBs]) no appreciable levels of PFOA or other PFAS chemicals investigated were detected in sweat despite their detection in serum. The authors conclude that sweating does not facilitate clearance of PFHxS, PFOS, or PFOA. In a case report study (Genuis et al., 2010, 2583643), excretion through sweat was also measured in a single male subject exposed to perfluorinated chemicals via inhalation exposure and subjected to treatment with bile sequesterants. With the exception of PHHxS, no other PFAS chemicals, including PFOA, were detected in sweat.

Thus far, no single study has conducted a comparative analysis of elimination of PFOA 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. 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 may limit 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 (phospholipophilicity) can further reduce the unbound fraction of PFOA as well as uptake into cells. As reported by Sanchez Garcia (2018), phospholipophilicity shows the highest correlation to cellular accumulation data compared to other measures of lipophilicity, raising the possibility that phospholipid binding affinity could distinguish between high and low accumulating compounds as well as half-life measures.
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.

D.4.5.2 Human Studies

There have been several studies of half-lives in humans all supporting a long residence time for serum PFOA with estimates measured in years rather than months or weeks. Using a linear mixed model, Bartell et al. (2010, 379025) determined an average half-life of 2.3 years based on a study of the decreases in human serum levels after treatment of drinking water for PFOA removal was instituted by the Lubeck Public Services District in Washington, West Virginia, and the Little Hocking Water Association (LHWA) in Ohio.

The results of this assessment showed a 26% decrease in PFOA concentration per year after adjustment for covariates and a half-life of 2.3 years [confidence interval (CI) = 2.1–2.4]. The only potential confounders determined to be significant were the treatment plant ($p = 0.03$) and homegrown vegetable consumption ($p < 0.001$). This confounder, as well as changes in the source of drinking water during the study could also have impacted the results.

In another study, the drinking water supply was contaminated with a mixture of perfluorinated chemicals when a soil-improver mixed with industrial waste was applied upriver to agricultural lands in Arnsberg, Germany {Brede et al., 2010, 3859855}. The PFOA levels in the finished drinking water were measured as 500–640 ng/L in 2006. PFOS and PFHxS also were present. The estimate for the human half-life was 3.26 years (geometric mean; range 1.03–14.67 years). Regression analysis of the data also suggested that the elimination rate might have been greater in younger subjects and older subjects.

Seals et al. (2011, 2919276) determined half-life estimates for 602 residents of Little Hocking, Ohio, and 971 residents of Lubeck, West Virginia, who were part of the C8 study but had relocated to a different area of the country. The half-life estimates for Little Hocking ranged from 2.5–3.0 years (average 2.9 years) and for Lubeck ranged from 5.9–10.3 years (average 8.5 years).

Based on their analysis, the authors suggested that, if their assumptions were correct, a simple first order elimination model might not be appropriate for PFOA given that the rate of elimination appeared to be influenced by both concentration and time. There was a difference in the CL for the two locations even though the range of years elapsed since relocation was the same for both communities. The authors identified three potential limitations of their analysis: the cross-sectional design, the assumption that exposure was uniform within a water district, and a potential bias introduced by exclusion of individuals with serum values < 15 ng/mL.

3M (Burris et al., 2000, 8568548, Burris et al., 2002, 6574114) conducted a half-life study on 26 retired fluorochemical production workers from their Decatur, Alabama, ($n = 24$) and Cottage Grove, Minnesota, ($n = 3$) plants. The mean serum elimination half-life of PFOA in these workers was 3.8 years (1,378 days, 95% CI, 1,131–1,624 days) and the median was 3.5 years (Olsen et al. 2005). No association was reported between the serum elimination half-life and with initial PFOA concentrations, age, or sex of the retirees, the number of years retired or working at the production facility, or medication use or health conditions.

Harada et al. (2005, 4564250) studied the relationship between age, sex, and serum PFOA concentration in residents of Kyoto, Japan. They found that females in the 20–50-year-old age group (all with regular menstrual cycles) had serum PFOA concentrations that were significantly lower than those in females over age 50 (all post-menopausal). Harada et al. (2005, 4564250)

also estimated the CL_R rate of PFOA in humans and found it to be only about 0.001% of the GFR. There was no significant difference in CL_R of PFOA with respect to sex or age group, and the mean value was 0.03 ± 0.013 ml/day/kg.

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 CL. The mean half-life for the sum of all PFOA isomers in younger females ($n = 12$) was 2.1 years (range 0.19–5.2 years) while that for all males and older females ($n = 31$) was 2.6 (range 0.0059–14 years); the medians were 1.8 and 1.7 years, respectively. The mean values for the four branched-chain isomers of PFOA were lower than the value for the linear chain, suggesting that resorption transporters might favor uptake of the linear chain over the branched-chain isomers. Older females and males have longer half-lives than young females, suggesting the importance of monthly menstruation as a pathway for excretion (Y. Zhang et al., 2013, 3859849).

The rate of serum PFOA 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 PFOA half-lives. Drinking water PFOA levels were 100 ng/L prior to closure of the waterworks facility and 1.0 ng/L in the unexposed community. The mean serum levels among the 106 participants 6 months after the end of exposure was 21.1 ± 14.7 ng/mL. The average decrease in PFOA was 26% of its previous value each year. The excretion rate constant after the end of exposure was 0.26 (95% CI: 0.24-0.28) and was higher in females (0.29) than males (0.25) but this did not reach significance. The mean half-life was 2.7 years and was also shorter in females (2.4 years) than in males (2.8 years). There was a high level of inter-individual variation in half-lives.

Fu et al. (2016, 3859819) determined the half-life of PFOA in 302 occupational workers 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 urine concentrations for PFOA among all workers were 4.3 and 1.9 ng/mL, respectively, whereas in serum, mean and median PFOA were 1052 and 427 ng/mL. The renal clearance rate for PFOA ranged from 0.00009 to 2.4 ml/kg/day (Geometric mean of 0.067 mg/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 of PFOA was set at 170 mL kg⁻¹ and 230 mL kg⁻¹ 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 PFOA estimated using daily clearance rate was 4.1 years (geometric mean value) and 4.0 years (geometric median value). However, when measured by annual decline rate, the

half-life of PFOA was estimated to be 1.7 years. The GM values of the half-lives of PFOA and PFOS for men here were 4.7, and 60.9 years (range 0.44–3663 years), respectively, while those in females were 3.1 and 8.0 years (range 0.76–30475 years). 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.

Worley and colleagues (2017, 3859800) calculated PFOA half-lives in subjects 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 ($p \leq 0.0001$) in both 2010 (16.3 ng/L) and 2016 (11.7 ng/L) relative to national averages reported by NHANES (3.07 ng/L in 2009-2010 and 1.94 ng/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 $r = 0.75$).

The half-life for PFOS was estimated to be 3.3 years, similar to the 3.9 years estimated for PFOA. For these calculations, the V_d values were scaled to bodyweight (values of 170 mL/kg bodyweight for PFOA and 230 mL/kg bodyweight for PFOS were assigned) When the authors varied the V_d and intake values by 20%, half-life values varied by several months (half-life estimates for PFOS ranged from 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 PFOA in the airport contaminated water was about 1000 times higher than those in the municipal communities (300ng/L at airport vs 0.3ng/L in municipal water). Specific gravity adjusted urine levels for PFOA was 0.031 ng/mL [median range of (0.010–0.13) as determined from the second to the fifth sampling periods.

Serum levels of PFOA in the first serum sample taken from all 26 employees was 9.1 ng/mL and the serum/water ratio was reported as 30. PFOA levels measured in paired serum and urine samples obtained from the second to the fifth sampling was reported as 10ng/mL and 0.031ng/mL respectively with an average urine/serum ratio of 0.0032. 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). Similar to Fu and colleagues (2016, 3859819), the half-life of PFOA was estimated as 1.77years.

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 D-36). 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 a route of elimination (Zhang et al (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 et al., 2020, 6781357; Worley et al., 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-36. Summary of PFOA Concentration in Blood and Urine in Relation to Half-life values in Humans

Study	Number of Subjects	Age Range	Primary Exposure Route	Exposure	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half Life	Considerations
Xu et al., 2020, 6781357 ^a	26 19 Males 7 Females	22-62 years	Oral, drinking water	210 ng/μL (linear) 88 ng/μL (branched) 300ng/μL Total**	median: 10 ng/mL (4.1-28 ng/mL)	median: 0.031 ng/mL range: 0.010-13 ng/mL (not creatinine adjusted)	1.77 y	<ul style="list-style-type: none"> • 1 woman was previously pregnant 2018 during sampling year • PFOA also measured in the private well of one airport employee living near the airport (PFOA concentration in well was lower than the airport at 0.53 ng/μL linear and < 0.3 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	NR	2010: GM ¹ 16.3 ng/mL (13.2-19.6 95% CI) 2016: GM 11.7 ng/mL (8.7-14.6, 95% CI)	2016 Creatinine adjusted: mean 0.031 ng PFAS/g creatinine median 0.024) ^b 2016 not adjusted for creatinine: mean 0.027 ng/mL median 0.022 ng/mL	3.9 y	<ul style="list-style-type: none"> • LOD was 0.01 μg/L, detection rate 95.6% • Clearance rate was not reported
Fu et al., 2016, 3859819	302 213 males 89 females	Males: 19-65 median 41 Females: 19-50 median 37	Occupational	NR	mean: 1052 ng/mL median 427 ng/mL, (2.5-32000ng/mL).	mean: 4.3 ng/mL median 1.9 ng/mL (LOD-53.6 ng/mL) (not creatinine adjusted)	Male: 4.7 y Females: 3.1 y Overall: 4.1 y	<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 • Clearance rate for PFOA = 0.062 mL/kg-day
Zhang et al., 2013, 3859849	86 47 males 37 females	22-68	Unspecified	NR	mean 3.1 ng/mL median 2.3 ng/mL (0.26-29 ng/mL)	mean 122 ng/g creatinine median 23 ng/g creatinine,	Young females: 2.1y Males and older females: 2.6y	<ul style="list-style-type: none"> • All participants had paired (whole blood/serum and urine). For young females menstrual clearance was

Study	Number of Subjects	Age Range	Primary Exposure Route	Exposure	Plasma/Serum Concentrations	Urinary Concentrations	Estimated Half Life	Considerations
						(3.5-1869 ng/g creatinine)		estimated and added to renal clearance. • Renal clearance rate for total PFOA: mean 0.30 mL/day/kg (young female), 0.77 mL/day/kg (male and older female)

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

^aMeasured concentrations in Drinking water at airport before and after mitigation measures. Authors state, “The geometric mean and median value for PFHxS, PFOA, and PFOS were 14.7 and 11.7, 4.1 and 4.0, 32.6 and 21.6 years, respectively, by the daily clearance rates, and they were 3.6, 1.7, and 1.9 years estimated by annual decline rates. The half-lives estimated by the limited clearance route information could be considered as the upper limits for PFAAs, however, the huge difference between two estimated approaches indicated that there were other important elimination pathways of PFAAs other than renal clearance in human.”

^bng/g reported in methods but in results reported as µg/g creatinine.

All human PFOA half-life values identified in the recent literature review are provided in Table D-37. First, and in contrast to the very large ranges of half-life values estimated for PFOS (0.61 to 60.9 years), PFOA half-life values fell within a more defined range from 0.53 years for branched PFOA in young females (Zhang et al., 2013, 3859849) to 22 years in a study of primiparous women in Sweden (Glynn et al., 2012, 1578498). Second, half-life values varied by geographical region. Using a population model, Gomis et al. (2017, 3981280) derived shorter half-life values for Americans relative to Australians. Because elimination should be the same at the population level, this variation may reflect the shorter time frame of biomonitoring data in Australia relative to the NHANES data set. Third, age and sex difference in PFOA half-lives have not been rigorously evaluated, though estimates in males are generally longer than those in females (Fu et al., 2016, 3859819; Gomis et al., 2017, 3981280; Li et al., 2017, 4238434) and exhibit an age-related increase (Genuis et al., 2014, 2851045; Zhang et al., 2013, 3859849). While most studies were conducted in adults and/or adolescents, at least one study examined PFOS half-lives in a Newborn Screening Programs (Spliethoff et al., 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 NSPs collected in the state of New York from infants born between 1997 and 2007 were analyzed for PFOA. The study authors determined the half-life of PFOA using the regression slopes for natural log blood concentrations versus the year 2000 and after. The calculated half-life for PFOS was 4.4 years. Fourth, linear isoforms exhibit longer half-lives than branched isoforms (Zhang et al., 2013, 3859849).

Table D-37. Summary of Human PFOA Half-Life Values

Study	Number of Subjects	Age Range ^a	Estimated Half-Life (years)	Subjects
Bartell et al., 2010, 379025	200 100 males 100 females	54.5 ± 15	2.3 y	Study of the decreases in human serum levels after treatment of drinking water for PFOA removal was instituted by the Lubeck Public Services District in Washington, West Virginia, and the Little Hocking Water Association (LHWA) in Ohio. Source waters for these systems had become contaminated with PFAS from the DuPont Works Plant in Washington, West Virginia, between 1951 and 2000.
Brede et al., 2010, 3859855	20 children 22 adult females 23 adult males	Children: 7.4-8.3 Females: 27-49 Males: 32-71	3.26 y	Subjects exposed to contaminated drinking water supply s in Arnsberg, Germany.
Burris et al., 2002, 6574114	9 7 males 2 females	61 (55-64)	4.37 y (range 1.50 to 14.49 y)	Second interim report with 9 retired fluorochemical production workers from the 3M Decatur, Alabama.
Costa et al., 2009, 1429922	53 males	20-63	5.1 y (range 2.6 - 9.7 y)	53 males working in a PFAA production facility in Italy from 1978 to 2007

Study	Number of Subjects	Age Range ^a	Estimated Half-Life (years)	Subjects
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: 4.7 y Females: 3.1 y Overall: 4.1 y based on annual decline rate Overall: 1.7 y	Occupationally exposed subjects working in one of the largest fluorochemical plants (Henxin Chemical Plant) in Yingcheng, Hubei province, China
Genuis et al., 2014, 2851045	53 Father 47 Mother 22 1st male child 19 2nd female child 17 3rd male child 16 4th male child 3	16-53	Father: 2.61 Mother: 2.61 1st Male child: 2.03 2nd Female child: 1.85 3rd Male child: 1.80 4th Male child: 1.59	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 4–5 year period.
Glynn et al., 2012, 1578498	413 females	19-41	22 y	Primiparous women 3 weeks after delivery in Uppsala County, Sweden 1996-2010 (the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas)).
Gomis et al., 2016, 3749264	6	35-60	2.4 y	six occupationally exposed ski waxers for whom direct and indirect exposures via inhalation were characterized.
Gomis et al., 2017, 3981280	Australia: A total of 24–84 pools per survey containing between 30-100 individual samples. USA: 2000 individuals were sampled throughout the USA	12+ (USA) <16 - >60 (Australia)	Australian men: 2 y American men: 2.4 y Australian women: 1.8 y American women: 2.1 y	Population based model using Australian biomonitoring studies from 2009-2014 (Toms et al. 2014, 2009) and the National Health and Nutrition Survey (NHANES) from 2003 -2011 in 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.
Li et al., 2017, 4238434	50 Males: 20 Females 30	15-50	Males: 2.8 y Females: 2.4 y	Subjects in Ronneby, Sweden, exposed to contaminated water through a municipal water source.
Seals et al., 2011, 2919276	602 residents of Little Hocking OH: 602 Lubeck WV: 971	<20 20-29 30-39 40-49 50-59 60-69 >70	2.9 y (Little Hocking) 8.5 y (Lubeck)	602 residents of Little Hocking, Ohio, and 971 residents of Lubeck, West Virginia, who were part of the C8 study but had relocated to a different area of the country.

Study	Number of Subjects	Age Range ^a	Estimated Half-Life (years)	Subjects
Splithoff et al., 2008, 2919368	240	Newborn infant (1-2 days)	4.4 y	New York State newborn screening program blood spot specimens from newborn infants
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	3.9y	Residentially exposed population from Lawrence, Morgan and Limestone Counties, Alabama recruited by ATSDR
Xu et al., 2020, 6781357	26 19 males 7 females	22-62 years	1.77 y	Subjects in Arvidsjaur, Sweden exposed to contaminated drinking water occupationally (working at the airport) and through residential drinking water
Zhang et al, 2013, 3859849	86 47 males 37 females	22-68	Young females: 2.1y Males and older females: 2.6y n-PFOA young females: 2.3 males and older females: 2.8 iso-PFOA young females: 1.4 males and older females: 2.5 4m-PFOA young females: 0.64 males and older females: 1.4 5m-PFOA young females: 0.53 males and older females: 1.3	Healthy volunteers in Shijiazhuang and Handan, Hebei province, China, in April–May 2010

PFOA = perfluorooctanoic acid; PFAS = perfluorinated alkylated substances; PFAS = Perfluoroalkyl acids.

^aData on age range presented in years (mean ± standard deviation, where applicable).

D.4.5.3 Animal Studies

D.4.5.3.1 Non-Human Primates

Butenhoff et al. (2004, 3749227) looked at the elimination half-life in monkeys treated for 6 months with 0, 3, 10, and 20 mg/kg/day via capsules. Elimination of PFOA from serum after cessation of dosing was monitored in recovery monkeys from the 10- and 20-mg/kg dose groups. For the two monkeys exposed to 10 mg/kg, serum PFOA elimination half-life was 19.5 ($r^2=0.98$) days and indicated first-order elimination kinetics. For three monkeys exposed to 20 mg/kg, serum PFOA elimination half-life was 20.8 days ($r^2=0.82$) and also indicated first-order elimination kinetics, although dosing was suspended at different time points because of weight loss. The data from NRC (2005), which were provided by Butenhoff et al. (2004b, 3749227), were about 21 days for females and 30 days for males.

D.4.5.3.2 Rats

Kemper (2003, 6302380) examined the plasma concentration profile of PFOA following gavage administration in sexually mature Sprague-Dawley rats. Male and female rats (four per sex per

group) were administered single doses of PFOA by gavage at DRs of 0.1, 1, 5, and 25 mg PFOA/kg. After dosing, plasma was collected for 22 days in males and 5 days in females. Plasma concentration versus time data were then analyzed using noncompartmental PK methods (Table D-38, Table D-39). To further characterize plasma elimination kinetics, animals were given oral PFOA at a rate of 0.1 mg/kg, and plasma samples were collected until PFOA concentrations fell below quantitation limits (extended time).

Plasma elimination curves were linear with respect to time in male rats at all dose levels. In males, plasma elimination half-lives were independent of dose level and ranged from approximately 138 hours to 202 hours. To further characterize plasma elimination kinetics, particularly in male rats, animals were given oral PFOA at a dose of 0.1 mg/kg, and plasma samples were collected until PFOA concentrations fell below quantitation limits (2,016 hours in males). The estimated plasma elimination half-life in this experiment was approximately 277 hours (11.5 days) in male rats.

Plasma elimination curves were biphasic in females at the 5-mg/kg and 25-mg/kg dose levels. In females, terminal elimination half-lives ranged from approximately 2.8 hours at the lowest dose to approximately 16 hours at the high dose. The estimated plasma elimination half-life in the extended time experiment was approximately 3.4 hours in females. Kemper et al. (2003, 6302380) reported half-lives of 6–8 days for male Sprague-Dawley rats (Table D-38) and 3–16 hours for females (Table D-39).

Table D-38. PK Parameters in Male Sprague-Dawley Rats Following Administration of PFOA

Parameter	Dose					
	0.1 mg/kg	1 mg/kg	5 mg/kg	25 mg/kg	1 mg/kg (IV)	0.1 mg/kg extended time
T _{max} (hr)	10.25 (6.45)	9.00 (3.83)	15.0 (10.5)	7.5 (6.2)	NA	5.5 (7.0)
C _{max} (µg/mL)	0.598 (0.127)	8.431 (1.161)	44.75 (6.14)	160.0 (12.0)	NA	1.08 (0.42)
Lambda z (1/hr)	0.004 (0.001)	0.005 (0.001)	0.0041 (0.0007)	0.0046 (0.0012)	0.004 (0.000)	0.0026 (0.0007)
T _{1/2} (hr)	201.774 (37.489)	138.343 (31.972)	174.19 (28.92)	157.47 (38.39)	185.584 (19.558)	277.10 (56.62)
AUC _{INF} (hr·µg/mL)	123.224 (35.476)	1194.463 (215.578)	6733.70 (1392.83)	25,155.61 (7276.96)	1249.817 (113.167)	206.38 (59.03)
AUC _{INF} /D (hr·µg/mL/mg/kg)	1096.811 (310.491)	1176.009 (206.316)	1221.89 (250.28)	942.65 (284.67)	1123.384 (100.488)	2111.28 (586.77)
Cl _p (mL/kg·hr)	0.962 (0.240)	0.871 (0.158)	0.85 (0.21)	1.13 (0.31)	0.896 (0.082)	0.51 (0.17)

AUC_{INF}: area under the plasma concentration time curve, extrapolated to infinity; AUC_{INF}/D: AUC_{INF} normalized to dose; Cl_p: plasma clearance; C_{max}: maximum plasma concentration; Lambda z: terminal elimination constant; T_{1/2}: terminal elimination half-life; T_{max}: time to C_{max}; NA = Not applicable.

Data presented as mean ± (standard deviation)

Table D-39. PK Parameters in Female Sprague-Dawley Rats Following Administration of PFOA

Parameter	Dose					
	0.1 mg/kg	1 mg/kg	5 mg/kg	25 mg/kg	1 mg/kg (IV)	0.1 mg/kg Extended Time
T _{max} (hr)	0.56 (0.31)	1.13 (0.63)	1.50 (0.58)	1.25 (0.87)	NA	1.25 (0.50)
C _{max} (µg/mL)	0.67 (0.07)	4.782 (1.149)	20.36 (1.58)	132.6 (46.0)	NA	0.52 (0.08)
Lambda z (1/hr)	0.231 (0.066)	0.213 (0.053)	0.15 (0.02)	0.059 (0.037)	0.250 (0.047)	0.22 (0.07)
T _{1/2} (hr)	3.206 (0.905)	3.457 (1.111)	4.60 (0.64)	16.22 (9.90)	2.844 (0.514)	3.44 (1.26)
AUC _{INF} (hr·µg/mL)	3.584 (0.666)	39.072 (10.172)	114.90 (11.23)	795.76 (187.51)	33.998 (7.601)	3.34 (0.32)
AUC _{INF/D} (hr·µg/mL/mg/kg)	31.721 (5.880)	38.635 (10.093)	20.78 (2.01)	29.54 (6.92)	30.747 (6.759)	34.39 (3.29)
Cl _p (mL/kg·hr)	32.359 (6.025)	27.286 (7.159)	48.48 (4.86)	35.06 (.88)	34.040 (9.230)	29.30 (3.06)

AUC_{INF}: area under the plasma concentration time curve, extrapolated to infinity; AUC_{INF/D}: AUC_{INF} normalized to dose; Cl_p: plasma clearance; C_{max}: maximum plasma concentration; Lambda z: terminal elimination constant; T_{1/2}: terminal elimination half-life; T_{max}: time to C_{max}; NA = not applicable.
Data presented as mean ± (standard deviation)

Gibson and Johnson (1979, 9641813) administered a single dose of ¹⁴C-PFOA averaging 11.4 mg/kg by gavage to groups of three male 10-week-old CD rats. The elimination half-life of ¹⁴C from the plasma was 4.8 days. NRC ([2005], cited in Butenhoff et al. [2004, 3749227]) reported half-lives of 4–6 days for male rats and 2–4 hours for female rats; there was no mention of the strains studied.

Toxicokinetic parameters informing half-lives were derived by comparing oral to intravenous (IV) dosing in rats (Kim et al., 2016, 3749289). Sprague-Dawley rats were administered 2 mg/kg PFOA 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). Half-life estimates were substantially longer than those observed by Kemper et al. (2003, 6302380) in Sprague-Dawley rats, as well in CD rats reported by Gibson and Johnson (1979, 9641813) As shown in Table D-40, Sex differences were also observed for other TK parameters including C_{max}, T_{max}, AUC (calculated from time 0 to infinity) and V_d indicating more rapid clearance of PFOA in females relative to males.

Table D-40. PK Parameters in Female Sprague-Dawley Rats Following Oral and IV Administration of PFOA

Parameter	1 mg/kg			
	Oral		IV	
	Male	Female	Male	Female
T _{max} (hr)	2.07 ± 0.21*	0.06 ± 0.004	8.92 ± 2.34	5.84 ± 0.38
C _{max} (µg/mL)	7.55 ± 0.51	5.41 ± 0.38	NA	NA
AUC (µg-day/mL)	24.81 ± 1.41	1.39 ± 0.06	21.10 ± 1.51*	1.63 ± 0.09
T _{1/2} (day)	1.83 ± 0.47	0.15 ± 0.01	1.64 ± 0.44*	0.19 ± 0.01
V _d	106.40 ± 8.90	153.83 ± 9.19	112.12 ± 29.41	171.37 ± 11.19

AUC: area under curve; C_{max}: maximum plasma concentration; T_{1/2}: terminal elimination half-life; T_{max}: time to C_{max}; V_d = volume of distribution.

Data presented as mean ± standard deviation.

*p < 0.05 between male and female.

Lou et al. (2009, 2919359) determined values of 21.7 days (95% confidence interval: 19.5–24.1) for male CD1 mice and 15.6 days (95% confidence interval: 14.7–16.5) for females for use in their pharmacokinetic model (see section 2.6.1). NRC ([2005], cited in Butenhoff et al. [2004b, 3749227]) provided values of 12 days for males and 20 days for females without any information on strains.

Depending on the experimental conditions, half-lives in rats ranged from 0.03 days in the initial period of high dose (40mg/kg) exposure in females (Dzierlenga et al., 2019, 5916078) to 13.4 days in males exposed to a relatively low dose of 0.4mg/kg (Benskin et al., 2009, 1617974). Rats exposed by the IV route exhibited shorter half-lives than rats administered the same dose by the oral gavage route (Kim et al., 2015, 2850129, Dzierlenga et al., 2019, 5916078). Similar to humans and mice, half-life estimates were shorter in females rats compared to males rats.

D.4.5.3.3 Mice

Half-life estimates (15.6 to 21.7 days) in the single mouse study {Lou, 2009, 2919359} were generally longer than those measured in rats.

A summary of animal half-life values identified in animals is shown in Table D-41. Values in both primates and rodents were much shorter than those estimated in humans as exemplified by values reported in days rather than in years. Values in cynomolgus monkeys ranged from 13.6 to 41.7 days {Butenhoff, 2004, 3749227}, 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 0.03 days in the initial period of high dose (40 mg/kg) exposure in females {Dzierlenga, 2019, 5916078} to 13.4 days in males exposed to a relatively low dose of 0.4mg/kg {Benskin, 2009, 1617974}. Rats exposed by the IV route exhibited shorter half-lives than rats administered the same dose by the oral gavage route {Kim, 2015, 2850129; Dzierlenga, 2019, 5916078}. Similar to humans and mice, half-life estimates were shorter in females rats compared to males rats. In contrast, female half-life values exceeded male values in cynomolgus monkeys suggesting species-specific factors impacting elimination across sexes. Similar to humans, PFOA isomers exhibited shorter half-lives compared to linear forms.

1 **Table D-41. Summary of Animal PFOA Half-life Values Identified in the Literature Review**

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex	Dose	Estimated Half Life ^a
Butenhoff et al., 2004, 3749227	Monkey, cynomolgus	IV	3–4 years	Male	10 mg/kg	13.6, 13.7, and 35.3 for 3 males
				Female	10 mg/kg	26.8, 29.3, and 41.7 for 3 females
Lou et al., 2009, 2919359	Mice, CD-1	Oral	70–80 days	Male	1 and 10 mg/kg	21.7
				Female	1 and 10 mg/kg	15.6
Benskin et al., 2009, 1617974	Rat, Sprague-Dawley	Oral	Adult (429 g)	Male	0.4 mg/kg n-PFOA (0.5 mg/kg PFOA)	n-PFOA: 13.4 iso-PFOA: 8.11 4m-PFOA: 4.32 5m-PFOA: 3.95 3m-PFOA: 6.26 tb-PFOA: 2.25 5,3/5,4m2-PFOA: 1.79 4,4m2-PFOA: 1.28 B8-PFOA: 9.10
Dzierlenga et al., 2019, 5916078	Rat, Sprague-Dawley	IV	8 weeks	Male	6 mg/kg - T1/2 initial phase	2.8 ± 1.4
					6 mg/kg - T1/2 terminal phase	10.3 ± 1.2
					6 mg/kg - T1/2 overall	6.4 ± 0.5
				Female	40 mg/kg - T1/2 initial phase	0.03 ± 0.02
					40 mg/kg - T1/2 terminal phase	0.22 ± 0.01
		Oral	8 weeks	Male	6 mg/kg - T1/2 overall	12.5 ± 0.7
					12 mg/kg - T1/2 overall	10.8 ± 0.5
					48 mg/kg - T1/2 overall	8.96 ± 0.42 hours
				Female	40 mg/kg - T1/2 initial phase	0.11 ± 0.02
					40 mg/kg - T1/2 terminal phase	1.23 ± 0.4
					40 mg/kg - T1/2 overall	0.11 ± 0.03
					80 mg/kg - T1/2 initial phase	0.16 ± 0.02

Study	Species and Strain	Exposure Route	Age or Lifestage	Sex	Dose	Estimated Half Life ^a
Kemper et al., 2003, 6302380	Rat, Sprague-Dawley	Oral	Sexually mature	Male	80 mg/kg - T1/2 terminal phase	1.82 ± 1.13
					80 mg/kg - T1/2 overall	0.16 ± 0.03
					320 mg/kg - T1/2 initial phase	0.06 ± 1.09
					320 mg/kg - T1/2 terminal phase	0.75 ± 0.11
					320 mg/kg - T1/2 overall	0.58 ± 4.20
				Female	0.1 mg/kg	8.4
					1 mg/kg	5.8
					5 mg/kg	7.3
					25 mg/kg	6.6
					1 mg/kg (IV)	5.8
					0.1 mg/kg extended	11.5
Kim et al., 2016, 2850129	Rat, Sprague-Dawley	IV	8-12 weeks	Male	1 mg/kg	1.64 ± 0.44
				Female	1 mg/kg	0.19 ± 0.01
		Oral	8-12 weeks	Male	1 mg/kg	1.83 ± 0.47
				Female	1 mg/kg	0.15 ± 0.01
				Male	48.63 mol/kg body weight	5.68 ± 0.99
				Female	48.63 mol/kg body weight	0.08 ± 0.03
Kudo et al., 2002, 2990271	Rat, Wistar	IV	9 weeks	Male	48.63 mol/kg body weight	5.68 ± 0.99
				Female	48.63 mol/kg body weight	0.08 ± 0.03

1 IV =intravenous injection.

2 ^aData presented in mean days ± standard deviation unless otherwise noted.

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}.

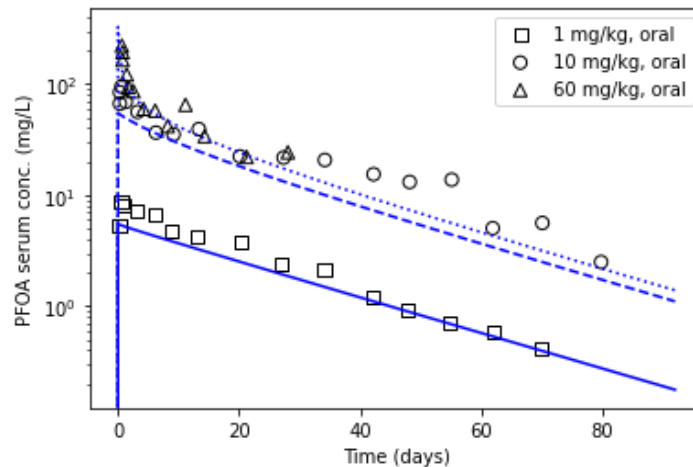


Figure E-1. Experimentally Observed Serum Concentrations {Lou, 2009, 2919359} and Median Predictions for a Single Oral Dose of 1, 10, or 60 mg/kg PFOA to Female CD1 Mice^a

^a 1 mg/kg oral dose represented by the squares and solid line; 10 mg/kg oral dose represented by the circles and dashed line; 60 mg/kg oral dose represented by the upward triangle and dotted line.

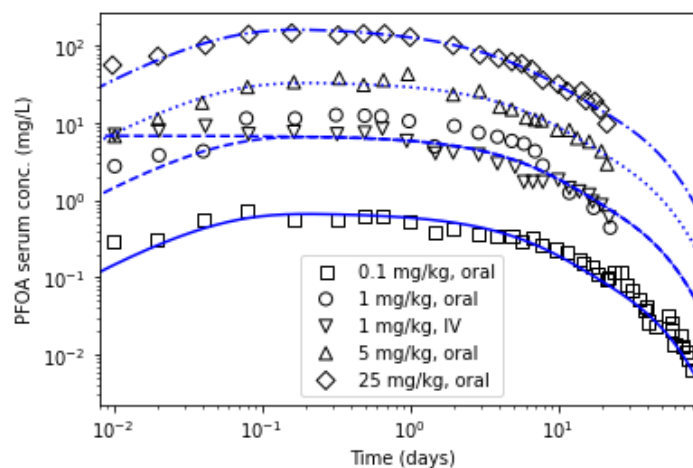


Figure E-2. Experimentally Observed Serum Concentrations {Kemper, 2003, 6302380} and Median Prediction for a Single IV Dose of 1 mg/kg or an Oral Dose of 0.1, 1, 5, or 25 mg/kg PFOA to Male Sprague-Dawley Rats^a

^a 1 mg/kg IV dose represented by the downward triangles and dashed line; 0.1 mg/kg oral dose represented by the squares and solid line; 1 mg/kg oral dose represented by the circle and dashed line; 5 mg/kg oral dose represented by the upward triangles and dotted line; 25 mg/kg oral dose represented by the diamonds and dash-dot line.

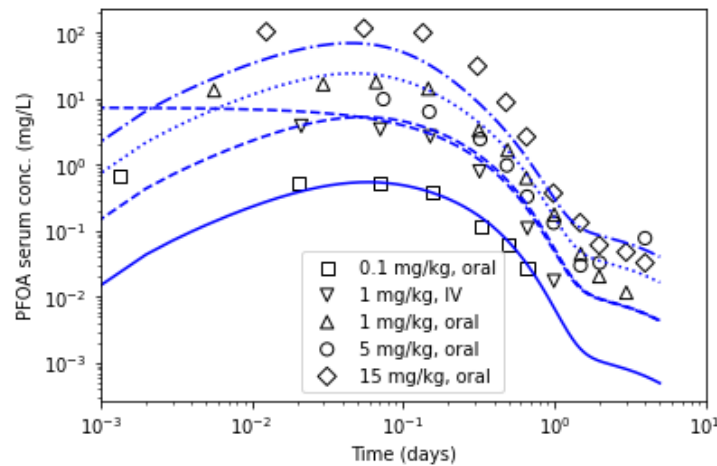


Figure E-3. Experimentally Observed Serum Concentrations {Kemper, 2003, 6302380} and Median Prediction for a Single IV Dose of 1 mg/kg or a Single Oral Dose of 0.1, 1, 5, or 15 mg/kg PFOA to Female Sprague-Dawley Rats^{a,b}

^a Change in slope from 1–10 days represents a transition to a “beta-phase” elimination in female rats.

^b 1 mg/kg IV dose represented by the downward triangles and dashed line; 0.1 mg/kg oral dose represented by the squares and solid line; 1 mg/kg oral dose represented by the circle and dashed line; 5 mg/kg oral dose represented by the upward triangles and dotted line; 15 mg/kg oral dose represented by the diamonds and dash-dot 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.

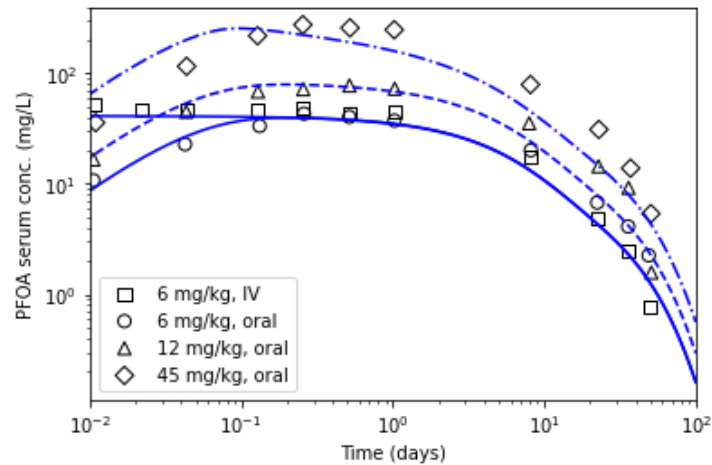


Figure E-4. Experimentally Observed Serum Concentrations {Dzierlenga, 2020, 5916078} and Median Predictions for a Single IV Dose of 6 mg/kg or a Single Oral Dose of 6, 12, or 45 mg/kg PFOA to Male Sprague-Dawley Rats^a

^a 6 mg/kg IV dose represented by the squares and solid line; 6 mg/kg oral dose represented by the circles and solid line; 12 mg/kg oral dose represented by the upward triangles and dashed line; 45 mg/kg oral dose represented by the diamonds and dash-dot line.

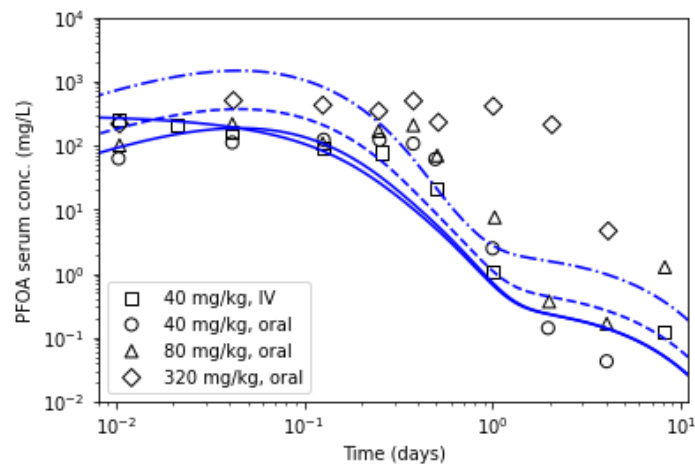


Figure E-5. Experimentally Observed Serum Concentrations {Dzierlenga, 2020, 5916078} and Median Predictions for a Single IV Dose of 40 mg/kg or a Single Oral Dose of 40, 80, or 320 mg/kg PFOA to Female Sprague-Dawley Rats^{a,b,c}

^a Change in slope from 1-10 days represents a transition to a “beta-phase” elimination in female rats.

^b The poor fit to 320 mg/kg reflects a dose that is outside the scope of the currently parametrized model.

^c 40 mg/kg IV dose represented by the squares and solid line; 40 mg/kg oral dose represented by the circles and solid line; 80 mg/kg oral dose represented by the upward triangles and dashed line; 320 mg/kg oral dose represented by the diamonds and dash-dot line.

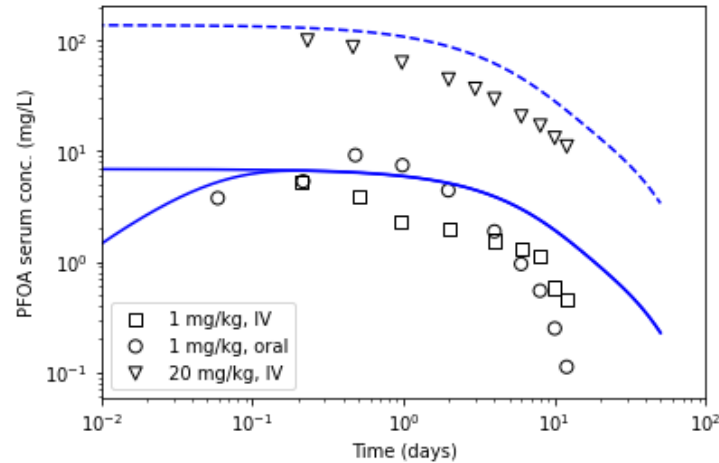


Figure E-6. Experimentally Observed Serum Concentrations and Median Predictions for a Single IV Dose of 1 mg/kg or an Oral Gavage Dose of 1 mg/kg PFOA {Kim, 2016, 3749289} or an IV Dose of 20 mg/kg PFOA {Kudo, 2002, 2990271} to Male Sprague-Dawley Rats^a

^a 1 mg/kg IV dose represented by the squares and solid line; 6 mg/kg oral dose represented by the circles and solid line; 20 mg/kg IV dose represented by the downward triangles and dashed line.

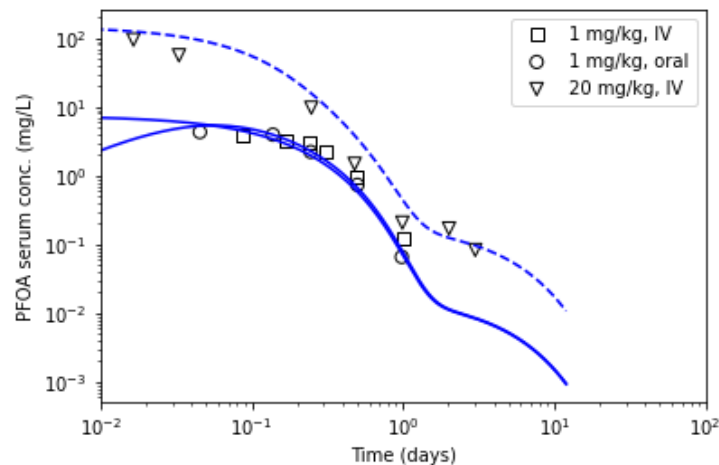


Figure E-7. Experimentally Observed Serum Concentrations and Median Predictions for a Single IV Dose of 1 mg/kg or an Oral Gavage Dose of 1 mg/kg PFOA {Kim, 2016, 3749289} or an IV Dose of 20 mg/kg PFOA {Kudo, 2002, 2990271} to Female Sprague-Dawley Rats^{a,b}

^a Change in slope from 1-10 days represents a transition to a “beta-phase” elimination in female rats.

^b 1 mg/kg IV dose represented by the squares and solid line; 6 mg/kg oral dose represented by the circles and solid line; 20 mg/kg IV dose represented by the downward triangles and dashed line.

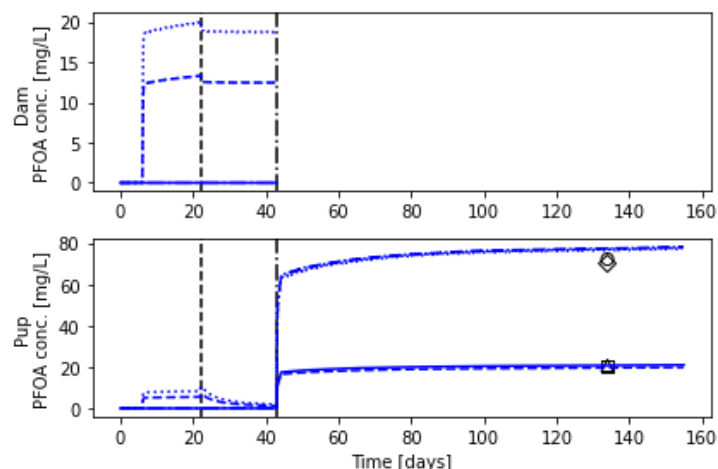


Figure E-8. Observed and Predicted PFOA Plasma Concentration in Female Sprague-Dawley Rats following Perinatal, Lactational, and Post-weaning Exposure during Study 1 of {NTP, 2020, 7330145}^{a,b}

^a Vertical black dashed and dash-dot lines represent the end of gestation and weaning, respectively.

^b Top panel represents dam concentrations (mg/L) from conception (t=0 days) to weaning (t=43 days) while bottom panel represents fetal/pup concentrations from conception (t=0 days) to post-natal week 16 (PNW16) during interim evaluation. Each simulation represents a dam daily dietary exposure of 0, 150, or 300 ppm coupled with either 300 ppm or 1,000 ppm daily dietary exposure to the pup post-weaning. Using the “dam/pup ppm” nomenclature, four total dosing scenarios are modeled: 0/300 ppm (square, solid line), 0/1000 ppm (circle, dot-dash line), 150/300 ppm (triangle, dashed line), and 300/1000 ppm (diamond, dotted line) with corresponding PNW16 pup plasma concentrations represented as color-matched circles. Dam concentrations only tracked through the end of weaning.

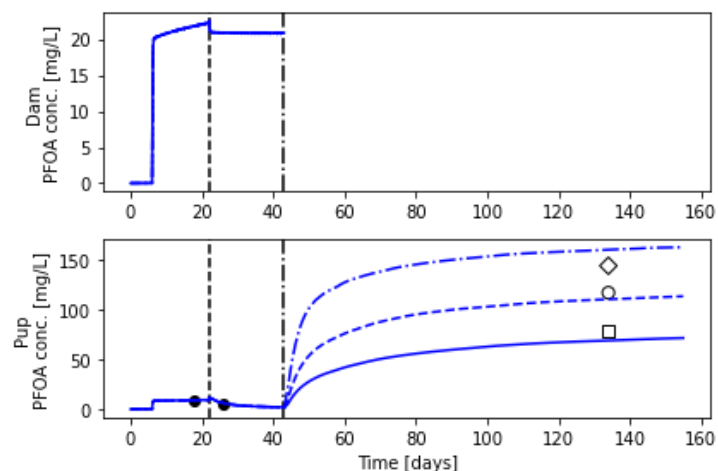


Figure E-9. Observed and Predicted PFOA Plasma Concentrations in Male Sprague-Dawley Rats following Perinatal, Lactational, and Post-weaning Exposure during Study 2 of {NTP, 2020, 7330145}^{a,b}

^a Vertical black dashed and dash-dot lines represent the end of gestation and weaning, respectively.

^b Top panel represents dam concentrations (mg/L) from conception (t=0 days) to weaning (t=43 days) while bottom panel represents fetal/pup concentrations from conception (t=0 days) to PNW16 during interim evaluation. Each simulation represents a dam daily dietary exposure of 300 ppm with 20 (solid line) 40 (dashed) and 80 (dot-dash) ppm daily dietary exposure to the pup post-weaning. Black circles represent fetal and pup concentrations at GD18 and PND 4 while the open square (20 ppm), open circle (40 ppm), and open diamond (80 ppm) represent the reported PFOA plasma concentrations in pup at PNW16. Dam concentrations only tracked through the end of weaning.