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

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Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water

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Contents

Figures

Tables

Acronyms and Abbreviations

AASLD	American Association for the Study of Liver Diseases
ABC	ATP-binding cassette transporter
ACG	American College of Gastroenterology
ADME	absorption, distribution, metabolism, and excretion
AF:CB	amniotic fluid and cord blood ratio
AFFF	aqueous film forming foam
AhR	aryl hydrocarbon receptor
ALP	alkaline phosphatase
ALSPAC	Avon Longitudinal Study of Parents and Children
ALT	alanine aminotransferase
APOB	apolipoprotein B
ApoC-III	apolipoprotein C-III
ASBT	apical sodium-dependent bile salt transporter
AST	aspartate aminotransferase
ATF	activating transcription factor
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BK	bradykinin
BM	bone marrow
BMD	benchmark dose
BMD_{10}	dose corresponding to a 10% change in response
BMDL	benchmark dose lower limits
$BMDL_{10}$	dose level corresponding to the 95% lower confidence limit of a 10% change
BMDS	Benchmark Dose Software
BMI	body mass index
BMR	benchmark response
BWT	birthweight
BW	body weight
C_{last7}	average concentration over the final week of study
CAD	coronary artery disease
CalEPA	California Environmental Protection Agency
CAMK	calcium/calmodulin dependent protein kinase
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service Registry Number
CAT	catalase
C_{avg}	average blood concentration
$C_{avg,pup,gest}$	area under the curve normalized per day during gestation
$C_{avg,pup,gest,lact}$	area under the curve normalized dose per day during gestation/lactation
$C_{avg,pup,lact}$	area under the curve normalized per day during lactation
CCL	Contaminant Candidate List
CD	celiac disease
CDC	Centers for Disease Control and Prevention
C-F	carbon-fluorine
CHD	coronary heart disease
CHDS	Child Health and Development Studies

CHF	congestive heart failure
CHO	Chinese hamster ovary
CI	confidence interval
CIMT	carotid artery intima-media thickness
C_{max}	maximum blood concentration
CRP	C-reactive protein
CSF	cancer slope factor
CSM	cholestyramine
CVD	cardiovascular disease
CYP	cytochrome P450 aromatase
CYTL	cytokine like
DBP	diastolic blood pressure
DCFDA	2,7-2,7-dichlorofluorescein diacetate
DDIT	DNA damage inducible transcript
DE	differentially expressed
DIPP	Diabetes Prediction and Prevention
DMR	differentially methylated region
DNA	deoxyribonucleic acid
DNBC	Danish National Birth Cohort
DPP	Diabetes Prevention Program
DPPOS	Diabetes Prevention Program and Outcomes Study
DTH	delayed-type hypersensitivity response
DWI-BW	body weight-based drinking water intake
EC	effect concentration
EC_{50}	half maximal effective concentration
ECM	extracellular matrix
ESC-CM	embryonic stem cell-derived cardiomyocyte
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ER	estrogen receptor
ERK	extracellular signal-regulated protein kinase
F_1	first generation
F_2	second generation
FGF	fibroblast growth factor
f_{oc}	soil organic carbon fraction
FXII	Hageman factor XII
GBCA	Genetic and Biomarkers study for Childhood Asthma
GD	gestational day
GH	growth hormone
GF	glomerular filtration
GGT	γ-glutamyltransferase
GI	gastrointestinal
glst	generalized least-squares for trend
GSSG	glutathione disulfide
GSH	glutathione
GSH-Px	glutathione peroxidase
HAWC	Health Assessment Workspace Collaborative
HDL	high density lipoprotein cholesterol
HED	human equivalent dose
HERO	Health and Environmental Research Online
HESD	Health Effects Support Document
HFD	high fat diet
HFMD	hand, foot, and mouth disease
HFPO	hexafluoropropylene oxide
Hib	*Haemophilus influenzae type b*

HIV	human immunodeficiency virus
HK	high-molecular-weight kininogen
HMOX	heme oxygenase
HMVEC	human microvascular endothelial cells
HNF	hepatocyte nuclear factor
HOME	Health Outcomes and Measures of the Environment
HR	Hazard Ratio
HRL	health reference level
HSA	human serum albumin
HUVEC	human umbilical cord endothelial cell
ICAM	intracellular adhesion molecule
iCOS	inducible co-stimulator
iCOSL	inducible co-stimulator ligand
IDL	intermediate density lipoprotein
IgE	immunoglobulin E
IGF	insulin-like growth factors
IgG	immunoglobulin G
IgM	immunoglobulin M
IHD	ischemic heart disease
IL	interleukin
IP	intraperitoneal
IPA	Ingenuity Pathway Analysis
IPCS	International Programme on Chemical Safety
IQR	interquartile range
IRIS	Integrated Risk Information System
IV	intravenous
JNK	c-JUN amino-terminal kinase
KC	Kupffer cell
KEGG	Kyoto Encyclopedia of Genes and Genomes
KKS	kallikrein-kinin system
K_H	Henry's Law Constant
KM	Kunming mice
$K_{mem/w}$	membrane/water partition coefficients
KO	knockout
K_{oc}	organic carbon-water partitioning coefficient
K_{ow}	octanol-water partition coefficient
LBW	low birthweight
LC	lethal concentration
LCM	liver capsular macrophages
LC-MS	liquid chromatography–mass spectrometry
LD	lactational day
LDL	low density lipoprotein cholesterol
L-FABP	liver fatty acid binding protein
LOAEL	lowest-observed-adverse-effect level
LOEC	lowest observed effect concentration
LOD	limit of detection
LPS	lipopolysaccharide
LSEC	liver sinusoidal endothelial cell
LXR	liver X receptor
LYZ	lysozyme
MAIT	mucosal invariant T
MALDI	Matrix-Assisted Laser Desorption/Ionization
MAM	mitochondria-associated endoplasmic reticulum membrane
MAPK	mitogen-activated protein kinase
MCLG	Maximum Contaminant Level Goal

MDA	malondialdehyde
MDH	Minnesota Department of Health
MDM	monocyte-derived macrophages
mEB	mouse embryoid body
MEF	mouse embryonic fibroblast
MeFOSAA	2-(N-Methyl-perfluorooctane sulfonamido) acetic acid
MEHP	mono-(2-ethylhexyl)phthalate
Me-PFOSA-AcOH	2-(N-Methyl-perfluorooctane sulfonamido) acetic acid
miRNA	micro ribonucleic acid
MMR	measles, mumps, and rubella
MOA	mode of action
mPLP	mouse prolactin-like protein
MRL	Minimum Reporting Level
mRNA	messenger ribonucleic acid
MRP	multidrug resistance-associated protein
MS	multiple sclerosis
MTTP	microsomal triglyceride transfer protein
MWCNT	multi-walled carbon nanotube
NAFLD	non-alcoholic fatty liver disease
NCBI	National Center for Biotechnology Information
NCEH	Neutral Cholesterol Ester Hydrolase
NCI	National Cancer Institute
NF	nuclear factor
NHANES	National Health and Nutrition Examination Survey
NK	natural killer
NOAEL	no-observed-adverse-effect level
NOD	non-obese diabetic
NOS	nitric oxide synthase
NPDWR	National Primary Drinking Water Regulation
NFR	nuclear factor-erythroid factor
NSC	neural stem cells
NT	not tested
NTCP	sodium/taurocholate cotransporting polypeptide
NTP	National Toxicology Program
OAT	organic anion transporter
OATP	organic anion transporting polypeptides
OECD	Organisation for Economic and Co-operation and Development
OR	odds ratio
OVA	ovalbumin
P_0	parental generation
PBL	peripheral blood leukocytes
PBPK	physiologically based pharmacokinetic
PcG	Polycomb group
PCM	peritoneal macrophages
PCNA	proliferating cell nuclear antigen
PDTC	pyrrolidine dithiocarbamate
PECAM-1	platelet endothelial cell adhesion molecule

PECO	Population, Exposure, Comparator, and Outcome
PFAA	perfluoroalkyl acids
PFAS	perfluoroalkyl and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFC	plaque forming cell
PFCA	perfluorinated carboxylic acids
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexanesulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFSA	perfluorosulfonic acid
PHA	phytohemagglutinin
P_{ion}	anionic permeability
PK	pharmacokinetic
P_{milk}	milk:blood PFOS partition coefficient
PND	postnatal day
PNW	postnatal week
POD	point of departure
POD_{HED}	point of departure human equivalent dose
POUNDS-Lost	Prevention of Obesity Using Novel Dietary Strategies Lost
PPAR	peroxisome proliferator activated receptor
ppm	parts per million
PR	progesterone receptor
PRR	pattern recognition receptor
PSA	prostate specific antigen
PTB	preterm birth
PTGS	prostaglandin-endoperoxide synthase
PWS	public water systems
PXR	pregnane X receptor
QA	Quality Assurance
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RAR	retinoic acid receptor
RfD	reference dose
R_{fm}	ratio of the concentrations in the fetus(es) and the mother during pregnancy
r^{i}_{milk}	species-specific milk consumption rate during lactation for the i^{th} week of lactation
RNS	reactive nitrogen species
ROS	reactive oxygen species
R_{PM}	ratio of PFOS in placenta relative to maternal serum
RSC	relative source contribution
RSV	respiratory syncytial virus
RXR	retinoid X receptor
SAB	Science Advisory Board
SBP	systolic blood pressure
SD	standard deviation
SDWA	Safe Drinking Water Act
SES	socioeconomic status
SGA	small for gestational age
SGP	sphingosine-1-posphate lyase
SHE	Syrian hamster embryo
SIRT	sirtuin
SOD	superoxide dismutase
SRBC	sheep red blood cell
T1D	type 1 diabetes
T-AOC	total antioxidant capacity
TBARS	thiobarbituric acid-reactive substances
TC	total cholesterol

TCR	T cell receptor
TG	triglycerides
THEMIS	thymocyte selection associated
TLR	toll-like receptor
TLT	TREM-like transcript cells
TNF	tumor necrosis factor
TNP	trinitrophenyl
TSCATS	Toxic Substance Control Act Test Submissions
TTE	transplacental transfer efficiencies
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UC	ulcerative colitis
UCMR 3	Third Unregulated Contaminant Monitoring Rule
UF	uncertainty factors
UF_A	interspecies uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL extrapolation uncertainty factor
UF_S	uncertainty factor for extrapolation from a subchronic to a chronic exposure duration
UF_{TOT}	total uncertainty factors
UV-vis	ultraviolet visible
V_d	volume of distribution
Vfil	filtrate volume
VLDL	very low-density lipoprotein cholesterol
WBC	white blood cell
WHO	World Health Organization
WNT	wingless-related integration site
WoS	Web of Science
WT	wild type
WTCHR	World Trade Center Health Registry
ZFL	zebrafish liver line

1 Background

1.1 National Primary Drinking Water Regulation for Per- and Polyfluoroalkyl Substances under the Safe Drinking Water Act

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), including perfluorooctane sulfonic acid (PFOS), under the Safe Drinking Water Act (SDWA). As part of the proposed rulemaking, EPA prepared *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* that described the derivation of oral cancer and noncancer toxicity values, a relative source contribution (RSC), and cancer classification, which could be subsequently used to derive an MCLG for PFOS. The agency sought peer review from the EPA Science Advisory Board (SAB) on key scientific issues related to the development of the MCLG, including the systematic review approach, oral toxicity values, RSC, and cancer classification.

The SAB provided draft recommendations on June 3, 2022 and final recommendations on August 23, 2022 {U.S. EPA, 2022, 10476098}, and EPA addressed those recommendations into the development of this updated assessment, *Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water*, which derives toxicity values and an MCLG for PFOS. To be responsive to the SAB recommendations, EPA has, for example:

- updated and expanded the scope of the studies included in the assessment;
- expanded the systematic review steps beyond study quality evaluation to include evidence integration to ensure consistent hazard decisions;
- separated hazard identification and dose-response assessment;
- added protocols for all steps of the systematic review and more transparently described the protocols;
- evaluated alternative pharmacokinetic models and further validated the selected model;
- conducted additional dose-response analyses using additional studies and endpoints;
- evaluated and integrated mechanistic information;
- strengthened the weight of evidence for cancer and rationale for the cancer classification;
- strengthened the rationales for selection of points of departure for the noncancer health outcomes; and
- clarified language related to the relative source contribution determination including the relevance of drinking water exposures and the relationship between the reference dose (RfD) and the relative source contribution.

1.2 Background on PFAS

PFAS are a large group of anthropogenic chemicals that share a common structure of a chain of linked carbon and fluorine atoms. The PFAS group includes PFOS, perfluorooctanoic acid (PFOA), and thousands of other chemicals. While the number of PFAS used globally in

commercial products in 2021 was approximately 250 substances {Buck, 2021, 9640864}, the universe of PFAS, including parent chemicals, metabolites, and degradants, is greater than 12,000 compounds (https://comptox.epa.gov/dashboard/chemical-lists/PFASMASTER). The Organisation for Economic Co-operation and Development (OECD) *New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs)*, published in 2018, includes over 4,700 PFAS {OECD, 2018, 5099062}.

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 many PFAS extremely persistent in the human body and the environment {Calafat, 2007, 1290899; Calafat, 2019, 5381304; Kwiatkowski, 2020, 7404231}. Due to their widespread use, physicochemical properties, persistence, and bioaccumulation potential, many PFAS co-occur in exposure media (e.g., air, water, ice, sediment) as well as in tissues and blood of aquatic and terrestrial organisms, including humans.

Based on structure, there are many families or classes of PFAS, each containing many individual structural homologues that can exist as either branched-chain or straight-chain isomers {Buck, 2011, 4771046}. These PFAS families can be divided into two primary categories: non-polymers and polymers. The non-polymer PFAS include perfluoroalkyl acids (PFAAs), fluorotelomer-based substances, and per- and polyfluoroalkyl ethers. PFOA and PFOS belong to the PFAA family of the non-polymer PFAS category and are among the most researched PFAS in terms of human health toxicity and biomonitoring studies (for review, see Podder et al. (2021, 9640865)).

1.3 Evaluation of PFOS Under SDWA

SDWA, as amended in 1996, requires EPA to publish a list every 5 years of unregulated contaminants that are not subject to any current proposed or promulgated NPDWRs, are known or anticipated to occur in public water systems (PWSs), and might require regulation under SDWA. This list is known as the Contaminant Candidate List (CCL). PFOS is included on the third CCL (CCL 3) {U.S. EPA, 2009, 1508321} and on the fourth CCL (CCL 4) {U.S. EPA, 2016, 6115068}.

After PFOS and PFOA were listed on the CCL 3 in 2009, EPA initiated development of health effects support documents (HESDs) for PFOA and PFOS that provided information to federal, state, tribal, and local officials and managers of drinking water systems charged with protecting public health when these chemicals are present in drinking water {U.S. EPA, 2016, 3603365; U.S. EPA, 2016, 3603279}. The two HESDs were peer-reviewed in 2014 and revised based on consideration of peer reviewers' comments, public comments, and additional studies published through December 2015. The resulting 2016 *Health Effects Support Document for Perfluorooctane Sulfonic Acid (PFOS)* {U.S. EPA, 2016, 3603365} described the assessment of cancer and noncancer health effects and the derivation of a noncancer RfD that served as the

basis for the non-regulatory 2016 *Drinking Water Health Advisory for Perfluorooctane Sulfonic Acid (PFOS)* {U.S. EPA, 2016, 3982043}.

SDWA requires EPA to make regulatory determinations for at least five CCL contaminants every 5 years. EPA must begin developing an NPDWR when the agency makes a determination to regulate based on a finding that a contaminant meets all three of the following criteria:

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

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

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 an NPDWR. The agency concluded that all three criteria were met—PFOA and PFOS may have adverse health effects; they occur in PWSs with a frequency and at levels of public health concern; and, 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}. As noted above in Section 1.1, EPA prepared *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 335-67-1) in Drinking Water* as part of this rulemaking.

In June 2022, EPA published an interim Drinking Water Health Advisory for PFOS {U.S. EPA, 2022, 10668548} to supersede the 2016 Drinking Water Health Advisory based on analyses of more recent data described in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1)in Drinking Water*, which showed that PFOS can impact human health at exposure levels much lower than reflected by the 2016 Drinking Water Health Advisory {U.S. EPA, 2016, 3982043; U.S. EPA, 2022, 10668548}.

1.4 Purpose of this Document

Consistent with SDWA Section 1412(b)(3)(A) and (B), the primary purpose of this draft document is to obtain public comment on EPA's toxicity assessment and proposed MCLG for PFOS by describing the best available science on health effects in order to derive an MCLG. To derive an MCLG, the latest science is identified, described, and evaluated, and then a cancer classification, toxicity values (i.e., a noncancer RfD and cancer slope factor (CSF)), and RSC for PFOS are developed (Section 2.3). The draft cancer and noncancer toxicity values, cancer classification, and RSC values derived in this assessment build upon the work described in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water*, the 2016 PFOS

HESD {U.S. EPA, 2016, 3603365}, and the previous 2016 Drinking Water Health Advisory {U.S. EPA, 2016, 3982043}.

In addition to documenting EPA's basis for the proposed MCLG, this document also serves the following purposes:

- Transparently describe and document the literature searches conducted and systematic review methods used to identify health effects information (epidemiological and animal toxicological studies and physiologically-based pharmacokinetic (PBPK) models) to update the literature.
- Describe and document screening methods, including the Populations, Exposures, Comparators, and Outcomes (PECO) criteria and the process for tracking studies throughout the literature screening.
- Identify epidemiological (i.e., human) and animal toxicological literature that report health effects after oral exposure to PFOS (and its associated salts), as outlined in the PECO criteria.
- Evaluate and document the available mechanistic information (including toxicokinetic understanding) associated with PFOS exposure to inform the interpretation of findings related to potential health effects in studies of humans and animals with focus on five main health outcomes (developmental, hepatic, immune, and cardiovascular effects, and cancer).
- Describe and document the study quality evaluations conducted for epidemiological and animal toxicological studies considered useful for point of departure (POD) derivation.
- Describe and document the data from *high* and *medium* confidence epidemiological and animal toxicological studies (as determined by study quality evaluations) that were considered for POD derivation; in cases of health effects with few available studies, data may be extracted from *low* confidence studies and used in the evidence syntheses. For dose-response assessment, only *high* and *medium* confidence studies were used to quantify health effects.
- Synthesize and document the adverse health effects evidence across studies, assessing health outcomes using a narrative approach. The assessment focuses on synthesizing the available evidence for five main health outcomes—developmental, hepatic, immune, and cardiovascular effects, and cancer—but also provides secondary syntheses for dermal, endocrine, gastrointestinal, hematologic, metabolic, musculoskeletal, nervous, ocular, renal, and respiratory effects; reproductive effects in males or females; and general toxicity.
- Develop and document strength of evidence judgments across studies (or subsets of studies) separately for epidemiological and for animal toxicological lines of evidence and integrate mechanistic analyses into judgments for the five main health outcomes.
- Develop and document integrated expert judgments across lines of evidence (i.e., epidemiological or animal toxicological lines of evidence) as to whether and to what extent the evidence supports that exposure to PFOS has the potential to be hazardous to humans. The judgments will be directly informed by the evidence syntheses and based on

structured review of an adapted set of considerations for causality first introduced by Austin Bradford Hill {Hill, 1965, 71664}.

- Describe and document the dose-response analyses conducted on the studies identified for POD derivation.
- Derive candidate RfDs and/or CSFs and select the RfD and/or CSF for PFOS and describe the rationale.
- Determine PFOS's cancer classification using a weight of evidence approach.
- Characterize hazards (e.g., uncertainties, data gaps).

1.5 Chemical Identity

PFOS is a PFAA that was used as an aqueous dispersion agent and emulsifier in a variety of water-, oil-, and stain-repellent products (e.g., agricultural chemicals, alkaline cleaners, carpets, firefighting foam, floor polish, textiles) {NLM, 2022, 10369707}. It can exist in linear- or branched-chain isomeric form. PFOS is a strong acid that is generally present as the sulfonate anion at typical environmental pH values. Therefore, this assessment applies to all isomers of PFOS, as well as nonmetal salts of PFOS that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body).

PFOS is stable in environmental media because it is resistant to environmental degradation processes, such as biodegradation, photolysis, and hydrolysis. In water, no natural degradation has been demonstrated, and it dissipates by advection, dispersion, and sorption to particulate matter. PFOS has low volatility in its 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 PFOS in arctic media and biota, including 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 PFOS are provided in Table 1-1. However, there is uncertainty in the estimation, measurement, and/or applicability of certain physical/chemical properties of PFOS in drinking water, including the K_{oc} {Li, 2018, 4238331; Nguyen, 2020, 7014622}, octanol-water partition coefficient (K_{ow}), and Henry's Law Constant (K_H) {NCBI, 2022, 10411459; ATSDR, 2021, 9642134}. For example, for K_{ow}, the Agency for Toxic Substances and Disease Registry (ATSDR) (2021, 9642134) reported that a value could not be measured because PFOS is expected to form multiple layers in octanol/water mixtures.

For a more detailed discussion related to the chemical and physical properties and environmental fate of PFOS, please see the PFAS Occurrence & Contaminant Background Technical Support Document {U.S. EPA, 2023, 10692764}, the 2016 PFOS Drinking Water Health Advisory {U.S. EPA, 2016, 3982043}, and the *Draft Aquatic Life Ambient Water Quality Criteria for Perfluorooctane Sulfonate (PFOS)* {U.S. EPA, 2022, 10668582}.

Table 1-1. Chemical and Physical Properties of PFOS

Property	PFOS, Acidic Form; Experimental Average	Source
Chemical Abstracts Service Registry Number (CASRN)[a]	1763-23-1	NLM, 2022, 10369707
Chemical Abstracts Index Name	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid	
Synonyms	perfluorooctane sulfonic acid; heptadecafluoro-1-octane sulfonic acid; PFOS acid	EPA CompTox Chemicals Dashboard
Chemical Formula	$C_8HF_{17}O_3S$	NLM, 2022, 10369707
Molecular Weight	500.13 g/mol	NLM, 2022, 10369707
Color/Physical State	Liquid	NLM, 2022, 10369707
Boiling Point	249°C	NLM, 2022, 10369707
Melting Point	> 400°C	ATSDR, 2021, 9642134 (potassium salt)
Vapor Pressure	0.002 mm Hg at 25°C	NLM, 2022, 10369707 (estimated)
Henry's Law Constant (K_H)	4.1E-04 atm-m^3/mol at 25°C	NLM, 2022, 10369707 (estimated from vapor pressure and water solubility)
K_{oc}	1,000 ± 5.0 L/kg (mean of values ± 1 standard deviation of selected values)	Zareitalabad et al., 2013, 5080561 (converted from log K_{oc} to K_{oc})
Log K_{ow}	4.49	NLM, 2022, 10369707 (estimated)
Solubility in Water	0.0032 mg/L at 25°C; 570 mg/L	NLM, 2022, 10369707 (estimated) ATSDR, 2021, 9642134 (potassium salt in pure water)

Notes: K_{oc} = organic carbon-water partitioning coefficient; K_{ow} = octanol-water partition coefficient.
[a] The CASRN given is for linear PFOS, but the toxicity studies are based on both linear and branched; thus, this assessment applies to all isomers of PFOS.

1.6 Occurrence Summary

1.6.1 Biomonitoring

The U.S. Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) has measured blood serum concentrations of several PFAS in the general U.S. population since 1999. PFOS and PFOA have been detected in up to 98% of serum samples taken in biomonitoring studies that are representative of the U.S. general population. Blood levels of PFOA and PFOS dropped 60% to 80% between 1999 and 2014, presumably due to restrictions on their commercial usage in the United States. Most PFOS production in the United States was voluntarily phased out by its primary manufacturer (3M) between 2000 and 2002, and in 2002 and 2007 EPA took regulatory action under TSCA to require that EPA be notified prior to any future domestic manufacture or importation of PFOS and 270 related PFAS {U.S. EPA, 2016, 3982043}. Manufacturers have since shifted to alternative short-chain PFAS, such as hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt (two "GenX chemicals"). Additionally, other PFAS were found in human blood samples from recent (2011–2016) NHANES surveys (e.g., perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoDA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonate (PFHxS), perfluorononanoic acid (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 replacement PFAS than for PFOS, PFOA, and other members of the carboxylic acid and sulfonate PFAS categories.

1.6.2 Ambient Water

Among the PFAS with established analytical methods for detection, PFOS (along with PFOA) is one of the dominant PFAS compounds detected in ambient water both in the U.S. and worldwide {Ahrens, 2011, 2657780; Benskin, 2012, 1274133; Dinglasan-Panlilio, 2014, 2545254; Nakayama, 2007, 2901973; Remucal, 2019, 5413103; Zareitalabad, 2013, 5080561}. Though it has a history of wide usage and is highly persistent in aquatic environments, current information on the distribution of PFOS in surface waters of the United States is somewhat limited; most published PFOS ambient water occurrence data focuses on regions with known PFAS use or occurrence. These regions are primarily freshwater systems in eastern states, including the Mississippi River, Great Lakes, Cape Fear Drainage Basin, and waterbodies near Decatur, Alabama and in northern Georgia {Jarvis, 2021, 9416544}. Additional monitoring has been conducted in areas of known aqueous film forming foam (AFFF) use.

In a recent review, Jarvis et al. (2021, 9416544) found that concentrations of PFOS in global surface waters ranged over eight orders of magnitude, generally in pg/L to ng/L concentrations, but sometimes reaching µg/L levels (range: 0.074–8,970,000 ng/L, arithmetic mean: 786.77 ng/L, geometric mean: 5.468 ng/L, median: 3.6 ng/L). Though these calculated concentrations are not necessarily representative of all the measured PFOS concentrations in U.S. surface waters, the majority of PFOS concentrations reported (approximately 91%) are less than 300 ng/L.

1.6.3 Drinking Water

Ingestion of drinking water is a potentially significant source of exposure to PFOS. Serum PFOS concentrations are known to be elevated among individuals living in communities with drinking water contaminated from environmental discharges.

Data from the third Unregulated Contaminant Monitoring Rule (UCMR 3) are currently the best available nationally representative finished water occurrence information for PFOS {U.S. EPA, 2017, 9419085; U.S. EPA, 2021, 7487276; U.S. EPA, 2023, 10692764}. UCMR 3 monitoring occurred recently (between 2013 and 2015) and analyzed 36,972 samples from 4,920 PWSs for PFOS. The minimum reporting level (MRL)[1] for PFOS was 0.04 µg/L. A total of 292 samples from 95 PWSs (out of 36,972 total samples from 4,920 PWSs) had detections of PFOS (i.e., greater than or equal to the MRL). PFOS concentrations for these detections ranged from 0.04 µg/L (the MRL) to 7 µg/L (median concentration of 0.06 µg/L; 90th percentile concentration of 0.25 µg/L).

Because PFOS and PFOA cause similar types of adverse health effects and their 2016 lifetime Health Advisory values were the same, EPA recommended an additive approach when PFOA and PFOS co-occur at the same time and location in drinking water sources {U.S. EPA, 2016,

[1] The minimum reporting level is the threshold at or above which a contaminant's presence or concentration is officially quantitated. In the case of many of EPA's nation-wide drinking water studies, the selected reporting level is known officially as the MRL. The MRL for each contaminant in each study is set at a level that EPA believes can be achieved with specified confidence by a broad spectrum of capable laboratories across the nation {U.S. EPA, 2021, 9640861}.

3603365; U.S. EPA, 2016, 3603279}. This approach was used 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} and the reported maximum summed concentration of PFOA and PFOS reported in UCMR 3 was 7.22 µg/L[2] and the median summed value was 0.05 µg/L. Summed PFOA and PFOS concentrations reported in UCMR 3 exceeded one-half the health reference level (HRL)[3] (0.035 µg/L) at a minimum of 2.4% of PWSs (115 PWSs) and exceeded the HRL (0.070 µg/L) at a minimum of 1.3% of PWSs (63 PWSs). Since the time of UCMR 3 monitoring, some sites where elevated levels of PFOA and PFOS were previously 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 across 25 states, tribes, or U.S. territories {U.S. EPA, 2017, 9419085}.

Data from more recent state monitoring efforts demonstrate occurrence in multiple geographic locations consistent with UCMR 3 monitoring {U.S. EPA, 2021, 7487276}. IN 2021, at the time of publication of the final regulatory determinations for PFOA and PFOS, the finished water data available from fifteen states collected since UCMR 3 identified at least 29 PWSs where the summed concentrations of PFOA and PFOS exceeded the EPA HRL {U.S. EPA, 2021, 7487276}. 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.

Likewise, Glassmeyer et al. (2017, 3454569) sampled source and treated drinking water from 29 drinking water treatment plants for a suite of emerging chemical and microbial contaminants, including 11 PFAS. PFOS was reported in source water at 88% of systems, with a median concentration of 2.28 ng/L and maximum concentration of 48.30 ng/L. Similarly, in treated drinking water, PFOS was detected in 80% of systems, with a median concentration of 1.62 ng/L and maximum concentration of 36.90 ng/L.

[2] Sum of PFOA + PFOS results rounded to 2 decimal places in those cases where a laboratory reported more digits.
[3] An HRL is a health-based concentration against which the agency evaluates occurrence data when making decisions about regulatory determinations. The HRL for PFOS that was used to evaluate UCMR 3 results was 0.070 µg/L (equal to the 2016 Drinking Water Health Advisory value).

2 Summary of Assessment Methods

This section summarizes the methods used for the systematic review of the health literature for PFOS, PFOA, and their related salts. The purposes of this systematic review were to identify the best available and most relevant health effects literature, to screen studies for quality, and to subsequently identify and consider studies that can be used for dose-response assessment. A detailed description of these methods is provided as a protocol in the Appendix (see PFOS Appendix).

The information that was gathered in the systematic review described in this document was used to update EPA's 2016 HESD for PFOS {U.S. EPA, 2016, 3603365} and to derive an MCLG to support a National Primary Drinking Water regulation under the Safe Drinking Water Act.

2.1 Introduction to the Systematic Review Assessment Methods

The methods used to conduct the systematic review for PFOS are consistent with the methods described in the draft and final *EPA ORD Staff Handbook for Developing IRIS Assessments* {U.S. EPA, 2020, 7006986; U.S. EPA, 2022, 10367891} (hereafter referred to as the Integrated Risk Information System (IRIS) Handbook) and a companion publication {Thayer, 2022, 10259560}. EPA's IRIS Handbook has incorporated feedback from the National Academy of Sciences (NAS) at workshops held in 2018 and 2019 and was well regarded by the NAS review panel for reflecting "significant improvements made by EPA to the IRIS assessment process, including systematic review methods for identifying chemical hazards" {NAS, 2021, 9959764}. Furthermore, EPA's IRIS program has used the IRIS Handbook to develop toxicological reviews for numerous chemicals, including some PFAS. Though the IRIS Handbook was finalized concurrently with this assessment, the alterations in the final IRIS Handbook compared to the draft version did not conflict with the methods used in this assessment. In fact, many of the NAS recommendations incorporated into the final IRIS handbook (e.g., updated methods for evidence synthesis and integration) were similarly incorporated into this assessment protocol {NAS, 2021, 9959764}. However, some of the study evaluation refinements recommended by NAS {2021, 9959764}, including clarifications to the procedure for evaluating studies for sensitivity and standardizing the procedure for evaluating reporting quality between human and animal studies, were not included in this assessment protocol, consistent with a 2011 NASEM recommendation not to delay releasing assessments until systematic review methods are finalized {NRC, 2011, 710724}. The assessment team concluded that implementing these minor changes in study quality evaluation would not change the assessment conclusions. Therefore, EPA considers the methods described herein to be consistent with the final IRIS Handbook and cites this version accordingly.

For this updated toxicity assessment, systematic review methods used were comparable to those in the IRIS Handbook for the steps of literature search, screening, study quality evaluation, data extraction, and the display of study quality results for all health outcomes through the 2020 literature searches {U.S. EPA, 2022, 10367891}. EPA then focused the subsequent steps of the systematic review process (synthesis of human, experimental animal, and mechanistic data; evidence integration; derivation of toxicity values) on health effects outcomes with the strongest weight of evidence (developmental, hepatic, immune, cardiovascular, and cancer) based on the

conclusions presented in EPA's preliminary analysis, *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water,* and consistent with the recommendations of the SAB {U.S. EPA, 2022, 10476098}.

This section provides a summary of methods used to search and screen the literature identified, evaluate the studies and characterize study quality, extract data, and identify studies that can be used for dose-response analysis. Extracted data are available in interactive visual formats (see Section 3) and can be downloaded in open access formats.

The systematic review protocol (see PFOS Appendix) provides a detailed description of the systematic review methods that were used. The particular focus of the protocol is the description of the problem formulation and key science issues guiding this assessment.

2.1.1 Literature Search

EPA assembled an inventory of epidemiological, animal toxicological, mechanistic, and toxicokinetic studies for this updated toxicity assessment based on three data streams: 1) literature published from 2014 through 2019 and then updated in the course of this review (i.e., through February 3, 2022) identified via literature searches of a variety of publicly available scientific literature databases, 2) literature identified via other sources (e.g., searches of the gray literature and studies shared with EPA by the SAB), and 3) literature identified in EPA's 2016 HESDs for PFOA and PFOS {U.S. EPA, 2016, 3603279; U.S. EPA, 2016, 3603365}.

The search strings for the new searches for this updated assessment focused on the chemical name (PFOA, PFOS, and their related salts) with no limitations on lines of evidence (i.e., human/epidemiological, animal, *in vitro*, *in silico*) or health outcomes. EPA conducted an updated literature search in 2019 (covering January 2013 through April 11, 2019), which was subsequently updated by a search covering April 2019 through September 3, 2020 (2020 literature search) and another covering September 2020 through February 3, 2022 (2022 literature search) using the same search strings used in 2019.

The publicly available databases listed below were searched for literature containing the chemical search terms outlined in the PFOS Appendix:

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

In addition to the databases above, other review efforts and searches of publicly available sources were used to identify relevant studies, as listed below:

- studies cited in assessments published by other U.S. federal, international, and/or state agencies (this included assessments by ATSDR and California Environmental Protection Agency (CalEPA)),
- studies identified during mechanistic or toxicokinetic synthesis (i.e., during manual review of reference lists of relevant mechanistic and toxicokinetic studies deemed relevant after screening against mechanistic- and ADME-specific PECO criteria), and

- studies identified by the SAB in their final report dated August 23, 2022 {U.S. EPA, 2022, 10476098}.

The details of the studies included from the 2016 HESD as well as the search strings and literature sources searched are described in the Appendix (see PFOS Appendix).

EPA relied on epidemiological and animal toxicological literature identified in the 2016 PFOS HESD to identify studies for this updated assessment on five major health outcomes, as recommended by SAB and consistent with EPA's preliminary analysis in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water*. The 2016 HESD for PFOS contained a summary of all relevant literature identified in searches conducted through 2013. The RfD derived in EPA's 2016 HESD was based quantitatively on animal toxicological studies whereas epidemiology studies were considered qualitatively, as a supporting line of evidence. This updated assessment includes the study quality evaluation of epidemiological studies that were identified and included in the 2016 HESD for the five health outcomes that had the strongest evidence. It also includes "key" animal toxicological studies from the HESD, which includes studies that were selected in 2016 for dose-response modeling. More details are provided in the Appendix (See PFOS Appendix).

All studies identified in the literature searches as well as those brought forward from the 2016 PFOS HESD were uploaded into the Health and Environmental Research Online (HERO) database (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2608) and are publicly available.

EPA has continued to monitor the literature published since February 2022 for other potentially relevant studies published after the 2022 literature search update Potentially relevant studies identified after February 2022 that were not recommended by the SAB in their final report are not included as part of the evidence base for this updated assessment but are provided in a repository detailing the results and potential impacts of new literature on the assessment (See PFOS Appendix A.3).

2.1.2 Literature Screening

This section summarizes the methods used to screen the identified health effects, mechanistic, and absorption, distribution, metabolism, excretion (ADME) literature. Briefly, PECO statements were established and detail the criteria used to screen all of the literature identified from literature searches in this assessment, prioritize the dose-response literature for dose-response assessment, and identify supplemental studies that may inform key science questions described in the protocol. The PECO criteria used for screening the dose-response, toxicokinetic, and mechanistic literature are provided in the Appendix (See PFOS Appendix).

Consistent with protocols outlined in the IRIS Handbook {U.S. EPA, 2022, 10367891}, studies identified in the literature searches and stored in HERO were imported into the Swift-Review software platform and the software was used to identify those studies most likely to be relevant to human health risk assessment. Studies captured then underwent title and abstract screening by at least two reviewers using DistillerSR or SWIFT ActiveScreener software, and studies that passed this screening underwent full-text review. Dose-response studies that met PECO inclusion

criteria following both title and abstract screening and full-text review underwent study quality evaluation as described below. Studies tagged as supplemental and containing potentially relevant mechanistic or ADME (or toxicokinetic) data following title and abstract and full-text level screening underwent further screening using mechanistic- or ADME-specific PECO criteria, and those deemed relevant underwent light data extraction of key study elements (e.g., extraction of information about the tested species or population, mechanistic or ADME endpoints evaluated, dose levels tested; see PFOS Appendix). Supplemental studies that were identified as mechanistic or ADME via screening did not undergo study quality evaluation.

2.1.3 Study Quality Evaluation for Epidemiological Studies and Animal Toxicological Studies

For study quality evaluation of the PECO-relevant human epidemiological and animal toxicological studies identified in the three literature searches (all health outcomes for the 2019 and 2020 searches; the five priority health outcomes for the 2022 search), epidemiological studies from the 2016 HESD that reported results on one or more of the five priority health outcomes, and key animal toxicological studies from the 2016 HESD, two or more quality assurance (QA) reviewers, working independently, assigned ratings about the reliability of study results (*good*, *adequate*, *deficient* (or *"not reported"*), or *critically deficient*) for different evaluation domains. These study quality evaluation domains are listed below and details about the domains, including prompting questions and suggested considerations, are described in the PFOS Appendix.

- Epidemiological study quality evaluation domains: participant selection; exposure measurement criteria; outcome ascertainment; potential confounding; analysis; selective reporting; and study sensitivity.
- Animal toxicological study quality evaluation domains: reporting; allocation; observational bias/blinding; confounding/variable control; reporting and attrition bias; chemical administration and characterization; exposure timing, frequency, and duration; endpoint sensitivity and specificity; and results presentation.

The independent reviewers performed study quality evaluations using a structured platform housed within EPA's Health Assessment Workplace Collaboration (HAWC; https://hawcproject.org/). Once the individual domains were rated, reviewers independently evaluated the identified strengths and limitations of each study to reach an overall classification on study confidence of *high*, *medium*, *low*, or *uninformative* for each PECO-relevant endpoint evaluated in the study. A study can be given an overall *mixed* confidence classification if different PECO-relevant endpoints within the study receive different confidence ratings (e.g., *medium* and *low* confidence classifications).

2.1.4 Data Extraction

Data extraction was conducted for all relevant human epidemiological and animal toxicological studies determined to be of *medium* and *high* confidence after study quality evaluation. Data were also extracted from *low* confidence epidemiological studies when data were limited for a health outcome or when there was a notable effect, consistent with the IRIS Handbook {U.S. EPA, 2020, 7006986}. Studies evaluated as being *uninformative* were not considered further and therefore did not undergo data extraction. All health endpoints were considered for extraction,

regardless of the magnitude of effect or statistical significance of the response relative to the control group. The level of detail in data extractions for different endpoints within a study could differ based on how the data were presented for each outcome (i.e., ranging from a narrative to a full extraction of dose-response effect size information).

Extractions were conducted using DistillerSR for epidemiological studies or HAWC for animal toxicological studies. An initial reviewer conducted the extraction, followed by an independent QA review by a second reviewer who confirmed accuracy and edited/corrected the extraction as needed. Discrepancies in data extraction were resolved by discussion and confirmation within the extraction team.

Data extracted from epidemiology studies included 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. Data extracted from animal toxicological studies included information on the experimental design and exposure duration, species and number of animals tested, dosing regime, and endpoints measured. Further information about data extraction can be found in the PFOS Appendix.

2.1.5 Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes. Evidence synthesis refers to the process of analyzing the results of the available studies (including their strengths and weaknesses) for consistency and coherence, often by evidence stream (e.g., human or animal) and health effect outcome. In evidence integration, the evidence across streams is considered together and integrated to develop judgments (for each health outcome) about whether the chemical in question poses a hazard to human health.

The evidence syntheses are summary discussions of the body of evidence for each evidence stream (i.e., human and animal) for each health outcome analyzed. The available human and animal health effects evidence were synthesized separately, with each synthesis resulting in a summary discussion of the available evidence. For the animal toxicological evidence stream, evidence synthesis included consideration of studies rated *high* and *medium* confidence. For the epidemiological evidence stream, evidence synthesis was based primarily on studies of *high* and *medium* confidence, including discussion of study quality considerations, according to the recommendations of the SAB {U.S. EPA, 2022, 10476098}. Inferences drawn from studies described in the 2016 PFOS HESD were considered when drawing health effects conclusions. Epidemiological studies were excluded from the evidence synthesis narrative if they included data that were reported in multiple studies (e.g., overlapping NHANES studies). Studies reporting results from the same cohort and the same health outcome as another study were considered overlapping evidence, and these additional studies were not discussed in the evidence synthesis narrative to avoid duplication or overrepresentation of results from the same group of participants. In cases of overlapping studies, the study with the largest number of participants and/or the most accurate outcome measures was given preference. Consistent with the IRIS Handbook {U.S. EPA, 2022, 10367891}, *low* confidence epidemiological studies and results were used only in a supporting role and given less weight during evidence synthesis and integration compared to *high* or *medium* confidence studies. *Low* confidence epidemiological

studies were included in evidence syntheses order to capture all of the available data for PFOS in the weight of evidence analyses.

For evidence integration, integrated judgments, that took into account mechanistic considerations for the five priority health outcomes (i.e., cancer, hepatic, immune, cardiovascular, and developmental), were drawn for each health outcome across human and animal lines of evidence. The evidence integration provides a summary of the causal interpretations between PFOS exposure and health effects based on results of the available epidemiological and animal toxicological studies, in addition to the available mechanistic evidence. Considerations when evaluating the available studies included risk of bias, sensitivity, consistency, strength (effect magnitude) and precision, biological gradient/dose-response, coherence, and mechanistic evidence related to biological plausibility.

The evidence integration was conducted according to guidance outlined in the IRIS Handbook and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* {U.S. EPA, 2020, 8642427}. The evidence integration included evidence stream evaluation in which the qualitative summaries on the strength of evidence from studies in animals and humans were evaluated, and subsequent inference across all evidence streams. Human relevance of animal models as well as mechanistic evidence to inform mode of action were considered. Evidence integration produced an overall judgment about whether sufficient or insufficient evidence of an association with PFOS exposure exists for each human health outcome, as well as the rationale for each judgment. The potential evidence integration judgments for characterizing human health effects are ***evidence demonstrates, evidence indicates (likely), evidence suggests, evidence inadequate,*** and ***strong evidence supports no effect.***

Details about evidence synthesis and integration are summarized in the Appendix (See PFOS Appendix).

2.2 Dose-Response Assessment

Evidence synthesis and integration enabled identification of the health outcomes with the strongest weight of evidence supporting causal relationships between PFOS exposure and adverse health effects, as well as the most sensitive cancer and noncancer endpoints. Studies were evaluated for use in POD derivation on the basis of study design, study quality evaluation, and data availability. For human evidence, all *high* or *medium* confidence studies were considered; for animal evidence, only animal toxicological studies with at least two PFOS exposure groups and of *high* or *medium* confidence were considered.

2.2.1 Approach to POD and RfD Derivation for Non-Cancer Health Outcomes

The current, recommended EPA human health risk assessment approach described in EPA's *A Review of the Reference Dose and Reference Concentration Processes*, which is a multistep approach to dose-response assessment, includes analysis of dose and response within the range of observation, followed by extrapolation to lower exposure levels {U.S. EPA, 2002, 88824}. For non-cancer health outcomes, EPA performed dose-response assessments to define points of departure (PODs) and extrapolated from the PODs to RfDs.

For PFOS, EPA performed benchmark dose (BMD) modeling of all animal toxicological studies considered for dose-response to refine the POD in deriving the RfD. The BMD modeling approach involves dose-response modeling to obtain BMDs (i.e., dose levels corresponding to specific response levels near the low end of the observable range of the data) and identifies the lower limits of the BMDs (BMDLs) to serve as potential PODs for deriving quantitative estimates below the range of observation {U.S. EPA, 2012, 1239433}. EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). BMDS fits mathematical models to the data and determines the dose (benchmark dose or BMD) that corresponds to a pre-determined level of response (benchmark response or BMR). For dichotomous data, the BMR is typically set at either 5 or 10% above the background or the response of the control group. For continuous data, a BMR of one half or one standard deviation from the control mean is typically used when there are no outcome-specific data to indicate what level of response is biologically significant {U.S. EPA, 2012, 1239433}. For dose-response data for which BMD modeling did not produce an adequate model fit, a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) was used as the POD.

For the epidemiological studies considered for dose-response assessment, EPA used multiple modeling approaches to determine PODs, depending upon the health outcome and the data provided in the studies. For the developmental, hepatic, and serum lipid dose-response studies, EPA used a hybrid modeling approach that involves estimating the incidence of individuals above or below a level considered to be adverse and determining the probability of responses at specified exposure levels above the control {U.S. EPA, 2012, 1239433} for cases in which EPA was able to define a level considered clinically adverse for these outcomes (see PFOS Appendix for details). EPA also performed BMD modeling and provided study LOAELs/NOAELs for the hepatic and serum lipid dose-response studies as sensitivity analyses of the hybrid approach. For the immune studies, where a clinically defined adverse level is not well defined, EPA used multivariate models provided in the studies and determined a BMR according to EPA guidance to calculate BMDs and BMDLs {U.S. EPA, 2012, 1239433}.

See the PFOS Appendix for additional details on the study-specific modeling.

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

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

Step 1a (for dose-response data from a study in an animal model): **Convert administered dose to an internal dose.** A pharmacokinetic model is used to predict the internal dose (in the animals used in the toxicity studies or in humans) that would correspond to 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 PFOS in blood are considered for all the internal dose-metrics.

Step 2: Conduct dose-response modeling. See above and the PFOS Appendix for study-specific details.

Step 3: Convert the POD to a human equivalent dose (HED) or point of departure human equivalent dose (POD_{HED}). The POD (a BMDL, NOAEL, or LOAEL) is converted to an HED following the method described in Section 4.1.3. Briefly, a pharmacokinetic model for human dosimetry is used to simulate the HED from the animal PODs from Step 2. Pharmacokinetic modeling 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 (see Section 4). Based on the available data, a serum PFOS concentration was identified as a suitable internal dosimetry target for the human and animal endpoints of interest.

Step 4: Select appropriate uncertainty factors (UFs) and provide rationale for UF selection. UFs are applied 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 epidemiological or animal toxicological database.

Step 5: Calculate the chronic RfD. The RfD is calculated by dividing POD_{HED} by the composite (total) UF.

$$\boldsymbol{RfD} = \left(\frac{POD_{HED}}{UF_C}\right)$$

where:

POD_{HED} = calculated from the BMDL, NOAEL, or LOAEL using the human pharmacokinetic (PK) model presented in Section 4.1.3.2.

UF_C = Composite (total) UF calculated by multiplying the selected individual UFs for 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, in accordance with EPA guidelines {U.S. EPA, 2002, 88824}.

2.2.2 Cancer Assessment

2.2.2.1 Approach for Cancer Classification

In accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, a descriptive weight of evidence expert judgment is made, based on all available animal, human, and mechanistic data, as to the likelihood that a contaminant is a human carcinogen and the conditions under which the carcinogenic effects may be expressed {U.S. EPA, 2005, 9638795}. A narrative is developed to provide a complete description of the weight of evidence and conditions of carcinogenicity. The potential carcinogenicity descriptors (presented in the 2005 guidelines) are:

- Carcinogenic to humans

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

More than one carcinogenicity descriptor can be applied if a chemical's effects differ by dose, exposure route, or mode of action (MOA)[4]. For example, a chemical may be carcinogenic to humans above but not below a specific dose level if a key event in tumor formation does not occur below that dose. MOA information informs both the qualitative and quantitative aspects of the assessment, including the human relevance of tumors observed in animals. MOA must be considered separately for each target organ.

2.2.2.2 Derivation of a Cancer Slope Factor

EPA's 2005 *Guidelines for Carcinogen Risk Assessment* recommends a two-step process for the quantitation of cancer risk. First, a model is used to fit a dose-response curve to the data, based on the doses and associated tumors observed. For animal toxicological studies, EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). For cancer data, BMDS fits multistage models and the model is used to identify a POD for extrapolation to the low-dose region based on the BMD associated with a significant increase in tumor incidence above the control. According to the 2005 guidelines, the POD is the lowest dose that is adequately supported by the data. The BMD_{10} (the dose corresponding to a 10% increase in tumors) and the $BMDL_{10}$ (the 95% lower confidence limit on that dose) are also reported and are often used as the POD.

In the second step of quantitation, the POD is extrapolated to the low-dose region of interest for environmental exposures. The approach for extrapolation depends on the MOA for carcinogenesis (i.e., linear or nonlinear). When coverage indicates that a chemical causes cancer through a mutagenic MOA (i.e., mutation of deoxyribonucleic acid (DNA)) or the MOA for carcinogenicity is not known, this extrapolation is performed by drawing a line (on a graph of dose vs. response) from the POD to the origin (zero dose, zero tumors). The slope of the line (Δresponse/Δdose) gives rise to the CSF, which can be interpreted as the risk per mg/kg/day. In addition, according to EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* {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 whether age-dependent adjustment factors are applied in the quantification of risk to account for additional sensitivity of children.

In cases for which a chemical is shown to cause cancer via an MOA that is not linear at low doses, and the chemical does not demonstrate mutagenic or other activity consistent with linearity at low doses, a nonlinear extrapolation is conducted. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* state that "where tumors arise through a nonlinear MOA, an oral RfD or inhalation reference concentration, or both, should be developed in accordance with EPA's established practice of developing such values, taking into consideration the factors

[4]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.

summarized in the characterization of the POD." In these cases, an RfD-like value is calculated based on the key event[5] for carcinogenesis or the tumor response.

Once a POD is determined, a PK model is used to calculate the HED for animal oral exposures (POD_{HED}). The CSF is then calculated by dividing the selected BMR by the POD_{HED}.

See the PFOS Appendix for additional details on the study-specific modeling.

2.3 MCLG Derivation

As provided in SDWA Section 1412(b)(4)(A), EPA establishes the MCLG at the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety. EPA assesses available science examining cancer and noncancer health effects associated with oral exposure to the contaminant. Consistent with the statutory definition of MCLG, EPA establishes MCLGs of zero for carcinogens classified as *Carcinogenic to Humans* or *Likely to be Carcinogenic to Humans*[6] for which there is insufficient information to determine that a carcinogen has a threshold below which there are no carcinogenic effects {U.S. EPA, 1998, 10442462; U.S. EPA, 2000, 10442463; U.S. EPA, 2001, 10442464}.

For nonlinear carcinogenic contaminants, contaminants that are suggestive carcinogens, and non-carcinogenic contaminants, EPA establishes the MCLG based on a toxicity value, typically an RfD, but a similar toxicity value (e.g., ATSDR Minimal Risk Level) may also be used when it represents the best available science. A noncancer MCLG is designed to be protective of noncancer effects over a lifetime of exposure with an adequate margin of safety, including for sensitive populations and life stages consistent with SDWA 1412(b)(3)(C)(i)(V) and 1412(b)(4)(A). The calculation of a noncancer MCLG includes an oral toxicity reference value

[5]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.

[6]The MCLG is derived depending on the available noncancer and cancer evidence for a particular chemical. Establishing the MCLG for a chemical has typically been accomplished in one of three ways depending upon a three-category classification approach {U.S. EPA, 1985, 9207; U.S. EPA, 1991, 5499}. The categories are based on the available evidence of carcinogenicity after exposure via ingestion. The starting point in categorizing a chemical is through assigning a cancer descriptor using EPA's current Guidelines for Carcinogen Risk Assessment {U.S. EPA, 2005, 6324329}. The descriptors in the 2005 Guidelines replaced the prior alphanumeric groupings, although the basis for the classifications is similar. In prior rulemakings, the agency typically placed Group A, B1, and B2 contaminants into Category I, Group C into Category II, and Group D and E into Category III based on the agency's previous cancer classification guidelines (i.e., Guidelines for Carcinogen Risk Assessment, published in 51 FR 33992, September 24, 1986 {U.S. EPA, 1986, 199530} and the 1999 interim final guidelines {U.S. EPA, 1999, 41631; U.S. EPA, 2001, 10442464}):

- Category I chemicals have "strong evidence [of carcinogenicity] considering weight of evidence, pharmacokinetics, and exposure {U.S. EPA, 1985, 9207; U.S. EPA, 1991, 5499}." EPA's 2005 cancer descriptors associated with this category are: "Carcinogenic to Humans" or "Likely to be Carcinogenic to Humans" {U.S. EPA, 2005, 6324329}. EPA's policy under SDWA is to set MCLGs for Category I chemicals at zero, based on the principle that any exposure to known or likely human carcinogens might represent some finite level of risk. In cases when there is sufficient evidence to determine a nonlinear cancer mode of action, the MCLG is based on the RfD approach described below.
- Category II chemicals have "limited evidence [of carcinogenicity] considering weight of evidence, pharmacokinetics, and exposure {U.S. EPA, 1985, 9207; U.S. EPA, 1991, 5499}." EPA's 2005 cancer descriptor associated with this category is: "Suggestive Evidence of Carcinogenic Potential" {U.S. EPA, 2005, 6324329}. The MCLG for Category II contaminants is based on noncancer effects {U.S. EPA, 1985, 9207; U.S. EPA, 1991, 5499}.
- Category III chemicals have "inadequate or no animal evidence [of carcinogenicity] {U.S. EPA, 1985, 9207; U.S. EPA, 1991, 5499}." EPA's 2005 cancer descriptors associated with this category are: "Inadequate Information to Assess Carcinogenic Potential" and "Not Likely to Be Carcinogenic to Humans" {U.S. EPA, 2005, 6324329}. The MCLG for Category III contaminants is based on noncancer effects.

such as an RfD, body weight-based drinking water intake (DWI-BW), and RSC as presented in the equation below:

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

Where:

RfD = chronic reference dose—an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure of the human population to a substance that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is equal to a POD_{HED} divided by a composite uncertainty factor.

DWI-BW = An exposure factor in the form of the 90th percentile body weight-adjusted drinking water intake value for the identified population or life stage, in units of liters of water consumed per kilogram body weight per day (L/kg bw-day). The DWI-BW considers both direct and indirect consumption of drinking water (indirect water consumption encompasses water added in the preparation of foods or beverages, such as tea or coffee). Chapter 3 of EPA's *Exposure Factors Handbook* {U.S. EPA, 2019, 7267482} provides DWI-BWs for various populations or life stages within the general population for which there are publicly available, peer-reviewed data such as NHANES data.

RSC = relative source contribution—the percentage of the total exposure attributed to drinking water sources {U.S. EPA, 2000, 19428}, with the remainder of the exposure allocated to all other routes or sources. The purpose of the RSC is to ensure that the level of a contaminant (e.g., MCLG value), when combined with other identified sources of exposure common to the population of concern, will not result in exposures that exceed the RfD. The RSC is derived by applying the Exposure Decision Tree approach published in EPA's *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* {U.S. EPA, 2000, 19428}. Further description of the RSC for PFOS can be found in the Appendix (see PFOS Appendix).

3 Results of the Health Effects Systematic Review and Toxicokinetics Methods

3.1 Literature Search and Screening Results

Studies referenced in this assessment are cited as "Author Last Name, Publication Year, HERO ID" and are available in EPA HERO: A Database of Scientific Studies and References. The HERO ID is a unique identifier for studies available in HERO. Additional study metadata are publicly available and can be obtained by searching for the HERO ID on the public-facing webpage available here: https://hero.epa.gov/.

The three database searches yielded 6,007 unique records prior to running SWIFT Review. Table 3-1 shows the results from database searches conducted in April 2019, September 2020, and February 2022.

Table 3-1. Database Literature Search Results

Database	Date Run: Results
WoS	4/10/2019: 3,081 results 9/3/2020: 1,286 results 2/2/2022: 1,021 results
PubMed	4/10/2019: 2,191 results 9/3/2020: 811 results 2/2/2022: 1,728 results
TOXLINE	4/10/2019: 60 results
TSCATS	4/11/2019: 0 results
Total number of references from all databases for all searches[a]	4/2019: 3,382 results 9/2020: 1,153 results 2/2022: 1,858 results
Total number of references after running SWIFT Review[a]	4/2019: 1,977 results 9/2020: 867 results 2/2022: 1,370 results
Total number of unique studies moved to screening [b]	**3,921**

Notes:
[a] The number of studies includes duplicate references across search dates due to overlap between search years.
[b] Duplicates across search dates removed.

The additional sources of literature outlined in Section 2.1.1 (i.e., assessments published by other agencies, studies identified during mechanistic or toxicokinetic syntheses, and studies identified by the SAB) yielded 200 unique records.

The 3,921 studies captured with the SWIFT Review evidence streams filters and the 200 records identified from additional sources yield a total of 4,121 unique studies. These 4,121 studies were moved to the next stage of screening (title and abstract screening using either DistillerSR or SWIFT ActiveScreener). Of the 4,121 unique studies, 918 moved on to full-text level review, 1,589 were excluded during title and abstract screening, and 1,614 were tagged as containing potentially relevant supplemental material. Of the 918 screened at the full-text level, 599 were considered to meet PECO eligibility criteria (See PFOS Appendix) and included relevant information on PFOS. The 599 studies that were determined to meet PECO criteria after full-text

level screening included 423 epidemiological (human) studies, 45 animal toxicological studies, 6 PBPK studies, and 125 studies that were not extracted (e.g., *low* confidence studies, meta-analyses, studies that did not evaluate effects on one of the priority health outcomes). An additional 16 PBPK studies were identified during the toxicokinetic screening for a total of 22 PBPK studies. Details of the literature search and screening process are shown in Figure 3-1.

The 423 epidemiological studies and 45 animal toxicological studies underwent study quality evaluation and were subsequently considered for data extraction as outlined in Sections 2.1.3 and 0 (see PFOS Appendix for more details). The results of the health outcome-specific study quality evaluations and data extractions are described in Sections 3.4 and 3.5.

Additionally, the 22 studies tagged as containing relevant PBPK models were reviewed by PK subject matter experts for inclusion consideration. The included studies are summarized in Section 3.3.2 and parameters described in these studies were considered for incorporation into the animal and human PK models, which are summarized in Section 4.1.3.

Finally, the 89 toxicokinetic and 301 mechanistic studies identified as relevant for PFOS moved on to a limited data extraction as described in the Appendix (see PFOS Appendix). The toxicokinetic studies pertaining to ADME are synthesized in Section 3.3.1. The mechanistic studies relevant to the 5 prioritized health outcomes are synthesized in Sections 3.4 and 3.5 and were considered as part of the evidence integration.

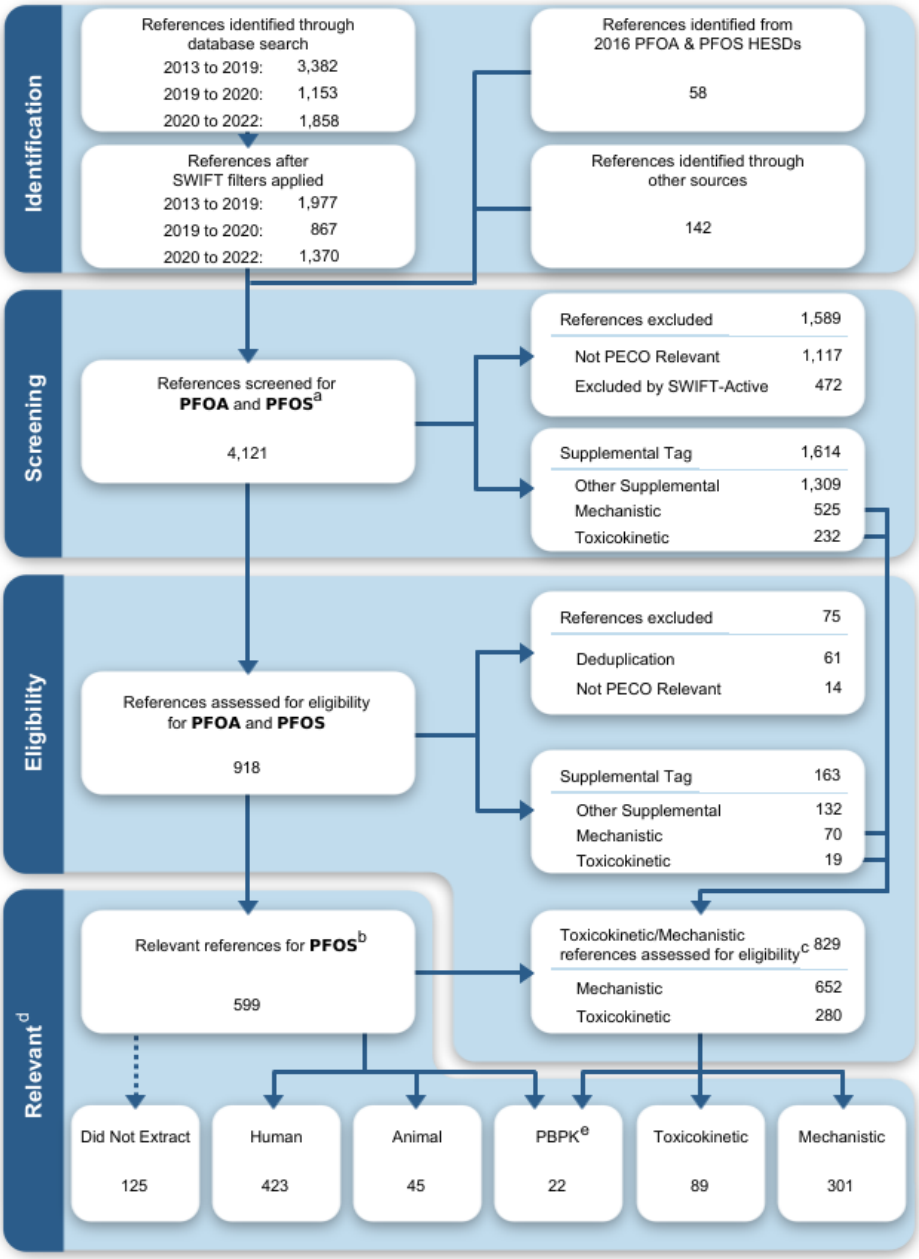


Figure 3-1. Summary of Literature Search and Screening Process for PFOS

Interactive figure and additional study details available on Tableau.
Interactive figure based on work by Magnuson et al. (2022, 10442900).
"Other sources" include assessments published by other agencies, studies identified during mechanistic or toxicokinetic syntheses, and studies identified by the SAB.
[a] Includes number of unique references after deduplication of studies captured with the SWIFT Review evidence streams filters and records identified from additional sources.
[b] Includes number of unique references considered to meet PECO eligibility criteria at the full text level and include relevant information on PFOS.
[c] Includes number of unique references identified during title/abstract screening, full text screening, and data extraction assessed for toxicokinetic and/or mechanistic eligibility.
[d] Only includes studies with relevant information on PFOS.
[e] Includes 6 PBPK studies determined to meet PECO criteria plus an additional 16 PBPK studies identified during the toxicokinetic screening.

3.1.1 Results for Epidemiology Studies of PFOS by Health Outcome

Of the 423 epidemiological studies that met the inclusion criteria, 179 studies had a cohort study design, 167 had a cross-sectional design, 40 had a case-control design, and 37 had other study designs (e.g., nested case-control). Epidemiological studies were categorized into 18 health outcomes. Most studies reported on the developmental (n = 90), cardiovascular (n = 85), metabolic (n = 74), or immune systems (n = 63). Studies that reported outcomes spanning multiple health outcomes were not counted more than once in the grand totals shown in Figure 3-2.

	Study Design				
Health System	Case-control	Cohort	Cross-sectional	Other	Grand Total
Cancer	5	3	3	5	16
Cardiovascular	5	18	56	6	85
Dermal	0	1	0	0	1
Developmental	6	58	19	7	90
Endocrine	1	8	20	7	36
Gastrointestinal	1	4	0	0	5
Hematologic	0	0	8	0	8
Hepatic	1	3	18	2	24
Immune	6	31	17	9	63
Metabolic	7	32	31	4	74
Musculoskeletal	0	0	6	2	8
Nervous	3	26	5	3	37
Ocular	0	0	1	0	1
Renal	1	3	16	0	20
Reproductive, Male	0	7	15	2	24
Reproductive, Female	10	23	19	3	55
Respiratory	1	3	1	0	5
Other	0	2	3	0	5
Grand Total	40	179	167	37	423

Figure 3-2. Summary of Epidemiology Studies of PFOS Exposure by Health System and Study Design[a]

Interactive figure and additional study details available on Tableau.

[a] A study can report on more than one health system. Column grand totals represent the number of unique studies and are not a sum of health system tags.

3.1.2 Results for Animal Toxicological Studies of PFOS by Health Outcome

Of the 45 animal toxicological studies that met the inclusion criteria, most studies had either short-term (n = 19) or developmental (n = 15) study designs and most were conducted in rats (n = 23). The rat studies had short-term (n = 12), developmental (n = 7), chronic (n = 2), reproductive (n = 2), and subchronic (n = 1) study designs. The remaining studies reported results for mice (n = 21) using developmental (n = 8), short-term (n = 7), subchronic (n = 5), or

reproductive (n = 1) study designs, monkeys (n = 1) using a chronic study design, or rabbits (n = 1) using a developmental study design. Animal toxicological studies were categorized into 13 health outcomes. Most studies reported results for the whole body (n = 25; i.e., systemic endpoints such as bodyweight), hepatic (n = 20), reproductive (n = 19), or developmental (n = 16) systems. Studies that reported outcomes spanning multiple health outcomes, study designs, or species were not counted more than once in the grand totals shown in Figure 3-3.

	Study Design & Species											
	Short-term		Subchronic		Chronic		Developmental			Reproductive		Grand Total
Health System	Mouse	Rat	Mouse	Rat	Monkey	Rat	Mouse	Rabbit	Rat	Mouse	Rat	
Cancer	0	0	0	0	0	1	0	0	0	0	0	1
Cardiovascular	1	2	2	0	1	1	0	0	2	0	1	10
Developmental	0	0	0	0	0	0	8	1	6	0	2	16
Endocrine	1	4	1	0	1	1	2	0	3	0	1	13
Hematologic	1	3	0	0	1	0	0	0	0	0	0	5
Hepatic	2	6	4	0	1	2	4	0	2	0	1	20
Immune	2	4	3	0	1	2	1	0	0	0	0	13
Metabolic	0	3	1	0	0	2	0	0	1	0	1	7
Nervous	2	6	1	0	0	1	1	0	2	0	1	14
Renal	0	3	3	0	1	2	2	0	0	0	0	10
Reproductive	2	3	1	1	1	0	4	1	3	1	2	19
Respiratory	1	1	1	0	0	0	0	0	0	0	0	3
Whole Body	4	7	4	1	1	2	2	1	2	0	2	25
Grand Total	7	12	5	1	1	2	8	1	7	1	2	45

Figure 3-3. Summary of Animal Toxicological Studies of PFOS Exposure by Health System, Study Design, and Species[a,b]

Interactive figure and additional study details available on Tableau.

[a] A study can report on more than one study design and species. Row grand totals represent the number of unique studies and are not a sum of study design and species tags.

[b] A study can report on more than one health system. Column grand totals represent the number of unique studies and are not a sum of health system tags.

3.2 Data Extraction Results

Data extracted from the 423 epidemiological studies are available via Tableau Public and data extracted from the 45 animal toxicological studies are available in the public HAWC site, displayed as exposure-response arrays, forest plots, and trees. See Sections 3.4 and 3.5 for health outcome-specific data extracted for synthesis development. Additionally, the limited data extractions from the ADME and mechanistic studies can be found via Tableau Public here and here, respectively.

3.3 Toxicokinetic Synthesis

As described in Section 3.1, EPA identified 89 and 22 studies containing information relevant to the toxicokinetics and PBPK modeling of PFOS, respectively. The results of these studies are described in the subsections below and additional information related to toxicokinetic characteristics of PFOS can be found in Appendix B.

3.3.1 ADME

PFOS is resistant to metabolic and environmental degradation due to its strong carbon-fluorine bonds. It is not readily eliminated and can have a long half-life in humans and animals. However, the toxicokinetic profile and the underlying mechanism for the chemical's long half-life are not completely understood. For PFOS, membrane transporter families appear to play an important

role in ADME, including organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), multidrug resistance-associated proteins (MRPs), and urate transporters. Transporters play a critical role in GI tract absorption, uptake by tissues, and excretion via bile and the kidney. Limited data are available regarding the transporters for PFOS; however, the toxicokinetic properties of PFOS suggest tissue uptake and renal resorption through facilitated uptake. Some inhibition studies suggest that PFOS transport could involve the same transporters as for PFOA, since PFOS and PFOA have similar chain lengths, renal excretion properties, and liver accumulation.

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

3.3.1.1 Absorption

Absorption data are available in laboratory animals for oral {Chang, 2012, 1289832} and inhalation {Rusch, 1979, 7561179} exposures, and extensive data are available demonstrating the presence of PFOS in human serum. Limited *in vitro* absorption data are available (see PFOS Appendix).

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

Uptake by cells may be influenced by interactions with lipids and serum proteins. PFOS exhibited higher levels of binding to lipids and phospholipids relative to PFOA, which correlated with uptake into lung epithelial cells {Sanchez Garcia, 2018, 4234856}. Phospholipophilicity correlated to cellular accumulation better than other lipophilicity measures. The extent to which PFOS phospholipophilicity influences absorption through the GI tract, lungs, or skin is unknown.

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

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

3.3.1.2 Distribution

3.3.1.2.1 PFOS Binding to Blood Fractions and Serum Proteins

Detailed study descriptions of literature regarding the distribution of PFOS in humans and animals are provided in the Appendix (see PFOS Appendix). Distribution of absorbed material requires vascular transport from the portal of entry to receiving tissues. Distribution of PFAS to plasma has been reported to be chain length-dependent {Jin, 2016, 3859825}. Increasing chain length (from C6 to C11) correlated with an increased mass fraction in human plasma. Among different kinds of human blood samples, PFOS 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 that median PFOS concentrations in plasma, serum, and whole blood were 5.24, 4.77, and 2.85 ng/mL, respectively. These findings suggest that the common practice of multiplying by a factor of 2 to convert the concentrations in whole blood to serum {Ehresman, 2007, 1429928} will not provide accurate estimates for PFOS.

PFOS is distributed within the body by noncovalently binding to plasma proteins. Many studies have investigated PFOS interactions with human serum albumin (HSA) {Zhang, 2009, 2919350; Salvalaglio, 2010, 2919252; Chen, 2009, 1280480; D'Alessandro, 2013, 5084740; Liu, 2017, 3856708}. *In vitro* analyses found that plasma proteins can bind PFOS in plasma from humans, cynomolgus monkeys, and rats {Kerstner-Wood, 2003, 4771364}. PFOS was highly bound (99.8%) to albumin and showed affinity for low-density lipoproteins (95.6%) with some binding to alpha-globulins (59.4%) and gamma-globulins (24.1%). HSA-PFOS intermolecular interactions are mediated through van der Waals forces and hydrogen bonds {Zhang, 2009, 2919350; Chen, 2009, 1280480}. Beesoon and Martin (2015, 2850292) determined that linear PFOS bound more strongly to calf serum albumin than the branched chain isomers in the order of 3m < 4m < 1m < 5m < 6m (iso) < linear. PFOS binding to HSA results in alterations in the albumin secondary structure and can diminish esterase activity {Liu, 2017, 3856708}, though the extent to which this affects the physiological functions of albumin is unknown. PFOS-mediated conformational changes may also interfere with albumin's ability to transport its natural ligands and pharmaceuticals, including vitamin B_2 (riboflavin) and ibuprofen {D'Alessandro, 2013, 5084740}, and may interfere with PFOS uptake into cells {Sheng, 2020, 6565171}.

Binding to albumin and other serum proteins may affect transfer of PFOS from maternal blood to the fetus {Gao, 2019, 5387135}. Since there is effectively a competition between PFOS binding in maternal serum vs. cord blood, lower cord blood albumin levels compared to maternal blood albumin levels are likely to reduce transfer from maternal serum across the placenta. Consistent with this hypothesis, Pan et al. (2017, 3981900) found that a high concentration of cord serum albumin was associated with higher PFOS transfer efficiencies, whereas high maternal serum albumin concentration was associated with reduced transfer efficiency.

3.3.1.2.2 PFOS Binding to Intracellular Proteins and Transporters

Within cells, PFOS has been shown to bind to liver fatty acid binding protein (L-FABP) {Luebker, 2002, 1291067; Zhang, 2013, 5081488; Yang, 2020, 6356370}. L-FABP is an intracellular lipid carrier protein that reversibly binds long-chain fatty acids, phospholipids, and an assortment of peroxisome proliferators {Erol, 2004, 5212239} and constitutes 2–5% of the cytosolic protein in hepatocytes.

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

3.3.1.2.3 Tissue Distribution in Humans and Animals

Evidence from human autopsy and surgical tissues demonstrates that PFOS distributes to a wide range of tissues, organs, and matrices throughout the body. It should be noted, however, that autopsy and surgical tissues may not accurately reflect PFAS tissue distribution in the living body {Cao, 2021, 9959613}. Blood and liver are major sites of PFOS accumulation {Olsen, 2001, 9641811}. Two studies measured PFOS levels in cerebrospinal fluid and serum {Harada, 2007, 2919450; Wang, 2018, 5080654} and in both studies, PFOS levels in cerebrospinal fluid were two orders of magnitude lower than in serum, suggesting that PFOS does not easily cross the adult human blood-brain barrier.

In a study of autopsy tissues collected within 24 hours of death, Pérez et al. (2013, 2325349) and found PFOS in the liver (104 ng/g), kidney (75.6 ng/g), lung (29.1 ng/g), and brain (4.9 ng/g), with levels below the limit of detection (LOD) in bone. PFOS also accumulates in follicular fluid {Kang, 2020, 6356899}, raising the possibility of reproductive toxicity in humans.

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

Several rodent studies identified high levels of PFOS in blood and liver across a range of dosing regimens and study durations. Whereas monkeys had nearly a 1:1 liver to serum ratio, rodent models were observed to accumulate far more PFOS in liver than serum {NTP, 2019, 5400978}. Plasma PFOS concentrations were generally similar in males and females. For example, in a 28-day toxicity study, dose-normalized plasma concentrations (μM/mmol/kg/day) in males and females were within 1.5-fold across the dose groups {NTP, 2019, 5400978}. Additional studies in rats and mice documented PFOS distribution to a wide range of tissues including kidney, heart, lungs, and spleen. Interestingly, in rodents, PFOS has been measured in moderate quantities in the brain and testicles, indicating that PFOS does cross the blood-brain and blood-testis barriers in rats {Qui, 2013, 2850956} and mice {Bogdanska, 2011, 2919253; Cui, 2009, 757868}.

3.3.1.2.4 Distribution During Reproduction and Development

Several studies in humans, rats, and mice quantified distribution of PFOS from pregnant females to placenta, cord blood, and amniotic fluid, which demonstrate pathways of distribution to and elimination from fetuses. Accumulation of PFOS in fetal tissues was found to vary by gestational age. New studies also confirm that distribution of PFOS from nursing mothers to their infants via

breastmilk correlates with duration of breastfeeding. Distribution is influenced by the chemical properties of PFAS including length, lipophilicity, and branching.

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

Several studies reported a strong positive correlation between maternal and cord serum levels of PFOS {Kato, 2014, 2851230; Porpora, 2013, 2150057}. The ratio of PFOS in cord serum relative to maternal serum ranged from 0.22 to 0.98 (see PFOS Appendix) and generally increased with gestational age {Li, 2020, 6505874}. Li et al. (2020, 6505874) also showed a 6% increase in branched PFOS accumulation compared to linear PFOS isomers. Zhao et al. (2017, 3856461) observed higher transplacental transfer efficiencies (TTEs) for 1m-, 4m-, 3+5m-, and m2-PFOS compared to n-PFOS. Together, these findings indicate that branched isomers of PFOS transfer more efficiently from maternal blood to cord blood compared to linear isomers. In addition to PFOS branching, maternal factors including exposure sources, parity, and other maternal demographics are postulated to influence observed variations in cord:maternal serum ratios {Eryasa, 2019, 5412430; Jusko, 2016, 3981718; Brochot, 2019, 5381552}.

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

PFOS also distributes widely in fetal tissues. Mamsen et al. (2017, 3858487) measured the concentrations of five PFAS in fetuses, placentas, and maternal plasma from a cohort of 39 pregnant women in Denmark. The concentration of PFOS decreased from maternal serum to fetal tissues as follows: maternal serum > placenta > fetal tissues. In a second study, PFAS levels were measured in embryos and fetuses at gestational weeks 7–42 and in serum from their matched maternal pairs {Mamsen, 2019, 5080595}. PFOS accumulated at higher levels in fetal tissues compared to other PFAS chemicals examined in fetal tissues and across trimesters. The concentration of PFAS in fetal tissues fluctuated across trimesters and did not follow any particular trend. For example, PFOS concentration in the liver was higher in the second trimester compared to the third trimester, and lowest in the lung in the second trimester compared to the first and third trimesters.

New studies also confirm that distribution of PFOS from nursing mothers to their infants via breastmilk correlates with duration of breastfeeding {Mondal, 2014, 2850916; Cariou, 2015, 3859840; Mogensen, 2015, 3859839; Gyllenhammar, 2018, 4778766}. Distribution is influenced by the chemical properties of PFAS including length, lipophilicity, and branching. In the Mondal study {Mondal, 2014, 2850916}, mean maternal serum PFOS concentrations were lower in

breastfeeding mothers vs. non-breastfeeding mothers. Conversely, breastfed infants had higher mean serum PFOS than infants who were never breastfed. Maternal serum concentrations decreased with each month of breastfeeding {Mondal, 2014, 2850916; Mogensen, 2015, 3859839}. Cariou et al. (2015, 3859840) reported that PFOS levels in breastmilk were approximately 66-fold lower relative to maternal serum and the ratio between breastmilk and maternal serum PFOS was 0.38 ± 0.16. The authors noted that the transfer rates of PFAS from serum to breastmilk were lower compared to other lipophilic persistent organic pollutants such as polychlorinated biphenyls.

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

3.3.1.2.5 Volume of Distribution in Humans and Animals

In humans, a single volume of distribution (V_d) value of 239 mL/kg has been uniformly applied for most PFOS studies {Thompson, 2010, 2919278}. Gomis et al. (2017, 3981280) used a V_d of 235 mL/kg by averaging V_d values estimated for both humans and animals. V_d values may be influenced by differences in distribution between males and females, between pregnant and non-pregnant females, and across serum, plasma, and whole blood.

V_d estimates derived in monkeys, mice, and rats vary by species, age, sex, and dosing regimen. For example, Huang et al. (2019, 5387170) calculated the apparent volume of central and peripheral distribution in rats. In this study, a two-compartment model was the best fit for male rats for both IV and gavage routes of administration and females dosed by the IV route, whereas a one-compartment model was the best fit for female rats dosed by oral gavage. V_d values in females after IV administration were lower than that observed in males in both the central and peripheral compartments. For the oral route, striking sex differences were noted between the central and peripheral compartments. While V_d values were quite similar in males for both compartments, they were notably higher in the central compartment compared to the peripheral compartment in females. Interestingly, another study found that for PFOS, a classical compartment model was not applicable {Iwabuchi, 2017, 3859701}. Rather, the body organs behaved as an assortment of independent one-compartments with a longer elimination half-life in liver than serum in the elimination phase. Further discussion on the V_d for PFOS can be found in Section 6.6.2.

3.3.1.3 Metabolism

Consistent with other reports and reviews {U.S. EPA, 2016, 3603365; ATSDR, 2018, 9642134; Pizzuro, 2019, 5387175}, the available evidence demonstrates that PFOS is not metabolized in humans, primates, or rodents.

3.3.1.4 Excretion

Excretion data are available for oral exposure in humans and laboratory animals. Most studies have investigated the elimination of PFOS in humans, cynomolgus monkeys, and rats. Available evidence supports urine as the primary route of excretion in most species, though fecal elimination is prominent in rats. In rats, hair is another route of elimination in both males and

females. In females, elimination pathways include menstruation, pregnancy (cord blood, placenta, amniotic fluid, and fetal tissues) and lactation (breast milk) (see PFOS Appendix).

3.3.1.4.1 Urinary and Fecal Excretion

Urinary excretion is considered the main route of PFOS excretion in humans. Zhang et al. (2015, 2851103) estimated a daily urinary excretion rate of 16% of the estimated total daily intake for PFOS for adults. Zhang et al. (2013, 3859849) calculated median renal clearance rates of 0.044 mL/kg/day in young women and 0.024 mL/kg/day in men and older women for total PFOS. In a later study, Fu et al. (2016, 3859819) estimated a urinary clearance rate 0.010 mL/kg/day (geometric mean for men and women). These studies showed that PFOS daily renal clearance values were significantly lower in males compared to females.

Several studies in rats suggest that the fecal route is as or more important than the urinary route of excretion for PFOS. In a study by Chang et al. (2012, 1289832), excretion in urine and feces were approximately equivalent when examined 24 and 48 hours after oral gavage administration of ^{14}C-PFOS. A study by Kim et al. (2016, 3749289) measured the amounts of unchanged PFOS excreted into the urine and the feces of male and female Sprague-Dawley rats for 70 days after a single dose of 2 mg/kg by oral or IV administration {Kim, 2016, 3749289}. PFOS levels in urine and feces were similar in both males and females, which correlated to similar half-life estimates for PFOS (26.44 and 28.70 days in males and 23.50 and 24.80 days in females by the oral and IV routes, respectively).

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

3.3.1.4.2 Renal and Enterohepatic Resorption

Early evidence of enterohepatic resorption of PFOS was revealed by Johnson et al. (1984, 5085553), who demonstrated that cholestyramine (CSM) treatment increased mean cumulative ^{14}C elimination in feces by 9.5-fold for male CD rats administered 3.4 mg/kg ^{14}C-PFOS. CSM is a bile acid sequestrant, and its facilitation of PFOS gastrointestinal clearance suggests enterohepatic circulation.

Several studies present evidence of enterohepatic excretion and potential resorption in humans {Genuis, 2010, 2583643; Harada, 2007, 2919450}. Harada et al. (2007, 2919450) estimated a biliary resorption rate of 0.97, which could contribute to the long half-life in humans. Genuis et al. (2010, 2583643) described a case report of excretion analyzed after inhalation PFOS exposure. After treatment with a bile acid sequestrant CSM for 1 week, PFOS serum levels decreased from 23 ng/g to 14.4 ng/g. Additionally, stool PFOS concentrations increased from undetectable before treatment (LOD = 0.5 ng/g) to 9.06 and 7.94 ng/g in the weeks after

treatment, suggesting that it may help with removing PFOS that gains access to the GI tract via bile.

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

3.3.1.4.3 Maternal Elimination Through Lactation and Fetal Partitioning

PFOS can readily pass from mothers to their fetuses during gestation and through breast milk during lactation. In conjunction with elimination through menstruation discussed in Section 3.3.1.4.4, females may eliminate PFOS through routes not available to males. The total daily elimination of PFOS in pregnant females was estimated to be 30.1 ng/day, higher than the 11.4 ng/day for PFOA {Zhang, 2014, 2850251}. The ratio of branched:total PFOS isomers in cord blood was 0.27 and was higher in cord blood compared to maternal blood and placenta. These findings suggest branched PFOS isomers may transfer to the fetus more readily than linear forms. In another study in humans {Zhang, 2013, 3859792}, the mean levels in the cord blood, placenta, and amniotic fluid were 21%, 56%, and 0.1%, respectively, of levels found in the mother's blood, demonstrating that cord blood, placenta, and amniotic fluid are additional routes of elimination in pregnant females. Blood loss during childbirth could be another source of excretion. Underscoring the importance of pregnancy as a life-stage when excretion is altered, Zhang et al., (2015, 2857764) observed that the partitioning ratio of PFOS concentrations between urine and whole blood in pregnant women (0.0004) was lower than the ratio found in non-pregnant women (0.0013) and may be affected by the increase in blood volume during pregnancy {Pritchard, 1965, 9641812}.

Mamsen and colleagues (2017, 3858487) measured placental samples and fetal organs in relation to maternal plasma levels of five PFAS in 39 Danish women {Mamsen, 2017, 3858487}. Fetal organ levels of PFOS were lower than in maternal blood. The average concentration of PFOS was 0.6 ng/g in fetal organs compared to 1.3 ng/g in the placenta and 8.2 ng/g in maternal plasma. Increasing fetal PFOS levels with fetal age suggest that the rate of elimination of PFOS from mother to fetus may increase through the gestational period.

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

PFOS was 0.38 in this study, much lower than the ratio of 0.78 for PFOA. However, the ratio between breast milk and maternal serum was 0.038, essentially the same as PFOA. Thus, PFOS exhibits a low transfer from maternal blood to cord blood and a 10-fold lower transfer from maternal blood to breast milk.

3.3.1.4.4 Other Routes of Elimination

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

Two studies supported an association between increased serum concentrations of PFOA and PFOS and early menopause {Knox, 2011, 1402395; Taylor, 2014, 2850915}. However, a re-analysis of these data {Ruark, 2017, 3981395} suggested that this association could be explained by reverse causality and more specifically, that pharmacokinetic bias could account for the observed association with epidemiological data. Also challenging the assumption that this is due to menstruation, Singer et al. (2018, 5079732) failed to find evidence of associations between menstrual cycle length and PFAS concentrations. Furthermore, Lorber et al. (2015, 2851157) suggested that factors other than blood loss, such as exposure to or disposition of PFOA/PFOS, may also help explain the differences in elimination rates between males and females. Curiously, studies providing direct measurements of PFOS in menstrual blood were not identified. However, for PFOS to be selectively retained from the blood lost through menstruation would require a specific mechanism for that process and no such mechanism has been demonstrated or proposed.

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

3.3.1.4.5 Half-Life Data

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

Human PFOS half-life estimates range from less than 1 year in a single male child of 16 years {Genuis, 2014, 2851045} to up to 60.9 years for males occupationally exposed in a facility in China {Fu, 2016, 3859819} (see PFOS Appendix). With one exception {Genuis, 2014, 2851045}, half-lives estimated for males are longer than those estimated for females and show an age-related increase {Zhang, 2013, 3859849}. Also, linear isomers exhibit longer half-lives

than branched isomers {Zhang, 2013, 3859849; Xu, 2020, 6781357}. While most studies were conducted in adults and/or adolescents, at least one study estimated a PFOS half-life of 4.1 years in newborns {Spliethoff, 2008, 2919368}.

Half-life estimates in humans rely on measured serum and/or urine concentrations. However, relatively few studies calculated PFOS half-lives along with measured intake and serum and urine PFOS concentrations {Xu, 2020, 6781357; Worley, 2017, 3859800; Fu, 2016, 3859819; Zhang, 2013, 2639569} (see PFOS Appendix). PFOS half-life values among these 4 studies varied dramatically from 1.04 years in Xu et al. (2020, 6781357) to 60.9 years in Fu et al. (2016, 3859819). These comparisons support principles suggested by the broader literature. First, sex related differences with males exhibiting much longer half-lives compared to females which may, at least in part, relate to menstruation as an important route of elimination in females (especially females of reproductive age) may relate, at least in part, to menstruation as an important route of elimination. Second, Xu et al. (2020, 6781357) suggest that linear PFOS molecules exhibit longer half-lives than branched forms, which may reflect differential affinities of linear vs. branched forms for resorption transporters. Third, the relationships between blood and urine concentrations are not obvious, underscoring the role of non-urinary routes of excretion and the difficulty in measuring renal resorption. Finally, only two studies estimated PFOS intake in subjects {Xu, 2020, 6781357; Worley, 2017, 3859800}. Altogether, there is insufficient data to correlate PFOS intake measurements to serum/plasma and urine concentrations. These factors, as well as age and health status of subjects, likely contribute to the variability in PFOS half-life estimates in humans.

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

3.3.2 Pharmacokinetic Models

Pharmacokinetic (PK) models are tools for quantifying the relationship between external measures of exposure and internal measures of dose. For this assessment, PK models were evaluated for their ability to allow for 1) cross-species PK extrapolation of animal studies of both cancer and noncancer effects and 2) 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 published PK modeling efforts and then present conclusions from analyses that assessed the utility of the models to predict internal doses for use in dose-response assessment.

Numerous PK models for PFOS have been developed and published over the years to characterize the unique ADME described in Section 3.3.1. 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 available 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 from the published literature. 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 available data on the physiologically relevant quantifications of blood flow and tissue perfusion. Determining additional, non-physiological parameters typically requires explicitly fitting the PBPK model to time-course concentration data. However, the number of parameters estimated through data fitting is generally fewer than for classical PK or modified compartmental models. A review of the available PK models regarding their ability to predict PFOS ADME is provided below.

3.3.2.1 Classical Compartmental Analysis

The most common approach for the prediction of serum levels of PFOS is to apply a relatively simple one-compartment model. This type of model describes the toxicokinetics of the substance with a single differential equation that describes the rate of change in the amount or concentration of the substance over time as a function of the exposure rate and the clearance rate. This type of model describes the relationship between exposure, serum concentration, and clearance and can be used to predict one of these values when the other two values are set. Additionally, because the model can produce predictions of changes in exposure and serum concentration over time, these models can be applied to fill the temporal gaps around or between measured serum concentrations or exposures.

Some examples of one-compartment models used to predict human 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 in developing children (measured at several time points) to assess the relative proportion of exposure that originates from dietary exposure. Zhang et al. (2019, 5080526) performed a similar study using community tap water measurements and serum concentrations to estimate the proportion of PFOS exposure that originates from drinking water.

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

One common modification of the one-compartment model is to perform a “steady-state approximation” (i.e., to assume that the rate of change of the serum concentration is zero). This scenario occurs when an individual experiences constant exposure, constant body habitus, and constant clearance over a timespan of several half-lives. Due to the long half-life of PFOS, steady state is a reasonable assumption for adults starting from the age of 25 and above.

However, the steady state approximation cannot be applied for ages younger than 21 years of age (EPA defines childhood as < 21 years of age; {U.S. EPA, 2021, 9641727}) due to ongoing development during childhood and adolescence. This growth dilutes the concentration of the chemical in the body and results in lower levels than would be seen in its absence. Even though pubertal development including skeletal growth typically ends several years prior to the age of 25, there is a period after growth ceases during which PFOS levels increase until the adult steady-state level is reached. The general acceptability of the steady-state assumption in adults has the caveat that pregnancy or breastfeeding will result in changes in serum concentration and will not be accounted for in the steady-state approximation.

When adopting a steady-state assumption, the rate of change in serum levels over time is zero. It follows that the ratio between exposure to the substance and clearance determines the serum concentration. This is the approach used in the 2016 PFOS HESD to determine the constant exposure associated with a serum concentration {U.S. EPA, 2016, 3603365}. A similar approach was used in the recent risk assessment performed by CalEPA {CalEPA, 2021, 9416932}. Publications reporting applications of similar models include the work of Zhang et al. (2015, 2851103) who used paired urine and serum data to estimate the total intake of PFOS and compared it to the rate of urinary elimination, and Lorber et al. (2015, 2851157) who examined the effects of regular blood loss due to phlebotomy on PFOS levels and extrapolated that finding to clearance via menstruation.

In animals, two classical PK models for PFOS have been published since the 2016 HESD. In Huang et al. (2019, 5387170), male and female Sprague-Dawley rats were dosed via oral gavage at 2 or 20 mg/kg, through multiple administrations of PFOS at 2 mg/kg/day for five days, or intravenously at 2 mg/kg. Following the administration of PFOS, rats were sacrificed from 5 minutes up to 140 days post-dosing to characterize the biphasic PK curve. Using plasma data from these exposure scenarios, Huang and coworkers developed a two-compartment model to characterize PK parameters of interest such as the alpha- and beta-phase half-life, central and peripheral compartment volumes, and total PFOS clearance. For each dosing scenario, a single set of PK parameters were fit, making extrapolation to other dosing scenarios difficult. However, the authors demonstrate no significant difference between males and females in beta-phase half-life and overall clearance which is in agreement with previous studies of PFOS PK in rats {Kim, 2016, 3749289}.

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

3.3.2.2 Modified Compartmental Models

In addition to the common one-compartment models described above, several models for humans 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 (e.g., 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 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 of change in infants. One such developmental model with a lactational component was used to predict the maternal serum concentrations and exposure from measurements of PFOS concentrations in breast milk {Abdallah, 2020, 6316215}. Verner et al. (2016, 3299692) presented another developmental model to predict PFOS serum concentrations in the mother and child and predict previous exposure using mother/child paired serum measurements at different times. This model included all the key aspects previously mentioned for developmental PK models. Another unique approach that extended the one-compartment framework was a publication by Shan et al. (2016, 3360127), who estimated the exposure to specific isomers of PFOS using measurements in food, tap water, and dust to estimate the isomeric profiles of the substances in human serum.

Pharmacokinetic models that can accommodate longer half-life values than would be predicted based on standard ADME concepts have been published as tools to estimate internal doses for humans, monkeys, mice, and rats {Andersen, 2006, 818501; Wambaugh, 2013, 2850932; Loccisano, 2011, 787186; Loccisano, 2012, 1289830; Loccisano, 2012, 1289833; Loccisano, 2013, 1326665; Chou, 2019, 5412429}. The underlying assumption for all the models is saturable resorption from the kidney filtrate, which consistently returns a portion of the excreted dose to the systemic circulation and prolongs both clearance from the body (e.g., extends half-life) and the time needed to reach steady state.

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

The renal-resorption model includes a central compartment that receives the chemical from the oral dose and a filtrate compartment for the glomerular filtrate from which resorption and transfer to the central compartment can occur. Transfer from the filtrate compartment to the central compartment decreases the rate of excretion. The resorption in the model was saturable, meaning that there was proportionally less resorption and greater excretion at high serum PFOS concentrations than at low concentrations. In addition to decreased renal excretion due to the renal resorption, excretion is also reduced in the model by implementing a constant proportion of PFOS that is bound to protein in plasma and is not available for renal filtration.

The model was parameterized using the body weight and urine output for cynomolgus monkeys {Butenhoff, 2004, 3749227} and a cardiac output of 15 L/h-kg from the literature {Corley, 1990, 10123}. A 20% blood flow rate to the kidney was assumed based on data from humans and dogs. Other parameters were assumed or optimized to fit the PK data for monkeys. In the IV time course data, some time and/or dose-dependent changes occurred in distribution of PFOS between the blood and tissue compartments, and these changes were less noticeable in the females; therefore, only the female data were used. The simulation captured the overall time course scenario but did not provide good correspondence with the initial rapid loss from plasma and the

apparent rise in plasma concentrations over the first 20 days. For oral dosing, the 0.15 mg/kg dose simulation was uniformly lower, and the 0.75 mg/kg dose simulation was higher than the data. When compared to PFOA, PFOS had a longer terminal half-life and more rapid approach to steady-state with repeated oral administration.

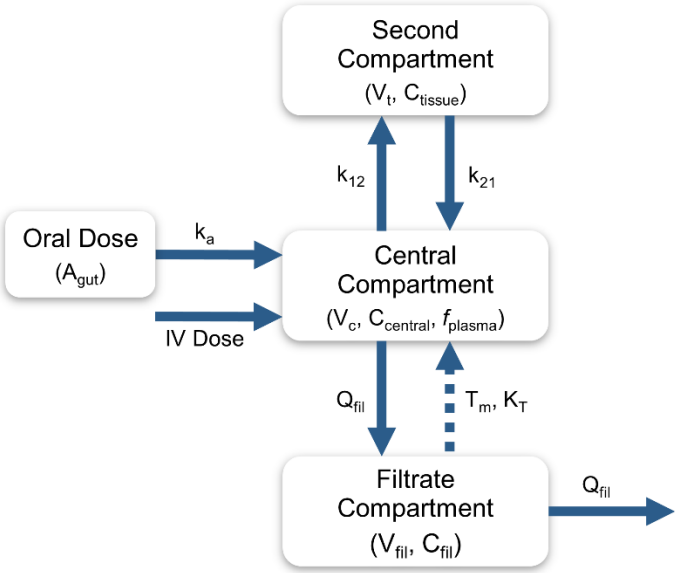


Figure 3-4. Schematic for a Physiologically Motivated Renal Resorption PK Model

Adapted from Wambaugh et al. (2013, 2850932).

Building on the work of other researchers, Wambaugh et al. (2013, 2850932) developed and published a PK model to support the development of an EPA RfD for PFOS {U.S. EPA, 2016, 3603365}. The model was applied to data from studies conducted in monkeys, rats, or mice that demonstrated an assortment of systemic, developmental, reproductive, and immunological effects. A saturable renal resorption term was used. This concept has played a fundamental role in the design of all of the published PFOS models summarized in this section. The model structure is depicted in Figure 3-4 (adapted from Wambaugh et al. (2013, 2850392)).

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 vs. 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 also assumed. Under this assumption, a single numeric value represents all individuals of the same species, sex, and strain. Body weight, the number of doses, and magnitude of the doses were the only parameters varied for different studies. Measurement errors were assumed to be log-normally distributed. Table 4-3. 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 Wambaugh et al. (2013, 2850932) analysis were derived from two *in vivo* PFOS PK studies. The monkey PK data were derived from Seacat et al. (2002, 757853) and Chang et al. (2012, 1289832). Data for the rats (male/females) and mice were both from Chang et al. (2012, 1289832). The data were analyzed within a Bayesian framework using a Markov Chain Monte Carlo sampler implemented as an R package developed by EPA to allow predictions across species, strains, and genders and identify serum levels associated with the NOAEL and LOAEL external doses. Prior distributions for the parameters were chosen to be vague, uniformed distributions, allowing them to be significantly informed by the data. The values were assumed to be log-normally distributed constraining each parameter to a positive value.

3.3.2.3 PBPK Models

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

The first PBPK model developed for this chemical was reported in a series of publications by Loccisano et al., which together describe the PK of PFOS in rats, monkeys, and humans, in both adult and developmental (for rat and human) scenarios {Loccisano, 2011, 787186; Loccisano, 2012, 1289830; Loccisano, 2012, 1289833; Loccisano, 2013, 1326665}. These models were developed based on an earlier "biologically motivated" model that served as a bridge between a one-compartment model and PBPK by implementing a tissue compartment (similar to a two-compartment model), an absorption compartment, and a renal filtrate compartment with saturable renal resorption {Tan, 2008, 2919374}. The 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 female development during puberty and menstrual clearance of PFOS to investigate the interaction between chemical levels and the timing of menarche, Ruark et al. (2017, 3981395) who added a detailed description of menopause to evaluate how that affects serum levels and the epidemiological association between early menopause and PFOS levels, Ngueta et al. (2017, 3860773) who implemented a reduction in menstrual clearance in individuals using oral contraceptives and the interaction between oral contraceptive use, endometriosis, and serum

PFOS levels, and Dzierlenga et al. (2020, 6315786; 2020, 6833691) who applied a model of thyroid disease {Dzierlenga, 2019, 7947729} to describe changes in PFOS renal clearance due to disease state.

In addition to this set of studies, Fabrega et al. (2014, 2850904) updated the model of Loccisano et al. (2013, 1326665) for humans by modeling a human population using regional food and drinking water measurements and human tissue data collected from cadavers in a region of Spain. The use of human tissue data is relatively rare due to the challenges in sourcing human tissue but may prove preferable to the assumption that human distribution is similar to distribution in an animal model. However, Fabrega et al. (2014, 2850904) estimated their tissue to blood partition coefficients from the ratio of tissue concentrations in the cadavers to the average serum concentrations in live volunteers who lived in the same region but were sampled several years earlier {Ericson, 2007, 3858652} and they provided 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, 5381552) presented the application of a PBPK model for PFOS with gestation and lactation phases to describe development and predicted maternal, infant, and breastmilk concentrations over a variety of scenarios including the prediction of maternal levels across multiple pregnancies.

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

Chou and Lin (2019, 5412429) developed a PFOS PBPK model for rat, mouse, monkey, and human. Using the model structure of Worley and Fisher (2015, 3223252), parameters were determined using a hierarchical Bayesian framework to pool datasets across studies for each species. This model reflects saturable resorption in the proximal tubule cells of the kidney and fecal elimination through the bile. While the Bayesian approach is ideal for handling multiple datasets, the method for implementing the Bayesian inference raises questions about the final posterior parameter distributions. Priors for the hierarchical model were determined using a least-squares fitting method on the most sensitive parameters as opposed to defining priors using information from previous studies and letting the data update those priors to determine the joint posterior distribution of the parameter space. In a subsequent study, Chou and Lin (2021, 7542658) added a gestation/lactation element to the model and parameterized the gestation/lactation components for rats and humans. This model structure used a three-compartment fetal model during gestation and a physiologically motivated PK model, similar to Wambaugh et al. (2013, 2850932) with renal resorption, for the infant. Using this model, the authors developed HEDs using interspecies extrapolation of the average serum concentration POD derived from the rat model. While the fits demonstrated good agreement with the evaluation dataset, parameters for only the rat are available for developmental endpoints.

3.4 Non-Cancer Health Effects Evidence Synthesis and Integration

3.4.1 Hepatic

EPA identified 23 epidemiological studies (30 publications)[7,8] and 25 animal toxicological studies that investigated the association between PFOS and hepatic effects. Of the epidemiological publications, 16 were classified as *medium* confidence, 6 as *low* confidence, and 7 were considered *uninformative* (Section 3.4.1.1). Of the animal toxicological studies, 3 were classified as *high* confidence, 17 as *medium* confidence, and 5 were considered *low* confidence (Section 3.4.1.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.1.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.1.1.1 Introduction and Summary of Evidence from the 2016 PFOS HESD

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

There are 6 epidemiological studies (7 publications)[8] from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and hepatic effects. Study quality evaluations for these 7 studies are shown in Figure 3-5.

[7] Multiple publications of the same data: Jain and Ducatman (2019, 5381566); Jain and Ducatman (2019, 5080621); Jain (2019, 5381541); Jain (2020, 6833623); Omoike et al. (2020, 6988477); Liu et al. (2018, 4238514); Gleason et al. (2015, 2966740) all use NHANES data from overlapping years.
[8] Olsen (2003, 1290020) is the peer-review paper of Olsen (2001, 10228462).

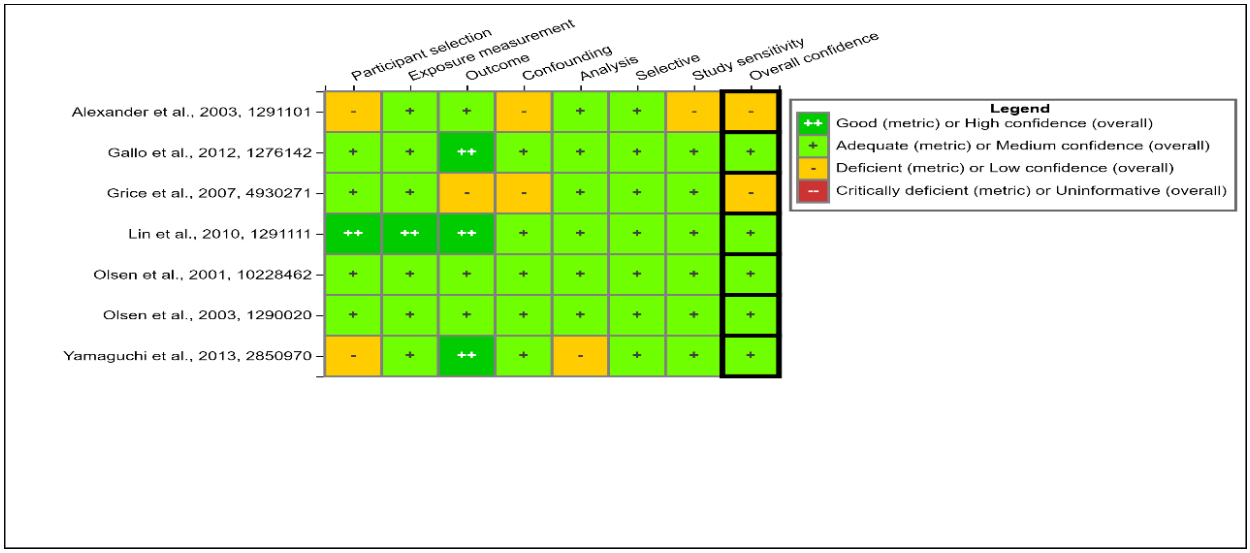


Figure 3-5. Summary of Study Evaluation for Pre-2016 Epidemiology Studies of PFOS and Hepatic Effects

Interactive figure and additional study details available on HAWC.

The 2016 PFOS HESD {U.S. EPA, 2016, 3603365} describes both cross-sectional and longitudinal studies that evaluated PFOS and liver enzymes in adults. Two available cross-sectional studies {Lin, 2010, 1291111; Gallo, 2012, 1276142} reported positive associations between PFOS exposure and ALT in adults of the general population (see PFOS Appendix). Lin et al. (2010, 1291111) examined 2,216 adults in NHANES (1999–2000, and 2003–2004) and observed that higher serum concentrations of PFOS were associated with abnormal liver enzymes increases in the U.S. general population. With each increase in log-PFOS, serum ALT and GGT concentrations (U/L) increased by 1.01 units (SE = 0.53) and 0.01 units (SE 0.03), respectively {Lin, 2010, 1291111}. When PFOA, PFHxS, and PFNA were simultaneously added in the fully adjusted regression models, one unit increase in serum log-PFOS concentration was associated with a decrease of 0.19 units (SE = 0.63, p-value = 0.769) in serum ALT concentration (U/L) and a 0.06 unit (SE = 0.03, p-value = 0.025) decrease in serum log-GGT concentration (U/L). The four PFAS were moderately correlated with one another, with PFOA and PFOS most strongly correlated (Spearman correlation coefficient of 0.68), and PFHxS and PFNA the least correlated (Spearman correlation coefficient of 0.24). Another *medium* confidence cross-sectional study (Yamaguchi, 2013, 2850970) conducted in Japan reported a positive correlation with ALT in addition to factors influencing PFOS exposure.

Gallo et al. (2012, 1276142) reported an analysis of data from the C8 Health Project, reflective of a highly exposed community. One of the largest studies of PFOS and ALT in adults, Gallo et al. (2012, 1276142) evaluated 47,092 adults from the C8 Study Project living in communities in Ohio and West Virginia impacted from a manufacturing-related PFOA-contaminated drinking water supply. Natural log transformed serum PFOS concentrations were associated with ln-ALT in linear regression models (regression coefficient: 0.020; 95% CI: 0.014, 0.026) and with elevated ALT in logistic regression models across deciles of PFOS (OR = 1.13; 95% CI: 1.07,

1.18). There was less consistent evidence of an association between PFOS and GGT or bilirubin in this study.

Both studies observed a slight positive association between serum PFOS levels and increased serum ALT values. The association between PFOS and increased serum GGT was less defined. Total or direct bilirubin showed no association with PFOS in either study. In the Gallo et al. (2012, 1276142) study, the cross-sectional design and self-reported lifestyle characteristics are limitations of the study, and while both Lin et al. (2010, 1291111) and Gallo et al. (2012, 1276142) showed a trend, it was not large in magnitude.

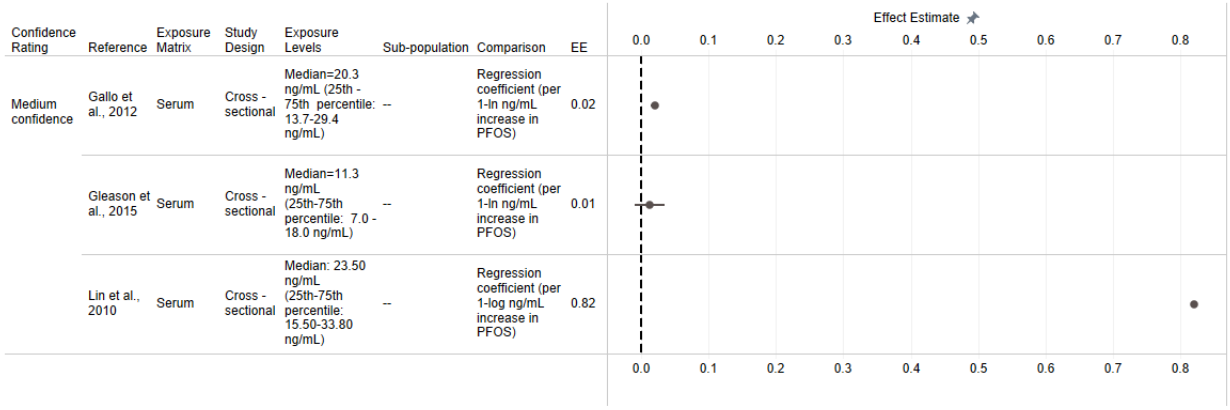


Figure 3-6. Overall ALT Levels from Pre-2016 HESD Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

Several cross-sectional occupational studies in PFOS production workers reported mostly null findings with respect to biomarkers of liver disease {Olsen, 2003, 1290020; Olsen, 2001, 10228462}.

Null or inconsistent associations were reported with GGT and bilirubin. There was no evidence of association with functional hepatic endpoints in these identified studies. No increases in deaths from cirrhosis of the liver were found in workers at the 3M facility in Decatur, Alabama {Alexander, 2003, 1291101}. At the same plant, nonsignificant increases in noncancerous liver disease (including cirrhosis) were observed with cumulative exposure to PFOS {Grice, 2007, 4930271}.

3.4.1.1.2 Study Quality Evaluation Results for the Updated Literature Review

There are 17 studies (23 publications)[9] from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and hepatic effects. Study quality evaluations for these 17 studies (23 publications) are shown in Figure 3-7.

[9] Multiple publications of the same data: Jain and Ducatman (2019, 5381566); Jain and Ducatman (2019, 5080621); Jain (2019, 5381541); Jain (2020, 6833623); Omoike et al. (2020, 6988477); Liu et al. (2018, 4238514); Gleason et al. (2015, 2966740) all use NHANES data from overlapping years.

Of these, 12 were classified as *medium* confidence, four as *low* confidence, and seven were considered *uninformative.* Of the informative studies, three cross-sectional {Nian, 2019, 5080307; van den Dungen, 2017, 5080340} and multiple publications of data from NHANES {Jain, 2019, 5381541; Liu, 2018, 4238514; Omoike, 2020, 6988477; Jain, 2019, 5080621; Jain, 2019, 5381566}, one prospective cohort in elderly adults {Salihovic, 2018, 5083555}, and one occupational cohort of fluorochemical plant workers {Olsen, 2012, 2919185} examined liver enzymes in adults. In addition, two of the cross-sectional studies {Rantakokko, 2015, 3351439, Liu, 2018, 4238396} examined functional liver endpoints in adults. In children and adolescents, four studies were available including one cohort {Mora, 2018, 4239224} and three cross-sectional studies {Khalil, 2018, 4238547; Jin, 2020, 6315720; Attanasio, 2019, 5412069}, with one examining function liver endpoints {Jin, 2020, 6315720}. All of the studies measured PFOS exposure using biomarkers in blood. The *uninformative* studies were excluded due to potential confounding {Abraham, 2020, 6506041; Jiang, 2014, 2850910; Predieri, 3889874; Sinisalu, 2020, 7211554}, lack of information on participant selection {Sinisalu, 2021, 9959547}, or use of PFAS as the dependent variable (in a publication with a more suitable analysis available {Jain, 2020, 6833623} or where the independent variable is a genetic variant and thus not affected by PFAS exposure {Fan, 2014, 2967086}).

Figure 3-7. Summary of Study Evaluation for Epidemiology Studies of PFOS and Hepatic Effects[a]

Interactive figure and additional study details available on HAWC.

[a] Multiple publications of the same data: Jain and Ducatman (2019, 5381566); Jain and Ducatman (2019, 5080621); Jain (2019, 5381541); Jain (2020, 6833623); Omoike et al. (2020, 6988477); Liu (2018, 4238514); Gleason et al. (2015, 2966740) all use NHANES data from overlapping years.

3.4.1.1.3 Synthesis of Hepatic Injury from the Updated Literature Review

Results for the eight studies that examined ALT are presented in the Appendix (see PFOS Appendix). Of the available informative studies that measured ALT in adults, statistically significant positive associations between ALT and PFOS (i.e., increases in ALT as a continuous measure with higher PFOS exposure levels) were observed in two of five studies {Salihovic, 2018, 5083555; Nian, 2019, 5080307} and multiple NHANES publications, including all the *medium* confidence studies. However, the positive associations in Jain et al. (2019, 5381541) were observed only in obese participants (Figure 3-8). In non-obese participants, associations were generally null, with an inverse association in non-obese participants with glomerular filtration (GF) stage of 3B/4. Among *low* confidence studies in adults, an inverse association was reported ($p < 0.05$) in Olsen et al. (2012, 2919185). However, this analysis differed from the other studies in that the exposure measure used was change in PFOS levels during the study period. In van den Dungen et al. (2017, 5080340), no association was observed.

In children and adolescents, positive associations were observed in girls in the fourth quartile in Attanasio (2019, 5412069) and in the *low* confidence study in obese children {Khalil, 2018, 4238547}. However, inverse associations were observed in Mora et al. (2018, 4239224), which may indicate that the associations in children are less consistent than in adults or that there are sex differences in children. Insufficient data were available to assess the potential for effect modification by sex.

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

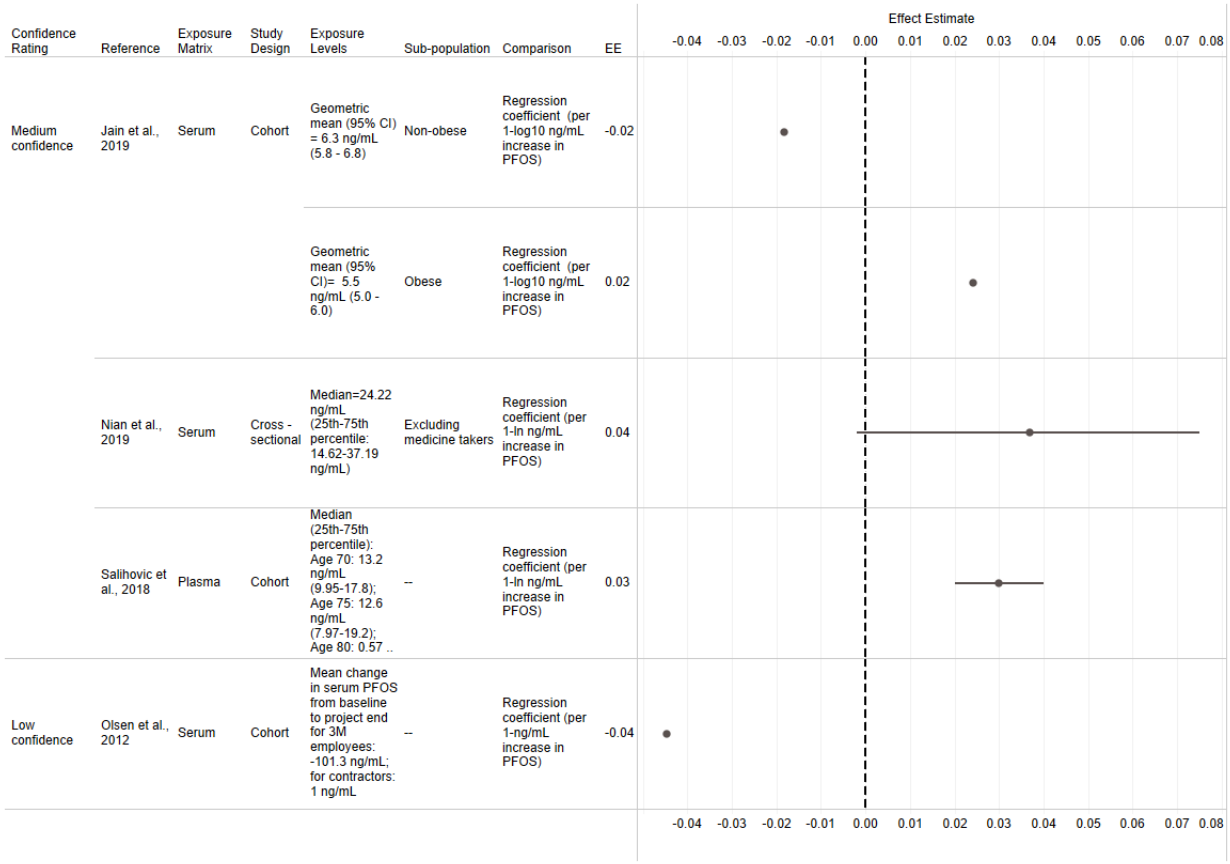


Figure 3-8. Overall ALT Levels from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

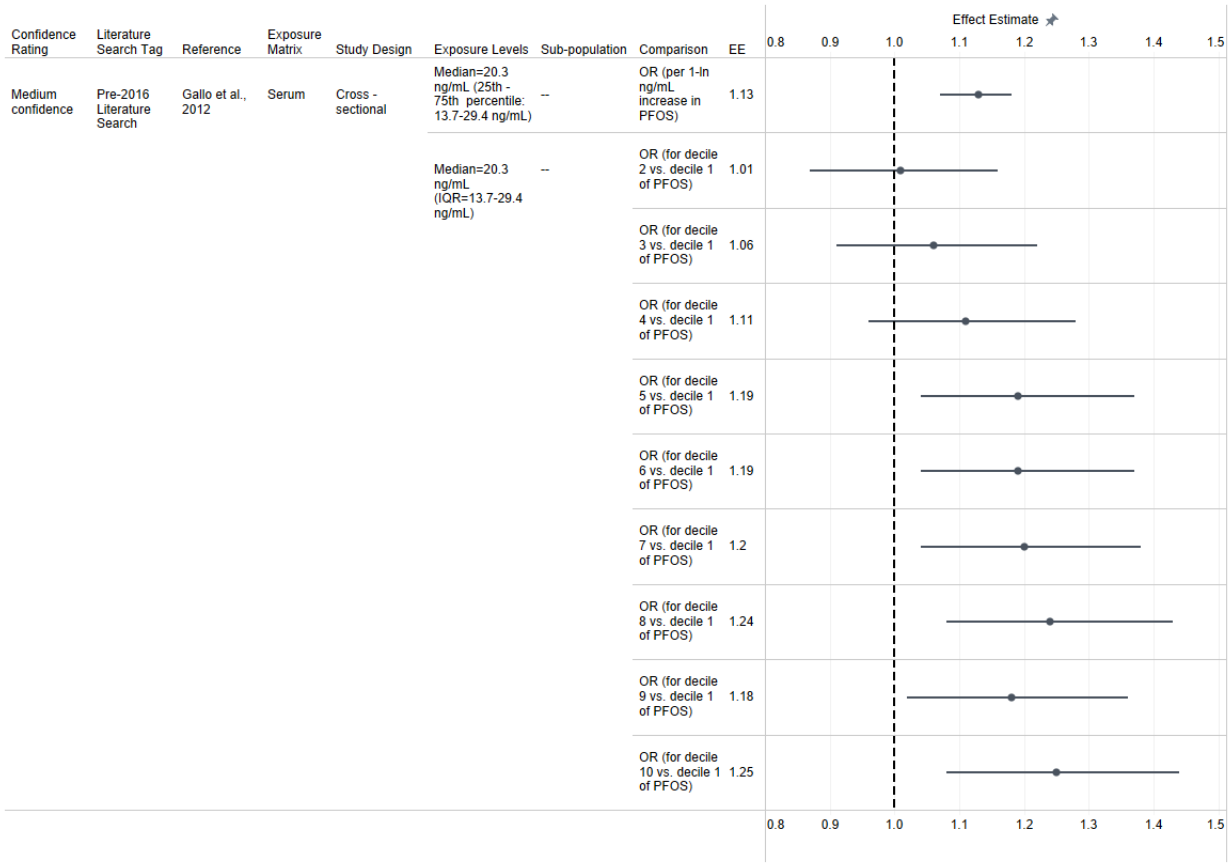


Figure 3-9. Odds of Elevated ALT Levels from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

For functional measures of liver injury, two *medium* confidence studies (one in adults and one in children and adolescents) examined histology endpoints. Both studies examined lobular inflammation. Rantakokko et al. (2015, 3351439) reported higher PFOS exposure levels were associated with reduced odds of lobular inflammation, whereas Jin et al. (2020, 6315720) reported the opposite, with OR of 2.9 for 2–4 foci *vs.* none, though the results in the latter study were non-monotonic and both were not statistically significant. Jin et al. (2020, 6315720) additionally reported higher odds (not statistically significant) of nonalcoholic steatosis ($p < 0.05$), ballooning, fibrosis, and portal inflammation. Lastly, Liu et al. (2018, 4238396) examined hepatic fat mass and found no correlation with PFOS exposure.

In summary, across studies in the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and the updated systematic review, there is generally consistent evidence of a positive association between exposure to PFOS and ALT. However, one source of uncertainty in epidemiology studies of PFAS is confounding across the PFAS, as individuals are exposed to a mixture of PFAS and it is difficult to disentangle the effects. This cannot be ruled out in this body of evidence given the attenuation of the association in Lin et al. (2010, 1291111), the only general

population study that performed multi-pollutant modeling. In addition, associations for other hepatic outcomes were less consistent, including for functional outcomes such as liver disease. Thus, while there is evidence of an association between PFOS and ALT, there is residual uncertainty.

3.4.1.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 6 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and 19 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and hepatic effects. Study quality evaluations for these 25 studies are shown in Figure 3-10 and Figure 3-11.

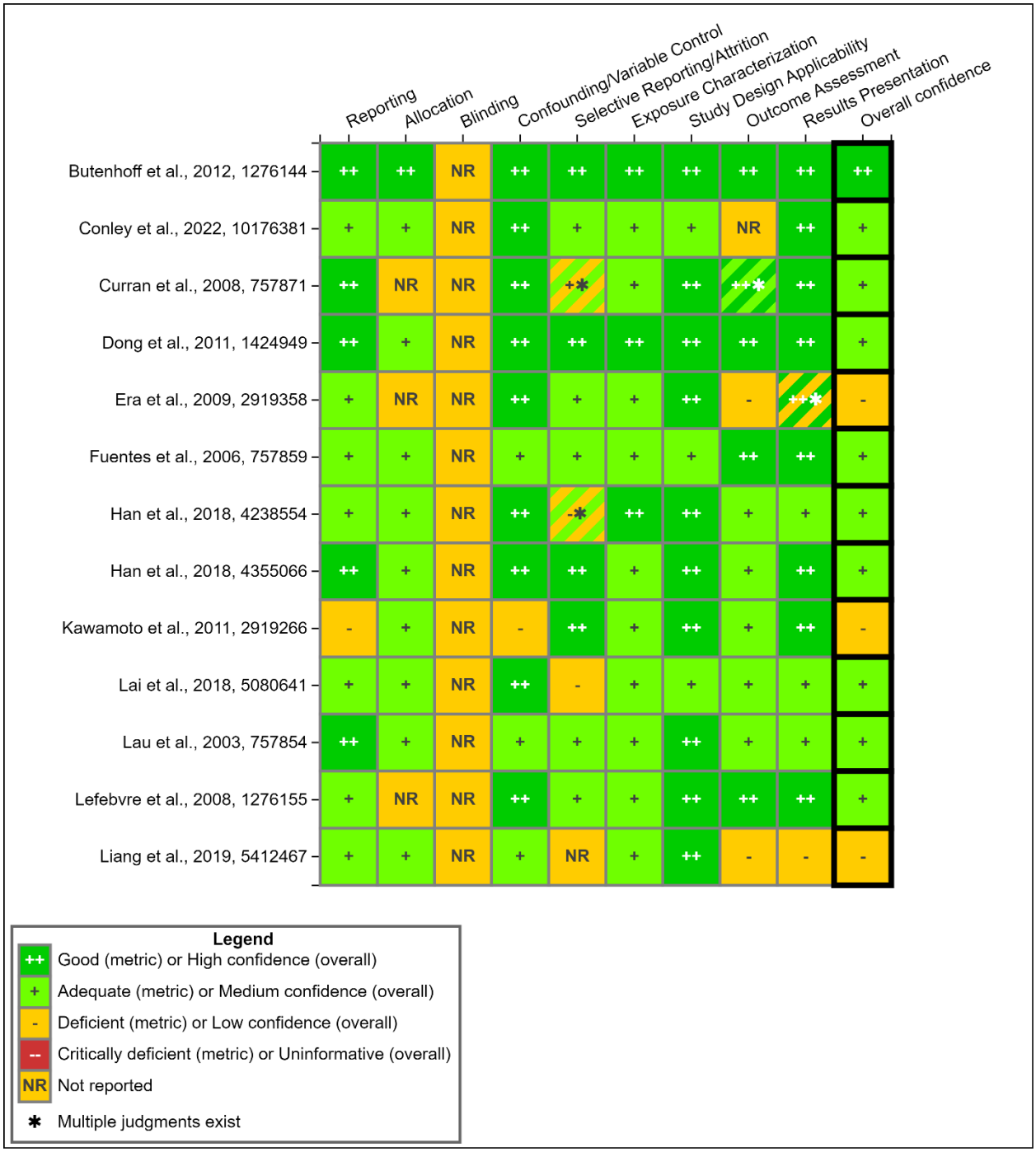


Figure 3-10. Summary of Study Evaluation for Animal Toxicological Studies of PFOS and Hepatic Effects[a,b]

Interactive figure and additional study details available on HAWC.
[a] Han et al. (2018, 4238554) and Wan et al. (2016, 3981504) reported on the same hepatic data as Han et al. (2018, 4355066).
[b] Lefebvre et al. (2008, 1276155) reported on the same hepatic data as Curran et al. (2008, 757871).

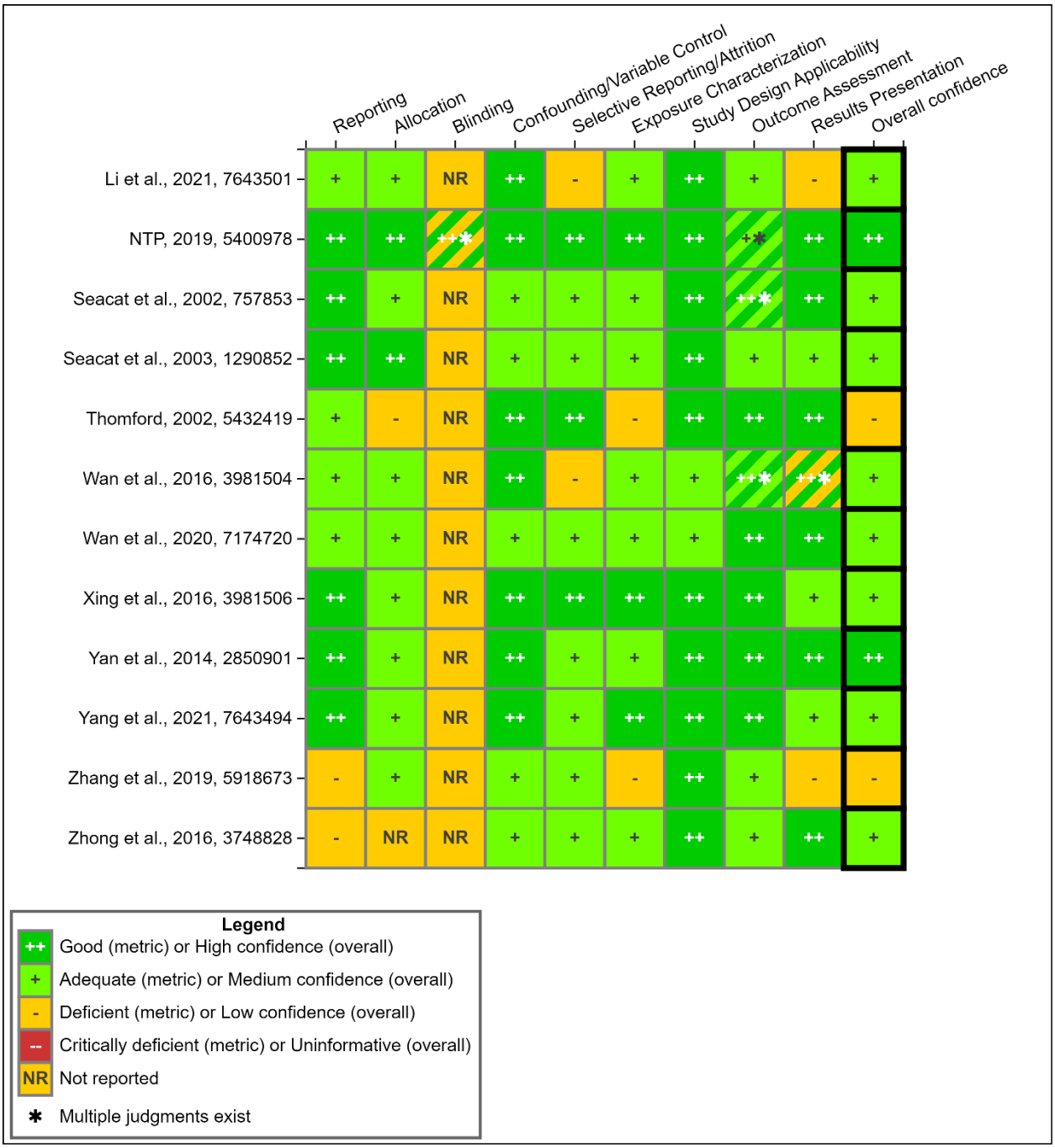


Figure 3-11. Summary of Study Evaluation for Animal Toxicological Studies of PFOS and Hepatic Effects (Continued) [a,b]

Interactive figure and additional study details available on HAWC.
[a] Han et al. (2018, 4238554) and Wan et al. (2016, 3981504) reported on the same hepatic data as Han et al. (2018, 4355066).
[b] Lefebvre et al. (2008, 1276155) reported on the same hepatic data as Curran et al. (2008, 757871).

Hepatic effects were observed in male and female mice, rats, and monkeys after varying oral exposure durations and PFOS doses. This includes effects such as increased absolute and relative

liver weight, altered clinical parameters indicating potential liver injury, and histopathological alterations of liver tissue. Data from numerous studies provide evidence confirming the liver as a target of PFOS toxicity.

3.4.1.2.1 Liver Weight

Significant increases in liver weight relative to body weight and absolute liver weight were observed in several strains of male and female mice exposed to 1.25–10 mg/kg/day PFOS for short-term, subchronic, and gestational durations {Lai, 2018, 5080641; Xing, 2016, 3981506; Yan, 2014, 2850901; Lau, 2003, 757854; Zhong, 2016, 3748828; Yang, 2021, 7643494; Dong, 2011 1424949}. In male BALB/c mice, significant increases in both relative and absolute liver weights were observed after 28-day exposure to PFOS doses of 1.25 and 5 mg/kg/day {Yan, 2014, 2850901}. Similarly, two short-term studies in male C57BL/6 mice reported significantly increased relative liver weights following exposures to 2.5 {Yang et al., 2021, 7643494} or 2.5–10 mg/kg/day PFOS {Xing, 2016, 3981506}. In a 60-day study in male C57BL/6 mice, Dong et al. (2011, 1424949) observed a dose-related increase in relative liver weights; at doses above 0.417 mg/kg/day PFOS, the increases were statistically significant compared to control. In a 7-week gavage study in female CD-1 mice, Lai et al. (2018, 5080641) reported significant increases in absolute and relative liver weights at 3 mg/kg/day PFOS but not 0.3 mg/kg/day.

Two developmental studies in CD-1 mice observed higher liver weights in the dams following gestational PFOS exposure {Fuentes, 2006, 757859; Wan, 2020, 7174720}. Fuentes et al. (2006, 757859) observed significantly increased absolute liver weights in dams exposed to 3 or 6 mg/kg/day PFOS and significantly increased relative liver weights in dams exposed to 6 mg/kg/day PFOS. The dams were exposed from GD 6–18 to 0, 1.5, 3, or 6 mg/kg/day PFOS. Similarly, Wan et al. (2020, 7174720) reported significantly increased relative liver weights in dams exposed to 3 mg/kg/day PFOS without changes in maternal body weight (absolute liver weight not reported). Dams were exposed to 0, 1, or 3 mg/kg/day PFOS from GD 4.5–17.5. There was a 10% increase in relative liver weight in the fetuses, but the increase was not statistically significant and may have been related to reduced fetal weight in this group.

Two additional developmental toxicity studies in mice indicate that relative liver weights of pups exposed to PFOS during gestation may increase and then subsequently return to control levels after prolonged cessation of exposure during postnatal development {Zhong, 2016, 3748828; Lau, 2003, 757854}. Zhong et al. (2016, 3748828) dosed C57BL/6J mouse dams with 0, 0.1, 1, or 5 mg/kg/day PFOS from GD 1–17. Relative liver weights of male and female pups in the 5 mg/kg/day group were significantly increased at postnatal week 4 (PNW 4), but returned to levels statistically indistinguishable from controls by PNW 8. Similarly, Lau et al. (2003, 757854) exposed pregnant CD-1 mice to 0, 1, 5, or 10 mg/kg/day PFOS from GD 1–17 and found significant increases in offspring liver weights in the 5 and 10 mg/kg/day dose groups at PNDs 0 and 7 but not PND 35.

Significant increases in relative and absolute liver weights were also observed in male and female rats exposed to 0.15–20 mg/kg/day PFOS for short-term, chronic, and gestational durations {NTP, 2019, 5400978; Curran, 2008, 757871; Seacat, 2003, 1290852; Lau, 2003, 757854; Cui, 2009, 757868; Wan, 2012, 1332470; Wan, 2016, 3981504; Han, 2018, 4355066}(Lefebvre et al. (2008, 1276155) reported the same results as Curran et al. (2008, 757871)). An increase in relative liver weight was observed with exposure as low as

0.15 mg/kg/day PFOS administered to female Sprague Dawley rats for 28 days {Curran, 2008, 757871; Lefebvre, 2008, 1276155}. In males from the same study, relative liver weight was significantly increased at 1.33 mg/kg/day. A similar study in Sprague Dawley rats found that relative and absolute liver weights were increased in both males and females dosed with ≥ 0.312 mg/kg/day PFOS for 28 days {NTP, 2019, 5400978}. In a 14-week feeding study, Seacat et al. (2003, 1290852) also observed similar responses in male and female Sprague Dawley rats, with significant increases in relative liver weight at the highest dose tested in each sex (1.33 and 1.56 mg/kg/day, respectively) and increased absolute liver weight (in males only) at 1.33 mg/kg/day.

In a developmental toxicity study, Lau et al. (2003, 757854) observed inconsistent alterations in liver weight across time points in Sprague-Dawley rat offspring exposed to 0, 1, 2, or 3 mg/kg/day PFOS from GD 2–GD 21. Significant increases in relative liver weight were observed in the 2 and 3 mg/kg/day dose groups at PND 5, but not PND 0 or PND 35. No significant changes in relative or absolute liver weights were observed in Sprague-Dawley rat dams following 5-day exposure (GD 14–18) to PFOS (0, 0.1, 0.3,1, 3, 10, or 30 mg/kg/day) {Conley, 2022, 10176381}.

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

3.4.1.2.2 Clinical Chemistry Measures

Increases in serum enzymes including ALT, alkaline phosphatase (ALP), AST, and GGT following PFOS exposure were observed across multiple species, sexes, and exposure paradigms (Figure 3-12 (mice), Figure 3-13 (male rats), Figure 3-14 (female rats)). These enzymes are often useful indicators of hepatic enzyme induction, hepatocellular damage, or hepatobiliary damage, as increased serum levels are thought to be due to hepatocyte damage resulting in release into the blood {U.S. EPA, 2002, 625713}. Alterations in serum enzyme levels are generally considered to reach biological significance and indicate potential adversity at levels ≥ 2-fold compared to controls (i.e., ≥ 100% change relative to control response) {U.S. EPA, 2002, 625713; Hall, 2012, 2718645}.

Two studies in male mice showed statistically and biologically significant increases in serum enzymes indicative of hepatic or hepatobiliary damage after oral PFOS exposure (Figure 3-12) {Yan, 2014, 2850901; Xing, 2016, 3981506}. Xing et al. (2016, 3981506) observed a dose-dependent increase in ALT in male C57BL/6J mice after 30 days of PFOS exposure; ALT levels were increased by 50% and 88% above control in the 5 and 10 mg/kg/day groups, respectively. In comparison, in a study of 28-day exposure to 0, 1.25, or 5 mg/kg/day PFOS in male BALB/c mice, Yan et al. (2014, 2850901) observed much larger increases in ALT in the 5 mg/kg/day group (> 700% change), though there was no apparent linear dose-response relationship observed across the two tested dose levels. Both Yan et al. (2014, 2850901) and Xing et al. (2016, 3981506) observed statistically but not biologically significant increases in AST with increasing PFOS dose (responses did not exceed 50% change from control at any dose level). Xing et al. (2016, 3981506) observed a similar statistically but not biologically significant increase in ALP level (53% change in the 10 mg/kg/day group). Yan et al. (2014, 2850901) also reported a large increase in ALP (321% change relative to control) in the 5 mg/kg/day dose

group. Interestingly, a statistically and biologically significant dose-dependent increase in GGT was observed by Xing et al. (2016, 3981506), with an increase of approximately 140% in the lowest dose group (2.5 mg/kg/day) and 535% in the highest dose group (10 mg/kg/day), indicating potential damage to the biliary system {U.S. EPA, 2002, 625713}.

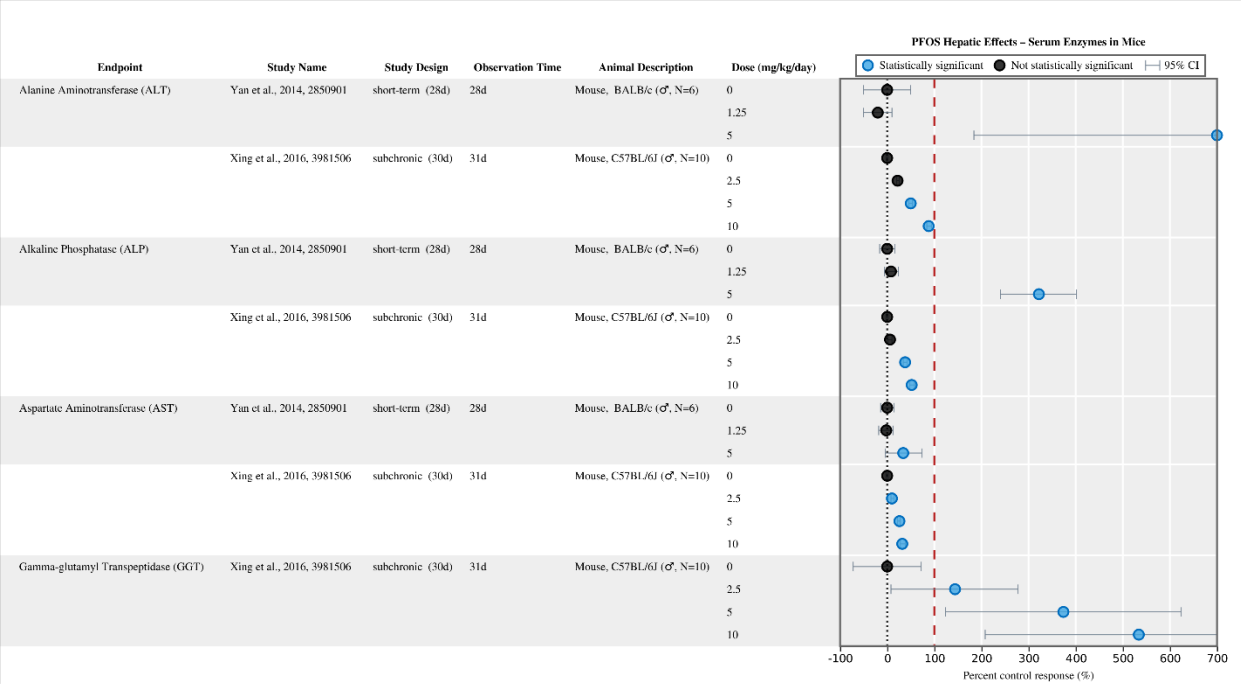


Figure 3-12. Percent Change in Serum Enzyme Levels Relative to Controls in Mice Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC and Tableau.
ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyl transpeptidase; d = day; CI = confidence interval.
[a] Results for Yan et al. (2014, 2850901) are presented for 3 dose levels (0, 1.25, and 5 mg/kg/day), and a statistically significant response of 756% occurred at the highest dose for the ALT endpoint alanine aminotransferase. The x axis has been truncated at 700% to allow results at lower doses for other studies and endpoints to be legible.
[b] The red dashed line indicates a 100% increase from the control response.

Multiple studies assessed serum liver enzymes in male and female Sprague Dawley rats exposed to PFOS for short-term and chronic exposure durations, or in dams following a developmental exposure paradigm (Figure 3-13, Figure 3-14) {Han, 2018, 4238554; Han, 2018, 4355066; Wan, 2016, 3981504; NTP, 2019, 5400978; Seacat, 2003, 1290852; Butenhoff, 2012, 1276144; Curran, 2008, 757871; Conley, 2022, 10176381}.

The National Toxicology Program (NTP) (2019, 5400978), Han et al. (2018, 4355066), and Curran et al. (2008, 757871) studies reported statistically significant increases in ALT levels in male rats exposed to PFOS for 28 days. However, these increases did not exceed 75% change at even the highest doses tested in each study (5, 10, and 6.34 mg/kg/day, respectively). Seacat et al. (2003, 1290852) similarly observed statistically but not biologically significant increases in ALT in male rats from the highest dose group (1.33 mg/kg/day) in a 14-week dietary PFOS study. Butenhoff et al. (2012, 1276144) did not observe consistent dose-related changes in ALT

levels in male rats exposed to PFOS via the diet for 4, 14, 27, or 53 weeks, though this study tested relatively low doses (approximately 0.02 to 1 mg/kg/day).

As with ALT levels, AST levels in male Sprague Dawley rats exposed to PFOS for varying durations were increased, but the increases did not exceed two-fold compared to controls. Han et al. (2018, 4355066) reported a statistically significant increase in AST in male rats dosed with 10 mg/kg/day PFOS for 28 days, but the increase was less than a 20% change from the control. Three other 28-day studies assessing AST levels in male rats either reported changes in AST that were not dose-dependent {NTP, 2019, 5400978} or not statistically significant between treated and control groups {Seacat, 2003, 1290852; Curran, 2008, 757871}. Butenhoff et al. (2012, 1276144) also did not observe statistically significant changes in AST levels in male rats exposed to PFOS via the diet for 4, 14, 27, or 53 weeks at doses up to 0.984 mg/kg/day.

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

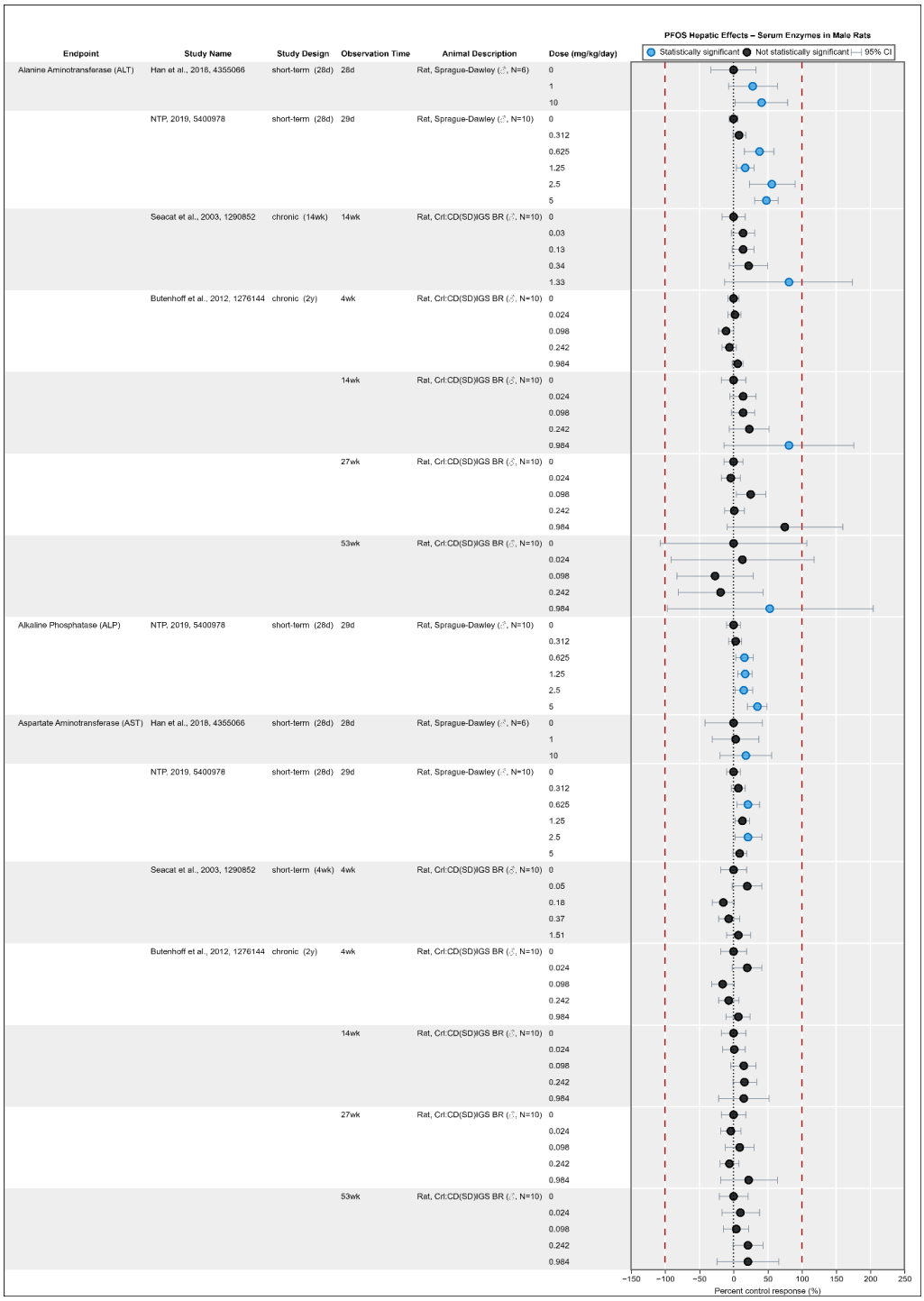


Figure 3-13. Percent Change in Serum Enzyme Levels Relative to Controls in Male Rats Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC and Tableau.
ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; d = day; w/wk = week; y = year; CI = confidence interval.
[a] Two publications Han et al. (2018, 4238554) and Wan et al. (2016, 3981504) reported on the same data as Han et al. (2018, 4355066) and are not shown in the figure.
[b] The red dashed lines indicate a 100% increase and decrease from the control response.

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

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

In a developmental exposure paradigm, Conley et al. (2022, 10176381) exposed Sprague-Dawley dams to PFOS (0, 0.1, 0.3,1, 3, 10, or 30 mg/kg/day) from GD 14–18, and no significant effects were observed on levels of ALT or AST in serum.

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

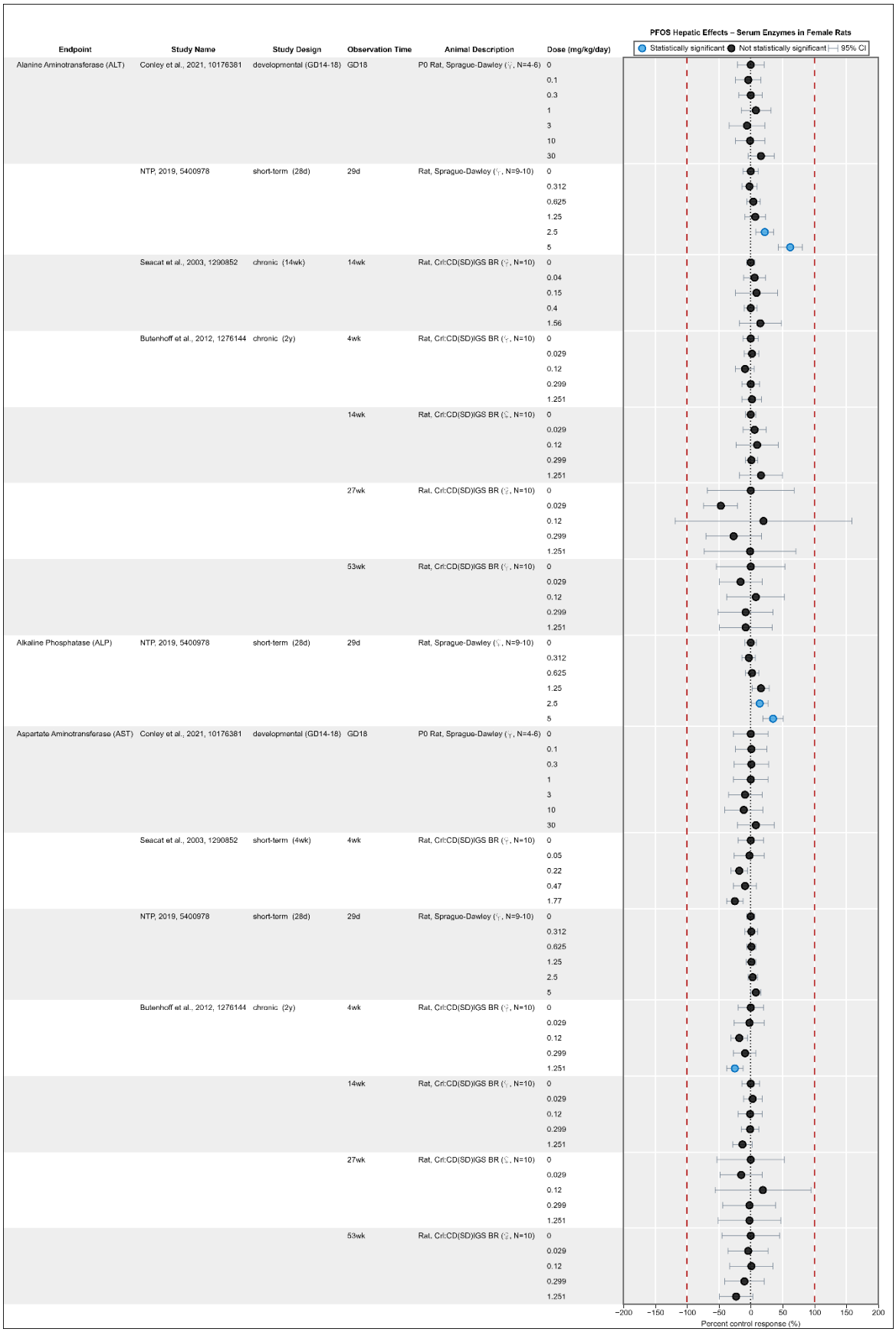

PFOS Hepatic Effects – Serum Enzymes in Female Rats
Statistically significant
Not statistically significant
95% CI
Endpoint
Study Name
Study Design
Observation Time
Animal Description
Dose (mg/kg/day)
Alanine Aminotransferase (ALT)
Conley et al., 2021, 10176381
developmental (GD14-18)
GD18
P0 Rat, Sprague-Dawley (♀, N=4-6)
0
0.1
0.3
1
3
10
30
NTP, 2019, 5400978
short-term (28d)
29d
Rat, Sprague-Dawley (♀, N=9-10)
0
0.312
0.625
1.25
2.5
5
Seacat et al., 2003, 1290852
chronic (14wk)
14wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.04
0.15
0.4
1.56
Butenhoff et al., 2012, 1276144
chronic (2y)
4wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.029
0.12
0.299
1.251
14wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.029
0.12
0.299
1.251
27wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.029
0.12
0.299
1.251
53wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.029
0.12
0.299
1.251
Alkaline Phosphatase (ALP)
NTP, 2019, 5400978
short-term (28d)
29d
Rat, Sprague-Dawley (♀, N=9-10)
0
0.312
0.625
1.25
2.5
5
Aspartate Aminotransferase (AST)
Conley et al., 2021, 10176381
developmental (GD14-18)
GD18
P0 Rat, Sprague-Dawley (♀, N=4-6)
0
0.1
0.3
1
3
10
30
Seacat et al., 2003, 1290852
short-term (4wk)
4wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.05
0.22
0.47
1.77
NTP, 2019, 5400978
short-term (28d)
29d
Rat, Sprague-Dawley (♀, N=9-10)
0
0.312
0.625
1.25
2.5
5
Butenhoff et al., 2012, 1276144
chronic (2y)
4wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.029
0.12
0.299
1.251
14wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.029
0.12
0.299
1.251
27wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.029
0.12
0.299
1.251
53wk
Rat, Crl:CD(SD)IGS BR (♀, N=10)
0
0.029
0.12
0.299
1.251
−200
−150
−100
−50
0
50
100
150
200
Percent control response (%)

Figure 3-14. Percent Change in Serum Enzyme Levels Relative to Controls in Female Rats Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC and Tableau.
ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; d = day; w/wk = week; y = year; CI = confidence interval.
[a] Two publications Han et al. (2018, 4238554) and Wan et al. (2016, 3981504) reported on the same data as Han et al. (2018, 4355066) and are not shown in the figure.
[b] The red dashed lines indicate a 100% increase or 100% decrease from the control response.

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

Levels of bilirubin, albumin, and bile salt/acids were also observed to be altered in several studies in mice, rats, and monkeys. However, these clinical chemistry measurements were generally altered at higher concentrations of PFOS than were serum enzymes, and changes were inconsistent across studies. Bilirubin (direct, indirect, or total) was either unchanged or increased in male rats exposed to ≥ 5 mg/kg/day PFOS and in female rats exposed to ≥ 2.5 mg/kg/day PFOS {NTP, 2019, 5400978; Curran, 2008, 757871; Seacat, 2003, 1290852}. Total bilirubin was decreased in male monkeys exposed to 0.75 mg/kg/day for 91–182 days, but there was no statistically significant response in female monkeys {Seacat, 2002, 757853}. Six studies examined albumin levels, but only two studies found significant alterations due to PFOS treatment {Yan, 2014, 2850901; NTP, 2019, 5400978; Seacat, 2003, 1290852; Butenhoff, 2012, 1276144; Curran, 2008, 757871; Conley, 2022, 10176381}. In male mice dosed with 1.25 or 5 mg/kg/day of PFOS for 28 days, albumin was significantly increased above control levels at both doses {Yan, 2014, 2850901}. In rats dosed with PFOS for 28 days, albumin was significantly increased in females dosed with 1.25–5 mg/kg/day and in males dosed with 5 mg/kg/day {NTP, 2019, 5400978}. Bile salt/acids were significantly increased in male rats exposed to 5 mg/kg/day PFOS and in female rats exposed to 2.5 and 5 mg/kg/day PFOS {NTP, 2019, 5400978}. In monkeys, serum bile acids were significantly increased in males, but not in females, dosed with 0.75 mg/kg/day PFOS {Seacat, 2002, 757853}.

3.4.1.2.3 Histopathology

Liver lesions were confirmed microscopically in male mice and male and female rats in several short-term and subchronic studies {Wan, 2012, 1332470; Xing, 2016, 3981506; Curran, 2008, 757871; Cui, 2009, 757868; Han, 2018, 4238554; Han, 2018, 4355066; Wan, 2016, 3981504; NTP, 2019, 5400978; Li, 2021, 7643501} and in two chronic studies of male and female rats and monkeys {Seacat, 2002, 757853; Butenhoff, 2012, 1276144}. Only three of these studies provided quantitative incidence data {NTP, 2019, 5400978; Butenhoff, 2012, 1276144; Curran, 2008, 757871).

Hepatocellular hypertrophy was shown to be significantly increased in male Sprague Dawley rats dosed with 2.5 and 5 mg/kg/day PFOS and in females dosed with 5 mg/kg/day PFOS for 28 days {NTP, 2019, 5400978} (Table 3-2). Cytoplasmic vacuolation and alterations were significantly increased in a dose-dependent manner in male and female rats, respectively, in the 2.5 (females only) and 5 mg/kg/day (males and females) exposure groups {NTP, 2019, 5400978}. Another 28-day study in Sprague Dawley rats observed higher incidence of hepatocellular hypertrophy in zone 3 of the liver in males exposed to 3.21 and 6.24 mg/kg/day PFOS, the two highest

concentrations; no incidence was seen in females {Curran, 2008, 757871} (Table 3-3). A higher incidence of cytoplasmic homogeneity in zone 3 of the liver was also observed in both males and females exposed to 3.21 and 6.24 mg/kg/day PFOS {Curran, 2008, 757871}. In the chronic study in Sprague Dawley rats {Butenhoff, 2012, 1276144; Thomford, 2002, 5029075}, hepatocellular hypertrophy was significantly increased in males exposed to 0.098–0.984 mg/kg/day of PFOS and in females exposed to 0.299–1.251 mg/kg/day for 103 weeks; a dose-response relationship was observed (Table 3-4).

Table 3-2. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by NTP (2019, 5400978)

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

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

Table 3-3. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by Curran et al. (2008, 757871)

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

Table 3-4. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by Thomford (2002, 5029075)

	Males				
	0 mg/kg/day	**0.024 mg/kg/day**	**0.098 mg/kg/day**	**0.242 mg/kg/day**	**0.984 mg/kg/day**
Hypertrophy, Hepatocellular, Centrilobular	0/50	2/50	4/50	17/50	29/50
Vacuolation, Hepatocellular Midzonal/Centrilobular	2/50	3/50	6/50	10/50	10/50
Hyperplasia, Bile Duct	19/50	20/50	25/50	24/50	25/50
Necrosis, Individual Hepatocyte	3/50	2/50	6/50	4/50	10/50
Altered Hepatocellular, Clear/Eosinophilic Cell	13/50	21/50	23/50	24/50	24/50
Degeneration, Cystic	5/50	15/50	19/50	17/50	22/50
	Females				
	0 mg/kg/day	**0.029 mg/kg/day**	**0.120 mg/kg/day**	**0.299 mg/kg/day**	**1.251 mg/kg/day**
Hypertrophy, Hepatocellular, Centrilobular	2/50	1/50	4/50	15/50	39/50
Hyperplasia, Bile Duct	21/50	25/50	19/50	17/50	27/50
Necrosis, Individual Hepatocyte	3/50	4/50	4/50	5/50	9/50
Infiltrate, Lymphohistiocytic	33/50	37/50	33/50	36/50	42/50
Infiltrate, Macrophage, Pigmented	2/50	3/50	5/50	6/50	20/50
Degeneration, Cystic	0/50	1/50	1/50	2/50	4/50

Butenhoff et al. (2012, 1276144) and Thomford (2002, 5029075) also observed a dose-dependent increase in cystic degeneration in male rats exposed to 0.024–0.984 mg/kg/day of PFOS (Table 3-4); this effect was observed at lower incidences in female rats, but also appeared to follow a dose-dependent positive trend. Lymphohistiocytic and macrophage infiltrate were increased in a dose-dependent manner in females exposed to 1.251 mg/kg/day. A dose-response relationship was also observed with hepatocellular single cell necrosis, which was increased in males and females exposed to 0.984 and 1.251 mg/kg/day PFOS, respectively {Butenhoff, 2012, 1276144; Thomford, 2002, 5029075}.

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

a dose response {Xing, 2016, 3981506}. Cytoplasmic vacuolation was also observed in one study of female mice exposed to 0.1 mg/kg/day PFOS for 60 days {Li, 2021, 7643501}. Male rats were used in multiple studies and this effect was observed at a range of exposures. Three studies from the same lab observed hepatocellular hypertrophy in male Sprague Dawley rats dosed with 1 mg/kg/day of PFOS for 28 days {Han, 2018, 4238554; Han, 2018, 4355066; Wan, 2016, 3981504}; however, none of the studies provided incidence data. Hepatocellular hypertrophy and centrilobular vacuolation were also observed in another 28-day rat study that was conducted with higher concentrations of PFOS (5 and 20 mg/kg/day) {Cui, 2009, 757868}. Hepatocellular hypertrophy was also observed in male and female cynomolgus monkeys exposed to 0.75 mg/kg/day PFOS for 182 days (incidence data not provided) {Seacat, 2002, 757853}.

Hepatocytic or cystic degeneration, inflammatory cell infiltration, and/or necrosis, were observed in several short-term and subchronic studies (28–30 days) in male mice and rats {Xing, 2016, 3981506; Cui, 2009, 757868; Han, 2018, 4238554; Han, 2018, 4355066; Wan, 2016, 3981504}. Livers of male C57BL/6J mice and Sprague Dawley rats dosed with PFOS concentrations ranging from 2.5–20 mg/kg/day for approximately 4 weeks showed focal or flakelike necrosis, hepatocytic degeneration, and/or inflammatory cell infiltration {Xing, 2016, 3981506; Cui, 2009, 757868}. Three publications from the same lab described hepatocyte degeneration and inflammatory infiltration in male Sprague Dawley rats dosed with lower concentrations of 1 mg/kg/day PFOS for 28 days {Han., 2018, 4238554; Han, 2018, 4355066; Wan, 2016, 3981504}. Hepatocytic degeneration and inflammatory cell infiltration were noted in a single study of female mice, with hepatocyte degeneration being observed in mice exposed to 0.1 mg/kg/day for 60 days and focal infiltration of inflammatory cells being observed in mice exposed to 1 mg/kg/day {Li, 2021, 7643501}. However, no quantification or statistical analyses were performed on these studies.

3.4.1.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse hepatic outcomes is discussed in Sections 3.2.2, 3.2.3, 3.2.5, 3.3.4, 3.3.5, and 3.4.1.1 of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 56 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to hepatic effects. A summary of these studies is shown in Figure 3-15.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Atherogenesis And Clot Formation	0	0	1	1
Big Data, Non-Targeted Analysis	9	0	5	14
Cell Growth, Differentiation, Proliferation, Or Viability	13	1	25	35
Cell Signaling Or Signal Transduction	13	1	15	25
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	16	0	10	24
Hormone Function	3	1	0	4
Inflammation And Immune Response	5	1	2	7
Oxidative Stress	6	0	7	12
Renal Dysfunction	1	0	0	1
Xenobiotic Metabolism	3	1	6	10
Other	3	0	0	3
Grand Total	30	2	30	56

Figure 3-15. Summary of Mechanistic Studies of PFOS and Hepatic Effects

Interactive figure and additional study details available on Tableau.

3.4.1.3.1 Nuclear Receptor Activation

3.4.1.3.1.1 Introduction

The ability of PFOS to mediate hepatotoxicity via receptor activation has been investigated for several receptor-signaling pathways, including that of the peroxisome proliferator-activated receptor (PPAR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), liver X receptor (LXR), and retinoic acid receptor (RAR). Activation of PPARα has been cited as a mechanism of action for PFAS, including PFOS, because of the association between increased liver weight and peroxisome proliferation downstream of PPARα activation in rats. However, increased hepatic lipid content in the absence of a strong PPARα response (i.e., activation of downstream target genes) is a characteristic of exposure to PFOS, and many of the genes activated by PFOS are associated with nuclear receptors other than PPARα, namely CAR and LXR {U.S. EPA, 2016, 3603365}. PPAR, PXR, CAR, LXR, and RAR are nuclear receptors that can form heterodimers with one another to induce transcription of linked genes, and therefore, the effects of PFOS on one or multiple receptors may contribute to mechanisms underlying hepatotoxicity {U.S. EPA, 2016, 3603365}. Additionally, hepatic effects observed with PFAS exposure including inflammation and necrosis cannot be fully explained by PPARα activation (Section 3.4.1.2.3). This updated assessment includes studies that have examined activation of PPARs (including PPARα, β/δ, and γ), CAR, PXR, LXR, and/or retinoid X receptor (RXR) activation, as well as the downregulation of hepatocyte nuclear factor 4-alpha (HNF4α) as potential mechanisms underlying the hepatic health effects induced by PFOS.

3.4.1.3.1.2 Receptor Binding and Activation

Receptor binding and activation assays have been conducted *in vitro* with the goal of examining the potential association between activation of PPARs, CAR, PXR, and LXR and PFOS-mediated hepatotoxicity. PPARs modulate gene expression in response to exogenous or

endogenous ligands and play essential roles in lipid metabolism, energy homeostasis, development, and cell differentiation {U.S. EPA, 2016, 3603365}.

Several studies used luciferase reporter assays to examine the activation of PPARα by PFOS *in vitro* with human and animal cell lines transfected with human or mouse PPARα with varying results {Wolf, 2014, 2850908; Rosenmai, 2018, 4220319; Takacs, 2007, 783393; Wolf, 2008, 716635; Behr, 2020, 6305866}. In COS-1 cells transfected with mouse PPARα, PPARα was activated in a concentration-dependent manner, with an approximate half maximal effective concentration (EC50) of 65 μM in one study {Wolf, 2014, 2850908} and a lowest observed effect concentration (LOEC) of 90 μM for PPARα activation in another study {Wolf, 2008, 716635}. However, a third study in transfected COS-1 cells found that PFOS activated mouse PPARα, with a significant increase in activity only at a concentration of 120 μM, but not at lower concentrations of 1-90 μM or at higher concentrations of 150 or 250 μM {Takacs, 2007, 783393}. In cell lines transfected with human PPARα, one study showed that PPARα was activated in COS-1 cells in a dose-dependent manner, with a LOEC of 30 μM {Wolf, 2008, 716635}. A second study in HEK293T cells showed that human PPARα was only activated (i.e., upregulated by approximately 1.5-fold) at the highest concentration of 100 μM {Behr, 2020, 6305866}. However, two additional studies reported that PFOS did not significantly increase the activity of human PPARα up to concentrations of 100 μM in HepG2 cells {Rosenmai, 2018, 4220319} or 250 μM in COS-1 cells {Takacs, 2007, 783393}. In every study that compared the ability of PFOS to activate PPARα with that of PFOA, PFOS was a weaker PPARα activator {Wolf, 2014, 2850908; Rosenmai, 2018, 4220319; Takacs, 2007, 783393; Wolf, 2008, 716635; Behr, 2020, 6305866}.

In vitro luciferase reporter assays have also been used to examine the ability of PFOS to activate other PPAR receptors, namely PPARγ and PPARβ/δ {Bagley, 2017, 4238503; Takacs, 2007, 783393; Zhang, 2014, 5081455; Behr, 2020, 6305866}. One study showed that PFOS significantly activates human PPARγ by 1.5-fold at 10 μM and by 3-fold at 100 μM in a luciferase assay in HepG2 cells {Zhang, 2014, 5081455}. The authors also performed a cell-free binding assay to show that PFOS binds to human PPARγ with a half maximal inhibitory concentration (IC50) of 13.5 μM and dissociation constant of 93.7 μM. Mouse and rat PPARγ were also activated at 100 μM with a luciferase reporter assay conducted in Chinese hamster ovary (CHO) cells {Bagley, 2017, 4238503}. However, two other studies did not observe activation of PPARγ by PFOS {Behr, 2020, 6305866; Takacs, 2007, 783393}: PFOS did not activate human PPARγ or PPARδ in HEK29 cells at concentrations of up to 100 μM {Behr, 2020, 6305866}, and neither human nor mouse PPARγ were activated by concentrations of up to 250 μM PFOS in COS-1 cells {Takacs, 2007, 783393}. This study conducted in COS-1 cells also examined activation of human and mouse PPARβ/δ and observed activation of mouse PPARβ/δ only at concentrations of 20 and 30 μM, but not at a lower concentration of 10 μM or at higher concentrations of 40-80 μM. Human PPARβ/δ was not shown to be activated by PFOS in this study. Furthermore, this study demonstrated that the activities of mouse PPARα, γ, and β/δ were more responsive than their human counterparts to positive control agonists and antagonists, demonstrating species-specific differences in receptor-activation {Takacs, 2007, 783393}. Given the discrepancies in the ability and magnitude of PFOS to activate either mouse or human PPAR receptors, the role of PPAR activation in mediating hepatotoxicity of PFOS is not fully understood.

Two studies examined the activation of CAR/PXR and/or LXR/RXR *in vitro* with luciferase reporter assays using HEK293 cells or CHO cells {Bagley, 2017, 4238503; Behr, 2020, 6305866}. No activation of human CAR, human PXR, rat PXR, rat LXRβ, human LXRα, or human RXRα was observed with concentrations of up to 100 µM PFOS. However, a luciferase reporter assay in HepG2 cells showed that PFOS activates human PXR with an EC_{50} of 7.87 µM {Zhang, 2017, 3604013}. Notably, these studies did not examine endogenous receptor activation, though other lines of evidence are available that evaluate endogenous receptor signaling *in vivo* and *in vitro*.

3.4.1.3.1.3 Receptor Signaling

3.4.1.3.1.3.1 In Vivo Models

PFOS can activate PPARα in rodents and humans. However, the extent to which activation of PPARα mediates hepatoxicity may be species-specific, and activation of other receptors may also contribute to toxicity {U.S. EPA, 2016, 3603365}. Indeed, several studies in Sprague Dawley rats have found evidence that PFOS may activate both PPARα and CAR/PXR in the liver {Dong, 2016, 3981515; NTP, 2019, 5400978; Martin, 2007, 758419; Elcombe, 2012, 1401466; Chang, 2009, 757876; Elcombe, 2012, 1332473}. In an acute/short term study, male rats were exposed to 10 mg/kg/day PFOS for 1, 3, or 5 days, and gene expression changes were assessed in their livers with an expression microarray {Martin, 2007, 758419}. Although PFOS exposure induced PPARα-regulated genes and pathway analysis revealed that PFOS clustered with PPARα agonists (e.g., bezafibrate, clofibric acid, and fenofibrate), the correlation between the gene response to PFOS and that of known peroxisome proliferators was weak (with a correlation coefficient of 0.26 for PFOS, in comparison to 0.76 for PFOA). Changes in cytochrome P450 3A (*Cyp3a*) genes were also observed, consistent with the activation of CAR/PXR.

Another transcriptomics study of the liver of rats exposed to 50 mg PFOS/kg diet for 28 days had similar results using an expression microarray {Dong, 2016, 3981515}. Upstream regulator analysis using Ingenuity Pathway Analysis (IPA, Qiagen) revealed that PFOS likely activated both PPARα and CAR/PXR, with alterations in 48 genes that have evidence of being regulated by PPARα in the IPA reference database (approximately 10% of all known genes in this pathway), and 29 genes from the reference database for the CAR/PXR pathway (approximately 14% of all known genes in this pathway). Two other studies support these results, reporting that genes regulated by either PPARα or CAR/PXR are altered by PFOS, according to qPCR analysis {NTP, 2019, 5400978; Chang, 2009, 757876}. In a developmental rat study, dams were dosed with 1 mg/kg/day PFOS from GD 0-19, and the expression of both PPARα- and CAR/PXR-regulated genes was found to be increased in liver samples from the dams on GD 20 and male offspring on PND 21; female offspring were not tested {Chang, 2009, 757876}. A 28-day study in male and female rats found increases in the expression of both PPARα-regulated genes (*Cyp4a1*, *Acox1*) and CAR-regulated genes (*Cyp2b1*, *Cyp2b2*) at all exposure concentrations tested (0.312–10 mg/kg/day) {NTP, 2019, 5400978}. However, there were apparent sex differences in this study; PPARα-regulated genes were increased by 2- to 31-fold in males and by 1.3- to 3-fold in females, while CAR-regulated genes were increased by 6- to 400-fold in males and 32- to 1,227-fold in females. Although *Acox1* was the least responsive gene in males, with increased expression in males exposed to 5 and 10 mg/kg/day and in females exposed to

0.312–10 mg/kg/day, the corresponding enzyme activity (acyl-CoA oxidase) was increased in males exposed to 5 and 10 mg/kg/day, but not in females.

Two studies in male rats provided additional evidence of PFOS activation of PPARα, CAR, and PXR through the use of enzymatic biomarkers {Elcombe, 2012, 1332473; Elcombe, 2012, 1401466}. In one study, rats were fed diets containing either 20 or 100 ppm (approximately 2 and 10 mg/kg/day, respectively) PFOS for 7 days, and livers were collected on days 1, 28, 56, and 84 post-exposure {Elcombe, 2012, 1332473}. In the second study, rats were fed the same dietary PFOS concentrations for up to 28 days, with livers collected on days 1, 7, and 28 of the exposure {Elcombe, 2012, 1401466}. PPARα, CAR, and PXR activities [as measured by lauric acid 12-hydroxylation (CYP4A activity), pentoxyresorufin- O-dependentylation (PROD; CYP2B activity), and testosterone 6B-hydroxylation (CYP3A activity), respectively] were found to be increased in the liver microsomes of rats exposed to PFOS at most time points and in both exposure concentrations tested. Liver palmitoyl CoA oxidase (ACOX activity), another marker of PPARα activity, was not changed after 7 days of exposure to PFOS {Elcombe, 2012, 1332473}, but was shown to be significantly increased at both concentrations after 28 days of exposure {Elcombe, 2012, 1401466}. However, in another study in male rats exposed to 0.643–2.205 mg/kg/day PFOS for 28 days or 14 weeks, ACOX activity was unchanged {Seacat, 2003, 1290852}.

Studies in various strains of wild-type (WT) mice also examined PPARα activation as a mechanism of PFOS-induced liver toxicity {Huck, 2018, 5079648; Wang, 2014, 2851252; Wan, 2012, 1332470; Bijland, 2011, 1578502; Rosen, 2009, 2919338; Lai, 2017, 3981375}. Through genetic studies and pathway analysis, changes in PPARα signaling or expression of PPARα and/or downstream target genes were found to be associated with PFOS exposure in several studies {Wang, 2014, 2851252; Wan, 2012, 1332470; Bijland, 2011, 1578502; Rosen, 2009, 2919338; Lai, 2017, 3981375}. However, these studies also found evidence of upregulation of other receptors such as PPARγ, CAR/PXR, or LXR/RXR. In one study, the authors concluded that the main mechanism of action of PFOS for observed changes in liver endpoints (increased absolute liver weight and histopathological changes including cytoplasmic vacuolization and steatosis) may be mitochondrial β-oxidation, which leads to the accumulation of free fatty acids and subsequent activation of PPARα {Wan, 2012, 1332470}. In another study, the authors did not report any changes in the expression of PPARα or a subset of the downstream target genes examined by qPCR (Acox1, Pdk4, Cpt1) in mice exposed to PFOS with or without high fat diet-induced hepatic steatosis {Huck, 2018, 5079648}. The authors suggested that alterations in PPARγ may be a mechanism of PFOS-induced liver hepatotoxicity, based on the fact that PPARγ gene expression was induced by PFOS in mice fed a normal diet. However, it should be noted that PPARγ gene expression was also up-regulated in the livers of mice fed a high fat diet in the absence of PFOS, and PPARγ was unchanged in mice exposed to PFOS and fed a high fat diet.

Two additional studies comparing 129S1/SvlmJ WT mice to *Pparα*-null mice support PPARα activation as a mechanism of PFOS-toxicity, but also support the hypothesis that other mechanisms, including the activation of CAR/PXR, may play a role {Rosen, 2010, 1274165; Rosen, 2017, 3859803}. The first study found that PPARα-regulated genes were altered in WT mice dosed with 10 mg/kg/day PFOS for 7 days {Rosen, 2010, 1274165}. However, other genes and pathways were affected in both WT and *Pparα*-null mice, including changes related to lipid

metabolism, inflammation, xenobiotic metabolism, and CAR activation (as indicated by upregulation of *Cyp2b10*) {Rosen, 2010, 1274165}. In a connected study, the authors reanalyzed their data using different expression analysis software than the initial analysis {Rosen, 2017, 3859803}. They found that only approximately 15% of the PFOS-responsive gene changes in the liver were PPARα-independent, including CAR activation. In both WT and *Pparα*-null mice, there were significant similarities in gene expression changes induced by PFOS in comparison to the CAR biomarker gene set and the CAR agonist phenobarbital {Rosen, 2017, 3859803}. Two gene expression compendium studies further analyzed these data using gene expression biomarker signatures built using microarray profiles from livers of WT, *Car*-null mice {Oshida, 2015, 2850125}, and *Pparα*-null mice {Oshida, 2015, 5386121}. These analyses found that both CAR and PPAR were activated by PFOS, and that CAR activation was generally more significant in *Pparα*-null mice. The authors concluded that CAR likely plays a subordinate role to PPARα in mediating the adverse hepatic effects of PFOS {Oshida, 2015, 2850125}.

Comparisons of 129S1/SvlmJ WT and *Pparα*-null mice also suggest that increases in liver weights may not be solely due to activation of PPARα. In the Rosen et al. {2010, 1274165} study, absolute and relative liver weights were significantly increased in both WT and *Pparα*-null mice exposed to 10 mg/kg/day PFOS for 7 days. The absolute liver weights were increased by 63% in WT mice and by 42% in *Pparα*-null mice, while relative liver weights were increased by 44% in both strains. Similarly, in a study of male C57BL/6 (H-2^b) mice and *Pparα*-null 129/Sv mice exposed to 0.005% and 0.02% PFOS in diet for 10 days, absolute liver weight in WT mice was increased by 95% and 122% in the 0.005% and 0.02% groups, respectively {Qazi, 2009, 1937260}. In *Pparα*-null mice, absolute liver weights were increased by 49% and 95% in the 0.005% and 0.02% groups, respectively. In a study by Abbott et al. (2009, 2919376), WT mice were dosed with 4.5–10.5 mg/kg/day PFOS and *Pparα*-null mice were dosed with 8.5 or 10.5 mg/kg/day from GD 15-18. The authors reported that gestational exposure to 10.5 mg/kg/day resulted in increased relative liver weights in both WT (14%) and *Pparα*-null (29%) mouse pups. WT and *Pparα*-null mouse dams showed 11% and 14% increases, respectively, in relative liver weights, though these increases were not statistically significant.

A zebrafish study supports the involvement of CAR/PXR and LXR/RXR in PFOS-mediated hepatic steatosis {Cheng, 2016, 3981479}. Gene expression of liver X receptor alpha (*nr1h3*), retinoic acid receptor alpha (*rara*), retinoid X receptor gamma b *(rxrgb*), and pregnane X receptor (*nr1l2*) was elevated in WT male zebrafish livers after exposure to 0.5 μM PFOS for 5 months, which was accompanied by increased relative liver weight and lipid droplet accumulation. In female zebrafish, only a slight increase in *nr1l2* and mild lipid droplet accumulation was observed; there was no change in relative liver weight.

In comparison to the nuclear receptors mentioned above, the involvement of the nuclear receptor HNF4α, a regulator of hepatic differentiation and quiescence, has been less frequently studied in PFOS-induced liver toxicity. Only one *in vivo* study examined compared gene expression changes in male WT mice exposed to 10 mg/kg/day PFOS for 7 days with genes regulated by HNF4α {Beggs, 2016, 3981474}. This study reported that 90 out of 681 genes (13%) altered by PFOS exposure were regulated by HNF4α. PFOS exposure was shown to decrease the protein expression of HNF4α in male WT mice. Increased relative liver weight in WT mice was also

observed in this study, and the authors concluded that hepatomegaly, along with other liver effects such as steatosis and hepatocellular carcinoma (which were not observed in this short-term study) may be mediated by PFOS-induced dysregulation of HNF4α.

3.4.1.3.1.3.2 In Vitro Models

In vitro genetic studies corroborate the *in vivo* findings in rodents that suggest PPARα contributes to the mechanism of PFOS hepatotoxicity but is likely not the only contributor {Rosen, 2013, 2919147; Bjork, 2009, 2325339; Louisse, 2020, 6833626; Song, 2016, 9959776}. Two studies conducted in primary rodent and human hepatocytes had conflicting results, with one study finding no clear pattern of the differential expression of genes associated with PPARα activation in either mouse or human hepatocytes {Rosen, 2013, 2919147}, and the other study finding evidence of PPARα activation by altered expression of PPARα signaling pathway genes in rat hepatocytes, but not in human hepatocytes, neither primary nor HepG2 cells {Bjork, 2009, 2325339}. In a third study in primary human hepatocytes, pathway analysis of gene expression changes induced by PFOS exposure were not significantly similar to those induced by known PPARα agonists, which is in contrast to changes following PFOA exposure {Beggs, 2016, 3981474}. However, transcripts associated with CAR/PXR activation were upregulated in human hepatocytes {Rosen, 2013, 2919147}. In contrast to the results from primary human hepatocytes, PFOS upregulated PPARα target genes in two human cell lines derived from the liver, HepaRG and HepG2 cells {Louisse, 2020, 6833626; Song, 2016, 9959776}. Gene expression patterns in PFOS-exposed HepG2 cells were also consistent with activation of LXR {Louisse, 2020, 6833626}. Another study in HepG2 cells, however, reported reduced gene expression of *PXR* and *LXR* following treatment with 10–100 µM PFOS for 24 hours, with the reduction in *PXR* being attenuated by 48 hours {Behr, 2020, 6505973}.

The involvement of HNF4α in PFOS-induced hepatotoxicity was examined in two *in vitro* studies, and the results support the findings of the *in vivo* study described above {Beggs, 2016, 3981474; Behr, 2020, 6505973}. In one study, protein levels of HNF4α were decreased in primary human hepatocytes after 48 and 98 hours of exposure to 10 µM PFOS {Beggs, 2016, 3981474}. A corresponding decrease in the expression of genes that are positively regulated by HNF4α (*CLDN1*, *CYP7A1*, *TAT*, and *ADH1B*) and increases in genes that are negatively regulated by HNF4α targets (*CCND1*, *AKR1B10*, and *PLIN2*) was observed. A study in HepaRG cells exposed to 1–100 µM PFOS for 24 or 48 hours corroborated these findings, as downregulations in both HNF4α and its target gene *CYP7A1* were observed {Behr, 2020, 6505973}.

3.4.1.3.1.4 Conclusions

Although activation of PPARα is a widely cited mechanism of liver toxicity induced by PFAS exposure, PFOS has been shown to activate a number of other nuclear receptors, including PPARγ, PPARβ/δ, CAR/PXR, and LXR/RXR. Many of these nuclear receptors, including CAR and PPARγ, are also known to play important roles in liver homeostasis and have been implicated in liver dysfunction, including steatosis {Armstrong, 2019, 6956799}. Therefore, PFOS exposure may lead to liver toxicity through the activation of multiple nuclear receptors in both rodents and humans.

3.4.1.3.2 Lipid Metabolism, Transport, and Storage

3.4.1.3.2.1 Introduction

The liver is the primary driver of lipid metabolism, transport, and storage. It is responsible for the absorption, packaging, and secretion of lipids and lipoproteins. Lipids are absorbed from digestion through biliary synthesis and secretion, where they are converted to fatty acids {Trefts, 2017, 10284972}. These fatty acids are then transported into hepatocytes, cells that make up roughly 80% of the liver mass, via a variety of transport proteins such as CD36, FATP2, and FATP5 {Lehner, 2016, 10284974}. Fatty acids can be converted to triglycerides, which can be packaged with high or very-low-density lipoproteins (HDL or VLDL, respectively) for secretion. Lipid handling for the liver is important for energy metabolism (e.g., fatty acid β-oxidation) in other organs and for the absorption of lipid-soluble vitamins. *De novo* cholesterol synthesis is another vital function of the liver {Huang, 2011, 10284973}. Cholesterol is important for the assembly and maintenance of plasma membranes. Dysregulation of any of these functions of the liver can have implications for metabolic and homeostatic processes within the liver itself and other organs and contribute to the development of diseases such as non-alcoholic fatty liver disease, steatosis, hepatomegaly, and obesity.

The liver is a major site of PFOS deposition and as such, not only influences hepatic lipid levels but can also alter gene expression for a variety of pathways involved in biological processes {U.S. EPA, 2016, 3603365}. PFAS have been shown to induce steatosis and increase hepatic triglyceride levels in rodents via inducing changes in genes directly involved with fatty acid and triglyceride synthesis. These include genes such as fatty acid binding protein 1 (*Fabp1*), sterol regulatory element binding protein 1 (*Srebp1*), VLDL receptor (*Vldlr*), and lipoprotein lipase (*Lpl1*) {Armstrong, 2019, 6956799}. These genes can be altered through PPARα and PPARγ induction pathways due to regulation of HNF4α. PFOS upregulates hepatic nuclear receptor genes directly involved in lipid metabolism (e.g., *Pxr and Rar*) and the β-oxidation of fatty acids (e.g., *acyl-CoA oxidase 1* (*Acox1*) and carnitine palmitoyltransferase 1A (*Cpt1a*)) {Lee, 2020, 6323794}. The responses of lipids, bile acids, and associated genes and processes to PFOS exposure are dose-, model-, and, for some responses, sex-dependent.

3.4.1.3.2.2 *In Vivo* Models

While the sections below focus on hepatic-specific measurements of lipids from the available literature, measurements of lipids in the serum are also important indicators of lipid homeostasis and alterations in lipid metabolism, transport, and storage due to PFOS exposure. Serum lipid metrics from both animal and epidemiological studies are reported in Section 3.4.3.2 and Section 3.4.3.1, respectively.

3.4.1.3.2.2.1 Rats

Two studies conducted in both male and female Sprague Dawley rats reported marked effects on lipid metabolism including sex-dependent effects of PFOS on hepatic outcomes {Bagley, 2017, 4238503; NTP, 2019, 5400978}.

In a study by Bagley et al. (2017, 4238503), male and female rats were exposed to 0 or 100 ppm of PFOS in their diet for three weeks. In males, the authors observed increased liver choline, an organic cation critical for the assembly/secretion of lipoproteins and the solubilization of cholesterol in bile; females fed PFOS diets had no change in liver choline levels. An increase in

hepatic free fatty acids, triglycerides, and liver lipid area percent was also observed in males fed PFOS, while a decrease was observed in females. This is indicative of hepatic steatosis occurring in males but not in females. Serum was collected from animals on days 2, 9, 16, and 23 during the three weeks of dietary PFOS exposure and subsequently analyzed for serum clinical chemistry. There were transient effects on the serum levels of enzymes related to lipid metabolism (e.g., lipase, lactate dehydrogenase) in the PFOS-fed groups. In comparison to controls, there was a reduction in lipase and lactate dehydrogenase in PFOS-fed males at all four of the timepoints tested. PFOS-fed females had similar reductions in lipase and lactate dehydrogenase concentrations at every timepoint except day 23. For days 2, 9, and 16, animals were not fasted prior to serum collection; on day 23, animals were instead fasted overnight, and serum was collected via exsanguination at necropsy. The gene expression of enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase (*Ehhadh*), one of the enzymes involved in peroxisomal β-oxidation, was upregulated to a larger degree in females than in males (4.1-fold vs. 3.7-fold). Similarly, stearoyl-CoA desaturase-1 (*Scd1*), involved in the conversion of oleic acid to stearate, was upregulated 9-fold in females (compared to 2-fold in males, a change that was not significantly different from the control males). While nuclear receptors (such as CAR, PXR, LXR-α, LXR-β, and PPAR-γ) are involved in lipid accumulation, and an upregulation of the mRNA for enzymes involved in this process (such as *Scd1*) would indicate their activation, there was no lipid accumulation in females. *Ehhadh* was increased in both sexes compared to controls. Together, this may indicate that steatosis in rats is not induced by activation of these nuclear receptors or transcription levels of protein involved in key steatosis pathways. The authors also investigated the effect of choline supplementation along with PFOS administration and found that the steatosis phenotype persisted in males. The authors hypothesize that increased efficiency of female hepatic cytosolic fatty acid binding protein results in greater mobilization from lipid to VLDL causing faster excretion into serum and thus adipose tissue. However, the authors note that this apparent sex difference in lipid accumulation warrants further study {Bagley, 2017, 4238503}.

NTP (2019, 5400978) used an oral dosing paradigm of 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg/day for 28 days and measured serum cholesterol and triglyceride concentrations (Section 3.4.3.2). Notably however, both males and females exhibited an increase in lipid metabolism/oxidation related genes (*Acox1*, *Cyp4a1*, *Cyp2b1*, and *Cyp2b2*). An increase in these genes indicates increases in PPARα and CAR activity.

In addition to the sex differences in liver lipid levels described Bagley et al. (2017, 4238503), Luebker (2005, 757857) reported that there may also be differences depending on the developmental stage. Female rats were exposed to 0, 0.4, 0.8, 1.0, 1.2, 1.6, or 2.0 mg/kg/day PFOS for 42 days (6 weeks) prior to mating through either GD 20 or LD 4. In the GD 20 group, dams were sacrificed and fetuses collected at GD 21, and liver cholesterol and triglycerides were measured in dams and fetuses exposed to 0, 1.6, or 2.0 mg/kg/day. In dams, liver cholesterol was significantly reduced at both doses of PFOS, whereas triglycerides were unchanged. No changes were observed in fetuses at this timepoint. In the LD 5 groups, dams and pups were sacrificed to measure liver cholesterol and triglycerides. In dams, liver cholesterol was unchanged at this time point, and liver triglycerides were significantly increased at 1.6 and 2.0 mg/kg/day. In pups, liver cholesterol was also unchanged; however, liver triglycerides were significantly decreased in pups exposed to 1.0–2.0 mg/kg/day in both sexes.

3.4.1.3.2.2.2 Mice

Several studies in a variety of mouse models were conducted to investigate the effects of PFOS on the transcription and translation of lipid metabolism and biliary pathways. The focus of these studies was to identify key regulators affected by PFOS exposure and the extent to which pathways were affected. To this end, the studies employed expression microarray, quantitative reverse transcription polymerase chain reaction (qRT-PCR), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Ingenuity Pathway Analysis (IPA), and other biochemical measures such as Western Blot and enzyme-linked immunosorbent assay (ELISA).

3.4.1.3.2.2.2.1 Biochemical and Related Histological Changes

Many biochemical changes occurred with lipids and bile within the liver as well as lipid transport out of the liver (serum/plasma values). In several mouse studies, triglycerides, total cholesterol, and/or LDL levels were altered in liver {Lai, 2018, 5080641; Liang, 2019, 5412467; Huck, 2018, 5079648; Xu, 2017, 3981352}. These changes often had potentially associated histopathological consequences, with steatosis and other lesions being observed in affected livers {Liang, 2019, 5412467; Huck, 2018, 5079648; Su, 2019, 5080481}.

In a 4-week study, decreased liver cholesterol was observed in male C57BL/6 mice dosed with 5 mg/kg/day PFOS {Xu, 2017, 3981352}; the mechanism of action was attributed to estrogen receptor ß (ERß) and is further described in Section 3.4.1.3.3. In a 7-week study, increased liver triglycerides were observed in female CD-1 mice exposed to 0.3 or 3 mg/kg/day PFOS {Lai, 2018, 5080641}. A yellowish appearance was also noted in the livers of the 3 mg/kg/day group, which the authors associated with lipid accumulation. The authors hypothesized that the increased hepatic triglycerides may be due to an impairment in lipid catabolism and/or lipid export.

A study in Kunming mice investigated lipid metabolism markers within pregnant mice and the offspring exposed prenatally {Liang, 2019, 5412467}. Lipid dysregulation was present in both mother and offspring. Specifically, the authors observed increased liver weight and triglyceride content at the 5 mg/kg/day dose of PFOS in both the mother and offspring. In maternal livers, hepatomegaly along with hepatic steatosis was observed. Further, the authors also found increased protein expression of CYP4A14 in offspring. This cytochrome P450 catalyzes the omega(ω)-hydroxylation of medium-chain fatty acids and arachidonic acid in mice and is a common indicator of PPARα activation. Authors also observed increases in CD36 protein levels, which has a direct effect on fatty acid uptake by hepatocytes, and decreased levels of the proteins apolipoprotein B (APOB), a cholesterol transporter, and FGF21 in the PND 1 mouse liver. Together, this evidence indicates that PFOS undergoes gestational transfer, impairing lipid homeostasis in the offspring.

In ICR mice exposed to 10 mg/kg/day PFOS for 21 days, lipid-based vacuolization was observed in the liver, which was accompanied by decreased fibroblast growth factor 21 (FGF21) protein concentration {Su et al. 2019, 5080481}. This hormone is produced by hepatocytes and regulates the metabolism of sugar and lipids through receptors in the hypothalamus. Interestingly, vitamin C showed a protective effect in the study, lowering the effect size of some of the increased parameters and reducing liver lesions. This indicates that nutritional status can mediate the hepatotoxicity of PFOS.

Beggs et al. (2016, 3981474) observed a decrease in hepatocyte nuclear factor alpha (HNF4α) protein, a master regulator of hepatic differentiation, in the livers of ten-week-old CD-1 mice exposed to 3 or 10 mg/kg/day PFOS by oral gavage for 7 days. HNF4α regulates liver development (hepatocyte quiescence and differentiation), transcription of specific liver genes, and lipid metabolism. This decrease in HNF4α protein occurred without a subsequent reduction in messenger ribonucleic acid (mRNA) levels but appeared to cause a subsequent upregulation of genes that are negative targets of HNF4α. For example, downstream proteins such as CYP7a1 and perilipin 2 (PLIN2) were reduced. HNF4α is considered an orphan receptor with various fatty acids as its endogenous ligands. These fatty acids maintain the structure of the receptor homodimer. PFOA and PFOS are analogous in structure to fatty acids and may also provide stabilization of the homodimer. The authors investigated the role of PFOS interaction with this protein via in silico docking models, which showed a displacement of fatty acids by PFOS and PFOA, possibly tagging HNF4α for degradation. Although the authors, do not directly look at liver pathology, they hypothesize that steatosis, hepatomegaly, and carcinoma in rodents may be a consequence of the loss of this protein and also presents a potential mechanism for PFOS induced hepatic effects in humans {Beggs, 2016, 3981474}.

3.4.1.3.2.2.2.2 Microarray Analyses and RT-PCR

Several studies observed perturbations in lipid transport, fatty acid synthesis, triglyceride synthesis, and cholesterol synthesis in PFOS-exposed mice {Das, 2017, 3859817; Rosen, 2017, 3859803; Su, 2019, 5080481; Liang, 2019, 5412467; Huck, 2018, 5079648}. Two of these studies, Das et al. (2017, 3859817) and Rosen et al. (2017, 3859803), investigated the effects of PFOS on lipid metabolism and homeostasis without the influence of PPARα using nullizygous models. After exposure to 3 or 10 mg/kg/day PFOS for 7 days, Das et al. (2017, 3859817) observed that a smaller subset of genes related to lipid homeostasis was activated in *Pparα*-null mice compared to WT mice. In addition, there were 3-to-4-fold reductions in the genes related to lipid homeostasis that were expressed in PFOS-exposed *Pparα*-null mice compared to WT mice, including carbohydrate response element binding protein (*Chrebp*), *Hnf4α*, Pparγ coactivator 1α (*Ppargc1a*), and sterol regulatory element binding transcription factor 2 (*Srebf2*). In *Pparα*-null mice, there was only a 2-fold decrease in *Hnf4α*, a 4-fold decrease in *Ppargc1a*, and a 3-fold increase in *Srebf1*. *Srebf* genes encode transcription factors that bind to the sterol regulatory element-1 motif that is found in the promoter of genes involved in sterol biosynthesis. This indicates that some of the effects on lipid metabolism are independent of, or only partially dependent on, PPARα as an upstream regulator.

The results from Das et al. (2017, 3859817) are concurrent with the findings in another study by the same authors {Rosen et al. 2017, 3859803}, which exposes WT and *Pparα*-null mice to 10 mg/kg/day PFOS for 7 days. PFOS exposure up-regulated genes related to fatty acid β-oxidation, lipid catabolism, lipid synthesis, and lipid transport in both strains; however, the increase in expression was several-fold lower in *Pparα*-null mice than in WT mice. In fact, the authors suggest that the transcriptome of the mice resembled that of mice treated with PPARγ agonists, thus suggesting a role for other PPAR receptors in the dysregulation of lipid synthesis that occurs with PFOS exposure. Xu et al. (2017, 3981352), in their investigations using *Erβ*-null mice (Section 3.4.1.3.3), found a difference in lipid metabolism and bile acid synthesis between *Erβ*-null and WT mice exposed to PFOS. In mice exposed to PFOS, mRNA levels of cholesterol-7a-hydroxylase (*Cyp7a1*), the rate limiting enzyme in the conversion of cholesterol to bile acid, was

downregulated in WT but not in *Erβ*-null mice, supporting a role for pathways independent of PPARα in hepatic lipid responses to PFOS exposure.

Genes involved in lipid homeostasis and regulation were found to be differentially expressed in mice exposed to PFOS {Su, 2019, 5080481; Liang, 2019, 5412467; Huck, 2018, 5079648}. Key regulators of fatty acid oxidation including *Cyp4a14* and *Cd36* were upregulated in the livers of PND 1 mice exposed during gestation to PFOS {Liang, 2019, 5412467}. Interestingly, genes related to hepatic export of lipids, such as *Apob* and *Fgf21*, were downregulated. Downregulation of these genes may play a role in the hepatic steatosis, hepatomegaly, and hepatocyte hypertrophy observed across multiple studies. A study using C57BL/6 mice dosed at 1 mg/kg/day PFOS in the diet for 6 weeks, found that a high fat diet (HFD) protected against PFOS-induced steatosis and hepatomegaly by inducing *Apoa1*, *Apoa2*, *Apob*, and the microsomal triglyceride transfer protein (*Mttp*) gene expression {Huck, 2018, 5079648}. *Srebf1*, a regulator of hepatic lipogenesis, was significantly induced in PFOS-exposed mice in the HFD group compared to those fed normal diets. Similarly, gene expression of *Cd36*, a major lipid importer, was induced by PFOS in mice fed normal diet but was suppressed in HFD groups, suggesting that co-administration of PFOS and HFD mitigates steatosis and hepatomegaly. Together, these results suggest that diet could be a mediating factor in PFOS toxicity and warrants consideration for evaluation of human hepatic effects.

3.4.1.3.2.2.2.3 Kyoto Encyclopedia of Genes and Genomes (KEGG) and Ingenuity Pathway Analyses (IPA)

KEGG and IPA tools (Qiagen) are useful for analysis and interpretation of large data sets generated from transcriptomic profiling. Two studies extensively utilized these tools to characterize the changes to liver lipid homeostasis. Much like in the studies described in the previous two subsections, many genes related to the synthesis of fatty acids, including lipid, fatty acid, triglyceride, linoleic acid and arachidonic acid metabolism, lipid transport, fatty acid biosynthesis, and triglyceride homeostasis were differentially expressed in mice administered PFOS {Beggs, 2016, 3981474; Lai, 2017, 3981375}.

Beggs et al. (2016, 3981474) exposed CD-1 mice to 0 or 10 mg/kg/day PFOS for 7 days. The pathway for hydroxylation of lipids was significantly dysregulated in the PFOS-exposed group. Lai et al. (2017, 3981375) exposed pregnant CD-1 mice to 0 or 0.3 mg/kg/day PFOS before mating through to embryonic day 18.5. Pathway enrichment analysis using KEGG and IPA to understand the signaling pathways and biological processes that were affected, as evidenced by differentially expressed genes, highlighted changes in fatty acid metabolism including the deregulation of the PPAR signaling pathway (not specific to any isoform), fat digestion and absorption, the biosynthesis of unsaturated fatty acids, and bile secretion in both the maternal and offspring livers.

3.4.1.3.2.2.3 Zebrafish

Zebrafish have been increasingly used as a model to investigate the toxicity of PFAS. Several studies have evaluated the toxicity of PFOS in zebrafish, specifically in regard to effects on lipid metabolism. Similar to the results in rodent models, fatty acid oxidation enzymes and related gene expression, as well as lipidosis, was increased in PFOS-treated animals {Cheng, 2016, 3981479; Khazaee, 2019, 5918850; Cui, 2017, 3981467; Du, 2014, 2851143}. The authors of these studies also reported increases in triglycerides, total cholesterol, and free fatty acid

receptors in liver samples from PFOS-exposed zebrafish. Interestingly, as seen in rodent models, there can be a temporal shift in the levels of proteins or genes involved in lipid metabolism, with PFOS exposure. Khazaee et al. (2019, 5918850) found that expression levels of the fatty acid binding protein 1-A gene fabp1a, which binds free fatty acids and their coenzyme A derivatives and is involved in their intracellular transport into the liver, varied over a 30-day period of exposure to 0.1 or 1 mg/L PFOS. Expression in the liver peaked at day 14 of exposure but being below control levels at day 30 of exposure. This suggests that lipid metabolism is dynamic, and the authors concluded that more research is needed to understand if a key time point exists for evaluating such gene expression changes versus examining such changes over time.

Sex-dependent differences were also observed in a few studies in PFOS-treated zebrafish {Cheng, 2016, 3981479; Cui, 2017, 3981467}. In one study in which zebrafish were exposed to 0.5 µM for 5 months beginning at 8 hours post-fertilization (hpf), males tended to have increased fatty accumulation and reduced hepatic glycogen storage compared to females {Cheng, 2016, 3981479}. In a 2-generation study, Cui et al. (2017, 3981467) observed that the offspring of zebrafish exposed to PFOS from 8 hpf until 180 days post-fertilization (dpf) tended to have increased expression of the leptin α (*lepa*) and insulin receptor α (*insr*) genes. Diacylglycerol O-acyltransferase 1 (*dgat1b*), a metabolic enzyme in triglyceride biosynthesis, and *apoa1*, which regulates cholesterol transport, were downregulated by PFOS exposure. The authors also noted that along with indicators of lipid dysregulation, there were morphologically different mitochondria, potentially exacerbating lipid homeostasis.

3.4.1.3.2.3 In Vitro Models

Two studies reported genetic profiles and pathway analyses in mouse and human hepatocytes to determine the effect of PFOS treatment on lipid homeostasis and bile synthesis. Rosen et al. (2013, 2919147) exposed mouse and human primary hepatocytes to 0-250 µM PFOS for 48 hours. Gene expression was evaluated using microarrays, IPA, and qRT-PCR. For PFOS-exposed murine hepatocytes, a much smaller group of genes was found to be altered compared to the whole liver (described in Section 3.4.1.3.4). These included genes associated with β-oxidation and fatty acid synthesis such as *Ehhadh* and *Fabp1*, which were both upregulated with PFOS exposure. In contrast to the transcriptome of primary mouse hepatocytes, in primary human hepatocytes, a relatively large group of genes related to lipid metabolism including *PLIN2* and *CYPT1A* were differentially expressed with PFOS exposure. The authors attribute some of these differences between mouse and human hepatocytes to a less robust activation of PPARα in humans. Further, many of the genes investigated were chosen to explore effects of PFOS exposure that are independent of PPARα activation but may include other nuclear receptors such as CAR, LXR, PXR and the aryl hydrocarbon receptor (AhR) (Section 3.4.1.3.1). Beggs et al. (2016, 3981474) exposed human primary hepatocytes to 0.01-100 µM PFOS for 48 or 96 hours, to determine pathways affected by PFOS exposure. PFOS treatment altered genes primarily associated with liver necrosis and carcinogenesis. However, pathways associated with lipid metabolism and bile synthesis (hydroxylation of lipids), including several CYP450 enzymes associated with lipid homeostasis such as *CYP2B6, CYP2C8, CYP3A4, CYP3A5, CYP4A11, CYP4A22*, and *CYP7A1* were also altered. Notably, CYP7A1 was among the top ten most downregulated genes with a fold change of -7.13 indicating potential limitations in the conversion of cholesterol to bile acid. Importantly, HNF4α, a master regulator of liver function, regulates many differentially expressed genes related to lipid metabolism which includes all the aforementioned CYP450s. Together these studies indicate PFOS-induced activation of CYP450

through a variety of PPARα-dependent and independent pathways. Interestingly, there may be crosstalk between some of these receptors. Beggs et al. (2016, 3981474) notes that HNF4α can regulate PPARα in mice.

There are several studies that investigated the effect of PFOS on lipid homeostasis using human cells such as HepG2, HepaRG, and HL-7702 cells. Various endpoints were also investigated in these cell lines such as mRNA expression through microarray and qRT-PCR assays; lipid, triglyceride, cholesterol, and choline content; and protein levels via ELISA or Western Blot.

In human hepatic cell lines such as HepaRG or HepG2, PFOS treatment correlated with suppression of gene expression for genes regulating cholesterol homeostasis. Louisse et al. (2020, 6833626) noted a concentration-dependent increase in triglycerides, a decrease of cholesterol, and downregulation of cholesterogenic genes, predominantly with the highest dose tested, in HepaRG cells exposed to 0-100 µM PFOS for 24 hours. Cellular cholesterol biosynthesis genes are regulated by SREBPs, which were also downregulated with PFOS exposure. In contrast, PPARα-responsive genes were upregulated with PFOS exposure, particularly at higher doses. Behr et al. (2020, 6505973) also exposed HepaRG cells to 0-100 µM PFOS for 24 or 48 hours. Similar to the results from Louisse et al. (2020, 6833626), at 24 hours, genes related to cholesterol synthesis and transport were downregulated at the highest dose except for several genes that were upregulated, including bile and cholesterol efflux transporters (*UGT1A1* and *ABCG1*), and genes involved in bile acid detoxification (*CYP3A4*). The gene profiles after 48 hours of exposure were similar, except at the high dose, which saw some attenuation of the response in cholesterol synthesis and transport. Cholesterol content was significantly higher in the supernatant at the highest dose of 100 µM but there was no significant difference after 48 hours between treated cells and controls, in line with the genetic data of some response attenuation.

Franco et al. (2020, 6507465) exposed HepaRG cells to 0.0001–1 µM. Interestingly, lipid levels were elevated with the lower PFOS concentrations and reduced with the higher PFOS concentrations. PFOS increased diglyceride levels in a dose-dependent manner except for a decrease that was observed at the highest concentration. In contrast, triglyceride levels were not significantly different from controls. This study provides evidence of potential non-monotonic dose-responses that could result from low-dose PFOS exposures, a potential area that may require further consideration.

While alterations in lipid metabolism have been reported, Das et al. (2017, 3859817) found that PFOS did not inhibit palmitate-supported respiration (i.e., mitochondrial metabolism) in HepaRG cells. There was no effect on oxidation or translocation of palmitoylcarnitine, an ester involved in the metabolism of fatty acids which plays a role in the tricarboxylic acid cycle.

3.4.1.3.2.4 Conclusions

As described in Section 3.4.3.2, serum lipid concentrations generally decrease with increasing PFOS doses in rodent bioassays. It is thought that the activation of PPARα, which is less robust in humans, mediates the effect seen in rodents. In the mechanistic evidence synthesized above, it appears that PFOS exposure in mammalian and non-mammalian species is associated with increased lipid accumulation within the liver. Interestingly, studies that measure both serum and liver lipid content generally follow this trend and report a decrease in serum lipids and an increase in liver lipid content; this effect may be contributing to the observed PFOS-induced

hepatomegaly and steatosis. Additional data on human liver lipid accumulation would clarify whether the effects on liver lipid contents in animal bioassays are mechanistically relevant to humans.

Effects on hepatic lipid metabolism can be observed through the influence of PFOS on not only PPARα, but other key regulators of hepatic lipid homeostasis such as HNF4α. Gene ontology using receptor null mice has shown that lipid homeostasis is complex and PFOS is likely acting on more than one key regulator. Other PPAR isoforms and hormone receptors such as ERβ play a role in regulating lipid and bile metabolism/catabolism, transport, and storage. While minor conflicts exist between some cell line studies, the evidence supports that PFOS causes lipid dyshomeostasis and contributes to liver dysfunction and disease, likely through the modulation of multiple nuclear receptors.

3.4.1.3.3 Hormone Function and Response

While much of the literature relevant to hormone function and response is focused on reproductive outcomes (See PFOS Appendix), recent literature has also shown a relationship between hepatic hormonal effects and PFOS exposure. For example, PFOS has been found to have estrogenic effects. Xu et al. (2017, 3981352) reported an induction of ERβ, but not estrogen receptor alpha (ERα), when wild-type (C57BL/6) male mice were dosed with 5 mg/kg/day PFOS via oral gavage for 4 weeks. To further explore this relationship, the authors investigated PFOS administration in male wild-type (WT) and Erβ-null mice. They observed no significant changes in either WT or *Erβ*- null mice in genes related to lipid metabolism and bile synthesis (3-hydroxy-3-methylglutaryl-CoA reductase [*Hmgcr*], scavenger receptor class B type I [*Srbi*], low-density lipoprotein [*Ldl*], ATP-binding cassette transporter [*Abca1*]) when following exposure to 5 mg/kg/day PFOS for 28 days by oral gavage. However, ATP-binding cassette sub-family G member 5 (*Abcg5*), a gene involved in sterol excretion, was increased due to PFOS exposure in WT mice but not in *Erβ*-null mice, while cholesterol 7α hydroxylase (*Cyp1a711*), the initiator of cholesterol catabolism, was reduced due to PFOS exposure in WT mice but not in *Erβ*-null mice. Further, liver cholesterol levels were significantly decreased in WT PFOS-treated animals but not in *Erβ*-null mice. This suggests that ERβ mediates PFOS hepatotoxicity via altered cholesterol and bile synthesis. To confirm induction of ERβ, the authors also investigated the response to PFOS exposure in HEPG2 cells. After exposing the cells to 0, 10, or 100 µmol/L of PFOS for 24 hours, the authors found that ERβ was induced at 10 µmol/L, but not at the highest dose, potentially indicating a non-monotonic dose response.

There is also *in vitro* evidence that in the liver, genes responsible for a response to hormone stimulus and hormone metabolism are altered with PFOS exposure {Popovic, 2014, 2713517; Song, 2016, 9959776}. Differentially expressed genes due to PFOS treatment in these studies encode proteins such as serine peptidase inhibitor, clade A, proprotein convertase subtilisin/kexin type 9, activin A receptor type IC, and insulin-like growth factor binding protein 7, all of which are associated with hormone stimulus and/or metabolism. However, it should be noted that these genes were more significantly altered with PFOA exposure; the authors indicated that while PFOS was more cytotoxic, PFOA exposure induced more gene alterations, suggesting that PFOS may be a relatively weak agonist or activator for the transcription factors or nuclear response elements involved in regulating their transcription {Song, 2016, 9959776}.

3.4.1.3.3.1 Conclusions

While there is a small number of studies regarding hormone function and response specifically within the liver, there is evidence that PFOS has the potential to perturb hormonal balance and hormonal metabolism in hepatic cells. There is also some evidence from one *in vivo* study in mice that PFOS hepatotoxicity may be partially modulated by ERβ. This could have implications for hormone function and responses in other organ systems and may also be important for mode of action considerations for hepatotoxicity.

3.4.1.3.4 Xenobiotic Metabolism

3.4.1.3.4.1 Introduction

Xenobiotic metabolism is the transformation and elimination of endogenous and exogenous chemicals via enzymes (i.e., cytochrome P450 [CYP] enzymes) and transporters (i.e., organic anion transporting peptides [OATPs]) {Lee, 2011, 3114850}. As described in Section 3.3.1.3, the available evidence demonstrates that PFOS is not metabolized in humans or other species. However, several studies have investigated how PFOS could alter activation of PXR/CAR as described in Section 3.4.1.3.1; subsequently, xenobiotic metabolism is altered via manipulation of the expression of key genes. For instance, the genes for OATP expression (i.e., *slco1d1* and *slco2b1*) in zebrafish or phase I and II biotransformation enzymes in human hepatocytes (i.e., *CYP3A4*), responsible for the transport or metabolism of xenobiotics, may be upregulated or downregulated following PFOS exposure.

Overall, results from both *in vivo* and *in vitro* model systems suggest that genes responsible for xenobiotic metabolism are upregulated as a result of PFOS exposure.

3.4.1.3.4.2 *In Vivo* Models

Four studies investigated xenobiotic metabolism endpoints with three studies using Sprague Dawley rats {Elcombe, 2012, 1401466; Curran, 2008, 757871; Chang, 2009, 757876} and the remaining study using *Pparα*-null and WT mice {Rosen, 2010, 1274165}. In a gestational and lactational exposure study, Chang et al. (2009, 757876) reported increased *Cyp2b2* expression in dams and male pups (2.8-fold and 1.8-fold, respectively). Elcombe et al. (2012, 1401466) also reported the induction of CYP2B1/2, in addition to CYP2E1 and CYP3A1 proteins, following test diets of 20 ppm or 100 ppm PFOS. Additionally, Curran et al. (2008, 757871) and Rosen et al. (2010, 1274165) reported upregulation of *Cyp4a22* and *Cyp2b10* expression.

Two studies examined xenobiotic metabolism endpoints, including CYP450 expression and CYP2B enzyme activity via the PROD biomarker response, in rats {Elcombe, 2012, 1332473; NTP, 2019, 5400978}. Sprague Dawley rats were exposed to 0, 20, or 100 ppm PFOS for a 7-day dietary treatment and then were assessed for CYP450 protein expression in the liver at recovery days 28, 56, and 84 {Elcombe, 2012, 1332473}. Total CYP450 concentration in liver microsomes was measured via carbon monoxide difference spectrum of ferrocytochrome P450. Across each dose group and recovery day, mean CYP450 concentrations were increased 123–189% compared to the control group. However, there was a non-linear PROD dose-response relationship; the 20 ppm group had decreased mean PROD activity across all recovery days, but the 100 ppm group had increased activity on recovery days 1 and 28, followed by similar activity on recovery day 56, then statistically significant decreased PROD activity by recovery day 84. NTP (2019, 5400978) also assessed Sprague Dawley rats following 28-day treatment of PFOS

(0, 1.25, 2.5, or 5 mg/kg/day) by gavage. Across all treatments of PFOS, females and males both had increased hepatic expression of *Cyp2b1*, *Cyp2b2*, and *Cyp4a1*.

One study examined the expression of genes related to xenobiotic metabolism in zebrafish {Jantzen, 2016, 3860109}. AB strain zebrafish embryos were exposed to PFOS from 3 to 120 hpf and evaluated at 180 dpf. Female zebrafish had significant reductions in *slco1d1* expression, while males had significant reductions in both *slco1d1* and *slco2b1* expression {Jantzen, 2016, 3860109}, which are the genes responsible for OATPs and significant in the transport of xenobiotics {Popovic, 2014, 2713517}. Jantzen et al. (2016, 3860109) noted that in their previous study, PFOS exposure from 5–14 dpf resulted in significantly reduced slco2b1 expression in zebrafish at 5 dpf but significantly increased expression at 14 dpf {Jantzen, 2016, 3860114}. While their current study reported alterations in gene expression long-term, further studies with additional time points are needed to elucidate the effect of PFOS exposure on OATP expression.

3.4.1.3.4.3 *In Vitro* Models

Gene expression of CYP enzymes responsible for xenobiotic metabolism were assessed in one study using primary human (e.g., *CYP2B6* and *CYP3A4* genes) and mouse (e.g., *Cyp1a1* and *Cyp3a11* genes) hepatocytes {Rosen, 2013, 2919147}. Results varied between human and mouse hepatocytes, with *CYP2B6* and *CYP3A4* expression upregulated in human hepatocytes, but not in mouse hepatocytes. The authors noted that the reasons for the differences in gene expression in the human and mouse hepatocytes were unclear; however, cell density, collection methods, and time in culture were possible factors, as these were not consistent between models.

Xenobiotic metabolism endpoints were assessed in five studies using hepatic cell lines, including HepG2 {Shan, 2013, 2850950; Song, 2016, 9959776} and HepaRG {Behr, 2020, 6505973; Franco, 2020, 6315712; Louisse, 2020, 6833626}. Franco et al. (2020, 6315712) assessed several phase I biotransformation enzymes following exposure to PFOS concentrations (0.0001, 0.001, 0.01, 0.1, or 1.0 µM) for 24 or 48 hours. Gene expression of phase I enzymes varied across concentrations and between the 24- and 48-hour exposures. For *CYP1A2*, after 24 hours, the two lowest concentrations resulted in significant increases in expression; however, after 48 hours, the two highest concentrations resulted in significant decreases (~10-fold) in expression. For *CYP2C19*, after 24 hours, there were no clear trends; however, after 48 hours, expression was significantly reduced across all concentrations {Franco, 2020, 6315712}.

Evidence varied for CYP3A4 induction, depending on the model and duration of exposure, as well as whether gene expression or enzyme activity was assessed {Franco, 2020, 6315712; Behr, 2020, 6505973; Louisse, 2020, 6833626; Shan, 2013, 2850950}. Franco et al. (2020, 6315712) reported that after 24 hours, there were no clear trends in *CYP3A4* expression. However, after 48 hours, *CYP3A4* expression was significantly reduced (up to five-fold) across all concentrations {Franco, 2020, 6315712}. Conversely, Behr et al. (2020, 6505973) and Louisse et al. (2020, 6833626) reported upregulation of CYP3A4 enzyme activity following 24- or 48-hour PFOS exposure (1, 10, 25, 50, and 100 µM) in HepaRG cells, while Shan et al. (2013, 2850950) reported no significant changes in CYP3A4 enzyme activity following PFOS exposure (0, 100, 200, 300, and 400 µM) in HepG2 cells.

Franco et al. (2020, 6315712) also assessed gene expression of two phase II enzymes, glutathione S-transferase mu 1 (*GSTM1*) and UDP-glucuronosyltransferase 1A1 (*UGT1A1*),

which were not significantly affected in differentiated HepaRG cells by exposure to PFOS after 24 or 48 hours. The authors noted that it was unclear how PFOS alters gene expression of phase I enzymes but not phase II enzymes. Further research is needed to determine whether altered gene expression occurs by interference with cytoplasm receptors, inhibition of nuclear translocation, or inhibition of the interaction of nuclear translocator complexes with DNA sequences {Franco, 2020, 6315712}.

Song et al. (2016, 9959776) analyzed expression of over 1,000 genes via microarray and gene ontology analysis in HepG2 cells exposed to PFOS. HepG2 cells were first exposed to 0-1,000 µM PFOS for 48 h to determine cell viability and cytotoxicity; an IC20 dose of 278 µM PFOS was determined from these results. HepG2 cells were then treated with 278 µM PFOS for 48 hours and used in microarray analysis. As a result of 278 µM PFOS treatment, 279 genes had ≥1.5-fold change in compared to the control group, including genes related to xenobiotic metabolism by cytochrome P450s such as flavin containing dimethylaniline monoxygenase 5 (*FMO5*), UDP glucuronosyltransferase family 1 member A6 (*UGT1A6*), glutathione S-transferase alpha 5 (*GSTA5*), alcohol dehydrogenase 6 (class V) (*ADH6*), and glutathione S-transferase alpha 2 (*GSTA2*).

3.4.1.3.4.4 Conclusions

Several studies are available that assessed xenobiotic metabolism endpoints as a response to PFOS exposure, including studies in rats {Elcombe, 2012, 1332473; NTP, 2019, 5400978}, zebrafish {Jantzen, 2016, 3860109}, primary hepatocytes {Rosen, 2013, 2919147}, or hepatic cell lines {Shan, 2013, 2850950; Song, 2016, 9959776; Behr, 2020, 6505973; Franco, 2020, 6315712; Louisse, 2020, 6833626}. Jantzen et al. (2016, 3860109) reported significant reductions in the expression of OATPs (*slco1d1* and *slco2b1*). While the majority of studies reported upregulation of gene expression of CYP enzymes {Elcombe, 2012, 1332473; NTP, 2019, 5400978; Franco, 2020, 6315712; Rosen, 2013, 2919147; Behr, 2020, 6505973; Louisse, 2020, 6833626; Song, 2016, 9959776}, direction and magnitude of change varied across doses and exposure times. Jantzen et al. (2016, 3860109) and Franco et al. (2020, 6315712) both noted the need for further studies to elucidate any potential relationships between PFOS exposure and xenobiotic metabolism.

3.4.1.3.5 Cell Viability, Growth and Fate

3.4.1.3.5.1 Cytotoxicity

Many *in vitro* studies have examined the potential for PFOS to cause cytotoxicity with various cell viability assays in both primary hepatic cell cultures {Khansari, 2017, 3981272; Xu, 2019, 5381556} and in hepatic cell lines {Louisse, 2020, 6833626; Rosenmai, 2018, 4220319; Shan, 2013, 2850950; Sheng, 2018, 4199441; Bagley, 2017, 4238503; Wielsøe, 2015, 2533367; Florentin, 2011, 2919235; Franco, 2020, 6315712; Ojo, 2020, 6333436; Franco, 2020, 6507465; Huang, 2014, 2851292; Oh, 2017, 3981364; Wan, 2016, 3981504; Cui, 2015, 3981517; Behr, 2020, 6505973; Song, 2016, 9959776}, with varying results depending on the exposure time and culturing methods. In mouse primary hepatocytes, cell viability was reduced by approximately 10% as determined by the CCK-8 assay after 24 hours of exposure to 10 µM PFOS {Xu, 2019, 5381556} and by 64%, as determined by a trypan blue exclusion assay in rat primary hepatocytes exposed to 25 µM PFOS for 3 hours {Khansari, 2017, 3981272}. However, another study in mouse and human primary hepatocytes reported that 100 µM PFOS did not induce

cytotoxicity after 48 hours, determined by a lack of treatment effect in genes related to cell damage such as heme oxygenase 1 (*HMOX1*), DNA damage inducible transcript 3 (*DDIT3*), and activating transcription factor 3 (*ATF3*) {Rosen, 2013, 2919147}.

Median lethal concentration (LC50) values in hepatic cell lines ranged from approximately 13 µM PFOS after for 24 or 48 hours of exposure in HepaRG cells {Franco, 2020, 6315712; Franco, 2020, 6507465}, to 45–65 µM after 24 or 48 hours of exposure in HepG2 cells {Wan, 2016, 3981504; Ojo, 2020, 6333436}, to 417 µM after 24 hours of exposure in HL-7702 cells {Sheng, 2018, 4199441}. However, two studies in HepG2 cells {Rosenmai, 2018, 4220319} and HepaRG cells {Louisse, 2020, 6833626} showed no effect on cell viability up to concentrations of 100 µM for 24 hours or 400 µM for 72 hours, respectively. A subset of these studies looked further into the mechanisms of cytotoxicity, including the induction of apoptotic pathways (Section 3.4.1.3.5.2.2).

3.4.1.3.5.2 Apoptosis

3.4.1.3.5.2.1 *In Vivo* Models

Apoptosis induced by PFOS exposure was assessed in five studies in male rats {Elcombe, 2012, 1332473; Elcombe, 2012, 1401466; Eke, 2017, 3981318; Wan, 2016, 3981504; Han, 2018, 4238554} and two studies in male mice {Xing, 2016, 3981506; Lv, 2018, 5080395}, with varying results. Two short-term dietary studies exposed rats to 20 or 100 ppm PFOS (equivalent to approximately 2 and 10 mg/kg/day, respectively), and apoptosis was assessed through the TUNEL assay {Elcombe, 2012, 1332473; Elcombe 2012, 1401466}. In one of these studies, rats were exposed for 7 days and allowed to recover for 1, 28, 56, or 84 days {Elcombe, 2012, 1332473}, while the other study exposed rats for 1, 7, or 28 days and collected liver directly after exposure {Elcombe, 2012, 1401466}. In the recovery study, at both PFOS exposure concentrations, a decreased apoptotic index was observed at all timepoints tested. In the 28-day study, the apoptotic index was decreased with 100 ppm PFOS at days 7 and 28, and increased at 20 ppm on day 7; no changes were observed at other timepoints. It should be noted that cell proliferation was markedly increased, particularly with the higher dose (100 ppm), in both studies (Section 3.4.1.3.5.3); increases in the total number of cells due to cell proliferation may confound certain metrics of apoptosis that do not report comparisons of the absolute number of apoptotic cells along with cell percentages.

Contrary to the dietary studies, three short-term gavage studies in rats showed an increase in expression of apoptotic genes (caspase 3 [*Casp3*] and caspase 8 [*Casp8*]) and proteins (e.g., cleaved poly-ADP-ribose polymerases [PARP], CASP3, and BCL2 associated X, apoptosis regulator [Bax]) in livers collected after administrations of up to 10 mg/kg/day PFOS for 28 days {Eke, 2017, 3981318; Wan, 2016, 3981504; Han, 2018, 4238554}. Similarly, two short-term gavage studies in male mice showed an increase in liver apoptosis {Xing, 2016, 3981506; Lv, 2018, 5080395}. Increased apoptosis in the liver, as determined via the TUNEL assay, was observed in male mice administered 2.5–10 mg/kg/day PFOS for 30 days {Xing, 2016, 3981506}. Increased apoptosis was also observed in liver tissue of male mice dosed with 10 mg/kg/day PFOS for 21 days, as measured by an increased expression of apoptotic-related proteins (tumor suppressor p53 [p53] and BAX) and a corresponding decrease in B cell leukemia/lymphoma 2 (BCL2) and by an increase in CASP3 enzyme activity {Lv, 2018, 5080395}.

Several studies further examined the mechanisms by which PFOS exposure may lead to apoptosis in the liver {Han, 2018, 4238554; Lv, 2018, 5080395; Xing, 2016, 3981506; Xu, 2020, 6316207; Oh, 2017, 3981364; Huang, 2014, 2851292; Yao, 2014, 2850398}. One rat study suggested that hepatic apoptosis was induced through mitochondrial damage, as shown by an increased level of cytoplasmic cytochrome c and decreased level of mitochondrial cytochrome c {Han, 2018, 4238554}. Two mouse studies concluded that hepatic apoptosis was induced by increases in oxidative stress, as evidenced by a decrease in antioxidant enzymes and a corresponding increase in lipid peroxidation {Lv, 2018, 5080395; Xing, 2016, 3981506}. In a third mouse study that examined microRNA (miRNA) expression in the liver, an increase in the expression of *miR-34a-5p*, which has been shown to recapitulate p53-mediated apoptosis, was observed {Yan, 2014, 2850901}.

3.4.1.3.5.2.2 *In Vitro* Models

In vitro, apoptosis has been examined in primary mouse hepatocytes and mouse and human cell lines after exposure to various concentrations of PFOS {Xu, 2019, 5381556; Xu, 2020, 6316207; Song, 2016, 9959776; Huang, 2014, 2851292; Oh, 2017, 3981364; Wan, 2016, 3981504; Cui, 2015, 3981517; Yao, 2016, 3981442}. PFOS was shown to increase the percentage of apoptotic cells {Xu, 2019, 5381556; Huang, 2014, 2851292; Oh, 2017, 3981364; Cui, 2015, 3981517; Yao, 2016, 3981442}, to increase the expression of proteins and genes in apoptotic pathways {Song, 2016, 9959776; Wan, 2016, 3981504}, or to increase CASP3 enzyme activity {Yao, 2016, 3981442}. Only one study in HL-7702 cells showed no change in the percentage of apoptotic cells {Cui, 2015, 3981568}.

In mouse primary hepatocytes, PFOS induced apoptosis through activation of Caspase 3, which was mediated by PFOS-induced mitochondrial membrane damage and increased intracellular calcium levels {Xu, 2020, 6316207}. One study in the Chang liver cell line suggested that apoptosis following exposure to PFOS may be caused by endoplasmic reticulum stress, mediated by the phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) {Oh, 2017, 3981364}. A study in human L-02 cells suggested that PFOS exposure may lead to apoptosis through the activation of p53 and myc proto-oncogene (myc) pathways {Huang, 2014, 2851292}. In two studies in HepG2 cells, PFOS exposure led to increases in apoptosis and alterations in autophagy, leading the authors to conclude that hepatotoxicity induced by PFOS exposure may be at least partially attributed to autophagy-dependent apoptosis {Yao, 2014, 2850398; Yao, 2016, 3981442}.

No *in vitro* study directly evaluated cellular necrosis, although one RNA-sequencing study in primary human hepatocytes found that PFOS exposure was associated with changes in gene expression that aligned with cell death and hepatic system disease, including necrosis, cholestasis, liver failure, and cancer {Beggs, 2016, 3981474}. Another RNA-sequencing study showed that PFOS induced genetic changes in WT zebrafish that were comparable to those seen in a zebrafish model of fatty liver disease; pathways involved in apoptosis of hepatocytes and focal necrosis of liver were upregulated {Fai Tse, 2016, 3981456}.

3.4.1.3.5.3 Cell Cycle and Proliferation

3.4.1.3.5.3.1 *In Vivo* Models

Alterations in cell proliferation and the cell cycle were also seen in many *in vivo* and *in vitro* studies {Thomford, 2002, 5029075; Elcombe, 2012, 1332473; Elcombe, 2012, 1401466; Han,

2018, 4355066; Huck, 2018, 5079648; Lai, 2017, 3981375; Beggs, 2016, 3981474; Cui, 2015, 3981568; Song, 2016, 9959776; Louisse, 2020, 6833626; Cui, 2015, 3981517}. Two short-term studies in male rats with PFOS doses of 20 or 100 ppm (approximately 2 and 10 mg/kg/day, respectively) found increased proliferation in the liver, as seen through increased BrdU staining, which was accompanied by increased liver weights {Elcombe, 2012, 1332473; Elcombe, 2012, 1401466}. In a third study in male rats dosed with 1 or 10 mg/kg/day PFOS for 28 days, proliferation in the liver was also observed, via an increase in the percentage of cells staining for proliferating cell nuclear antigen (PCNA) and expression of proliferation-related proteins (PCNA, c-JUN, c-MYC, and CCND1) {Han, 2018, 4355066}. Increased liver weight at 10 mg/kg/day was also observed. These results in short-term studies are in contrast to one chronic dietary study in male and female rats which did not identify significant increases in cell proliferation (as determined with PCNA or BrdU immunohistochemistry) after 4, 14, or 52 weeks of dietary PFOS administration {Thomford, 2002, 5029075}. However, the study authors noted that a biologically significant and test-compound related mild increase in proliferation was observed at week 4 in two out of five females in both of the highest dose groups. The biological significance was defined as having twice the mean of the controls and being greater than that of the highest control. Notably, this study did not use concentrations of PFOS greater than approximately 1 mg/kg/day.

Similarly, in mice exposed to 10 mg/kg/day PFOS for 7 days, proliferation in the liver, as seen through PCNA staining, was increased {Beggs, 2016, 3981474}; increased relative liver weights were also observed. However, no changes in PCNA positive cells or PCNA protein expression was observed in a second study in mice exposed to 1 mg/kg PFOS in their diet for 6 weeks {Huck, 2018, 5079648}. Using RNAseq, one study examined the fetal livers of mice exposed gestationally to 0.3 mg/kg/day PFOS and showed a positive association between PFOS exposure and pathways involved in the alteration of liver cell and hepatocyte proliferation {Lai, 2017, 3981375}.

3.4.1.3.5.3.2 *In Vitro* Models

In one study in primary rat hepatocytes, increased proliferation, as seen by an increased percentage of EdU-positive cells, was observed with PFOS exposures of 50 µg/mL for 24 hours {Han, 2018, 4355066}. A study in human HL-7702 cells found increased proliferation with 50–200 µM PFOS exposures for 48 or 96 hours using the MTT assay; they also reported an association between PFOS exposure and proteomic changes that correlated with increased proliferation {Cui, 2015, 3981568}. This same study found that approximately half of the proteins changed with PFOS exposure were involved in the cell cycle. Using flow cytometry, Cui et al. (2015, 3981568) further found that in HL-7702 cells, 50–200 µM PFOS for 48 or 96 hours decreased the percentage of cells at the G1/G0 (non-dividing) phases of the cell cycle while increasing the percentage of cells at the S phase (DNA synthesis); the percentage of cells at G2/M phase (interphase growth/mitosis) was increased at the 100 µM exposure after 48 hours of exposure but was decreased at the 200 µM exposure after 48 and 96 hours. Another study in a zebrafish liver cell line (ZFL) also used flow cytometry to examine changes in the cell cycle after PFOS exposure {Cui, 2015, 3981517}. In corroboration with the study in HL-7702 cells, PFOS concentrations of 27.9 and 56.8 µg/mL for 48 hours were shown to decrease the percentage of cells at the G1/G0 phases while increasing the percentage of cells at G2/M and S phases. In addition, two microarray studies in hepatic cell lines found that PFOS exposures ranging from

100-278 µM for 24 or 48 hours were associated with pathways involved in the regulation of cellular proliferation or the cell cycle {Song, 2016, 9959776; Louisse, 2020, 6833626}.

Several *in vitro* and *in vivo* studies mention pathways through which PFOS may be inducing proliferation. The RNAseq study of fetal livers of mice exposed gestationally to 0.3 mg/kg/day PFOS described above suggested that proliferation may be induced by PFOS activating RAC and Wnt/β-catenin signaling pathways {Lai, 2017, 3981375}. Additionally, in two studies, PFOS has been shown to decrease the expression of HNF4α {Behr, 2020, 6505973; Beggs, 2016, 3981474}, a regulator of hepatic differentiation and quiescence that has been suggested as a mediator of steatosis following PFOS exposure {Armstrong, 2019, 6956799}. In one study by Beggs et al. (2016, 3981474) (as described in Section 3.4.1.3.1.3), the authors concluded that PFOS may be causing cellular proliferation by down-regulating positive targets of HNF4α, including differentiation genes, and by inducing the expression of negative targets of HNF4α, including pro-mitogenic genes such as CCND1 and protein levels of stem cell markers such as NANOG, leading to hepatocyte de-differentiation.

3.4.1.3.5.4 Conclusions

Although some results were conflicting, there is generally strong evidence that PFOS exposure can disrupt the balance between cell proliferation and cell death/apoptosis. Out of the multitude of studies examining cell proliferation both *in vivo* and *in vitro*, only a single *in vivo* study showed that PFOS did not alter hepatic cellular proliferation, with increased cell proliferation observed in all other studies. Although most *in vitro* studies suggested that PFOS could induce apoptosis, several *in vivo* studies showed that PFOS either did not alter or decreased apoptosis.

Disruption in cell cycle and the reduction of HNF4α were the most frequently cited mechanisms of proliferation induced by PFOS. This increase in proliferation in the liver could be linked to increased liver weights, steatosis, and cancer. Similarly, many pathways were implicated in PFOS-mediated apoptosis, including mitochondrial dysfunction, endoplasmic reticulum stress, and alterations in autophagy.

3.4.1.3.6 Inflammation and Immune Response

The liver is an important buffer between the digestive system and systemic circulation and is thus exposed to compounds that are potentially immunogenic that result in protective immune and inflammatory responses. Kupffer cells constitute the majority of the liver-resident macrophages and make up one third of the non-parenchymal cells in the liver. Kupffer cells phagocytose particles, dead erythrocytes, and other cells from the liver sinusoids and play a key role in preventing immunoreactive substances from portal circulation from entering systemic circulation {Dixon, 2013, 10365841}. While Kupffer cells can be protective in drug- and toxin-induced liver toxicity, dysregulation of Kupffer cell-mediated inflammatory responses is associated with a range of liver diseases, including steatosis. Other liver-resident immune cells include natural killer (NK) cells, invariant NKT cells, mucosal associated invariant T (MAIT) cells, γδT cells, and memory CD8+T cells {Wang et al., 2019, 10365737}. The non-immune cells of the liver, liver sinusoidal endothelial cells (LSECs), hepatocytes, and stellate cells, also participate in immunity. They can express pattern recognition receptors and present antigens to T cells {Robinson, 2016, 10284350}. However, the impact of PFOS on the immune function of these cell types has not been thoroughly investigated.

3.4.1.3.6.1 *In Vivo* and *In Vitro* Models

Investigations into the liver immune response has been reported in an epidemiological study in the C8 Health Project cohort {Bassler, 2019, 5080624}, rat models {Han, 2018, 4355066; Han, 2018, 4238554}, mouse models {Lai, 2017, 3981375; Su, 2019, 5080481}, and *in vitro* models {Han, 2018, 4355066; Song, 2016, 9959776}. Bassler et al. (2019, 5080624) collected 200 serum samples from participants of the C8 Health Project to analyze mechanistic biomarkers of non-alcoholic fatty liver disease (NAFLD) and test the hypothesis that PFAS exposures are associated with increased hepatocyte apoptosis and decreased pro-inflammatory cytokines. PFOS levels were significantly correlated with decreases in serum levels of two pro-inflammatory cytokines, tumor necrosis factor α (TNFα) and IL-8. The authors state that these results are consistent with other findings that PFAS are immunotoxic and downregulate some aspects of the immune responses, but paradoxically result in increased apoptosis, which may subsequently result in progression of liver diseases including NAFLD.

In 6-week-old male Sprague Dawley rats gavaged with 0, 1, or 10 mg/kg/day PFOS for 28 days, changes in immune related end points in the liver were measured through western blot, qRT-PCR, histopathology, and ELISA {Han, 2018, 4355066; Han, 2018, 4238554}. In contrast to the C8 Panel study in humans {Bassler, 2019, 5080624}, the authors reported dose-dependent increases in both serum TNFα and hepatic *Tnfα* mRNA levels, indicating an increased pro-inflammatory response to PFOS exposure. Likewise, in a histopathological analysis of the liver of these PFOS-exposed animals, the authors noted intense inflammatory infiltrates in the periportal area and an increase in inflammatory foci. Han et al. (2018, 4355066) also reported increased TNFα in the free supernatant and *Tnfα* mRNA in primary Kupffer cells treated with 100 µM PFOS for up to 48 hours. These increases were not linear over time; supernatant levels and hepatic mRNA levels appeared to peak at 24 hours and 1 hour, respectively. Altered supernatant TNFα concentrations were not observed in similarly treated primary hepatocytes. Similar effects were also reported by Han et al. (2018, 4355066) for interleukin-6 (IL-6), which is a contributor to inflammatory responses in cells. Dose-dependent increases in IL-6 levels were observed in rat serum and increases in Il-6 mRNA were observed in rat liver tissue after the 28-day *in vivo* exposure. The authors also reported increased IL-6 free supernatant concentrations and mRNA levels in primary Kupffer cells treated with 100 µM PFOS for up to 48 hours. In the primary Kupffer cells, supernatant IL-6 levels and mRNA levels peaked at 1 and 6 hours of treatment, respectively. No changes in IL-6 concentrations were observed in supernatant from primary hepatocytes treated with 100 µM PFOS for up to 48 hours. In activation/inhibition assays targeting the c-JUN amino-terminal kinase (JNK), IκB, and nuclear factor-κB (NF-κB) signaling pathways in Kupffer cells (all of which are associated with cellular stress and/or immune/inflammatory responses) PFOS exposure induced JNK and IκB phosphorylation and NF-κB activity. Han et al. (2018, 4355066) further reported partial mediation of the TNF-α and IL-6 response in Kupffer cells co-treated with PFOS and either a NF-κB or JNK inhibitor, indicating that these two pathways are at least partially responsible for hepatic inflammatory responses to PFOS. In addition to cytokine levels, Han et al. (2018, 4355066) used the F4/80 antibody as a macrophage marker and found dose-dependent increases in F4/80+ cells of the livers of rats treated with either 1 or 10 mg/kg/day PFOS for 28 days. The authors suggest that the increase in hepatic macrophages may be a result of Kupffer cell activation.

In mice, the observed changes were similar to the rat data in that inflammatory markers and pathways were upregulated with PFOS exposure. In one study conducted in male ICR mice,

TNFα and IL-6 were significantly increased in serum of mice treated with 10 mg/kg/day PFOS for 21 days {Su, 2019, 5080481}. The authors also observed increased TNFα positive liver cells. In prenatally exposed CD-1 mouse offspring whose dams were treated with 0 or 0.3 mg/kg/day PFOS the day after mating until embryonic day 18.5, there was an upregulation of inflammatory pathways in the PFOS exposed fetuses {Lai, 2017, 3981375}. Using IPA, the authors identified numerous inflammatory genes that were upregulated in the fetal liver tissue. KEGG pathway analysis highlighted the deregulation of adipocytokines, pro-inflammatory cytokines produced by adipocytes, and TGFβ signaling. Interestingly, activation of TGFβ is associated with anti-inflammatory responses, immunosuppression, and tumor promoting pathways.

In another study investigating the hepatic effects of PFOS *in vitro*, Song et al. (2016, 9959776) saw much of the same effects using human liver hepatocellular carcinoma line, HepG2. After exposing these cells to 278 µM PFOS (the IC_{20} dose) for 48 hours, through KEGG pathway analyses, the authors reported that genes related to immune response were the fifth most differentially expressed biological process out of the 189 processes with altered genetic profiles. Within the immune response, 17 genes were differentially expressed, including those related to the TNF signaling pathway, as well as genes involved in the KEGG pathways of nucleotide-binding and oligomerization domain (NOD)-like receptor signaling, cytokine-cytokine receptor interactions, and the complement and coagulation cascade system.

3.4.1.3.6.2 Conclusions

While there are not many studies investigating the immunotoxicity of PFOS specifically related to the liver, evidence presented from various methods and biomarkers strongly indicate that PFOS can disrupt normal hepatic immunological function. However, the immune response to PFOS exposure in humans does not appear to be consistent with rodent and *in vitro* models. While a single study in the C8 Health Project cohort suggests that immunosuppression may be involved in the progression of NAFLD and potentially other types of liver disease, studies in rats, mice, primary hepatic (Kupffer) cells, and immortalized cell lines suggest that pro-inflammatory immune responses generally result from PFOS exposure. Specifically, there is evidence that activation through the JNK/NF-κB pathways may stimulate the production of pro-inflammatory cytokines such as TNFα and IL-6. Although further assessment of human populations and in human cell lines may be needed to understand the differences in responses between humans and laboratory models, both lines of evidence suggest PFOS exposure can alter the hepatic immune and inflammatory responses.

3.4.1.3.7 Oxidative Stress and Antioxidant Activity

3.4.1.3.7.1 Introduction

Oxidative stress, caused by an imbalance of reactive oxygen species (ROS) production and detoxification processes, is a key part of several pathways, including inflammation, apoptosis, mitochondrial function, and other cellular functions and responses. In the liver, oxidative stress contributes to the progression and damage associated with chronic diseases, such as alcoholic liver disease, non-alcoholic fatty liver disease, hepatic encephalopathy, and Hepatitis C viral infection {Cichoz-Lach, 2014, 2996796}. Indicators of oxidative stress include but are not limited to increased oxidative damage (e.g., malondialdehyde (MDA) formation); increased reactive oxygen species (ROS) production (e.g., hydrogen peroxide and superoxide anion); altered antioxidant enzyme levels or activity (e.g., superoxide dismutase (SOD) and catalase

(CAT) activity); changes in total antioxidant capacity (T-AOC); changes in antioxidant levels (e.g., glutathione [GSH] and glutathione disulfide [GSSG] ratios); and changes in gene or protein expression (e.g., nuclear factor-erythroid factor 2-related factor 2 [Nrf2] protein levels). PFOS has been demonstrated to induce these indicators of oxidative stress, inflammation, and cell damage.

3.4.1.3.7.2 *In Vivo* Models

Several studies in rats and mice assessed hepatic oxidative stress in response to PFOS exposure. In male Sprague-Dawley rats, a positive association between markers of oxidative stress, potentially due to decreased antioxidant capacity, and oral PFOS exposure (1 or 10 mg/kg/day of for 28 days) was reported {Wan, 2016, 3981504; Han, 2018, 4238554}. In hepatocytes extracted from dosed rats, Wan et al. (2016, 3981504) found decreased Nrf2 total protein levels and decreased activated Nrf2 in the nuclei at 10 mg/kg/day PFOS. Nrf2 is known for its role as a regulator of antioxidant response elements and is generally activated upon oxidant exposure. Additionally, liver lysates from rats at the highest PFOS dose showed decreases in expression of both heme oxygenase-1 (*Hmox1*) and NAD(P)H quinone dehydrogenase 1 (*Nqo1*) genes, both of which are associated with antioxidant, anti-inflammatory, and/or stress responses, revealing an inhibition of the Nrf2 signaling pathway following PFOS exposure. Results from Han et al. (2018, 4238554) also provide evidence of increased hepatic oxidative stress following PFOS exposure. PFOS-exposed rats had significant dose-dependent increases in ROS, as measured by the 2,7-dichlorofluorescein diacetate (DCFDA) fluorescent probe, and significant increases in hepatic inducible nitric oxide synthase (*iNos*) and *Cyp2e1* mRNA expression, key producers of oxidants in the cell. MDA levels, an indicator of lipid peroxidation, were also significantly increased at both 1 and 10 mg/kg/day. Simultaneously, significant decreases were observed in CAT and SOD activities in liver tissues. Antioxidants typically responsible for returning cells to their homeostatic state were altered in the liver following PFOS exposure, including decreases in GSH levels, increases in GSSG levels, and a decrease in the GSH/GSSG ratio. A decrease in this ratio generally indicates an imbalance of the oxidation-reduction (redox) state of the cell.

Four additional studies examined indicators of oxidative stress in male mice {Rosen, 2010, 1274165; Liu, 2009, 757877; Xing, 2016, 3981506; Lv, 2018, 5080395}. Rosen et al. (2010, 1274165) found exposure to PFOS in mice downregulated genes associated with oxidative phosphorylation. In their assessment of Kunming (KM) mice that were administered PFOS via subcutaneous injection, Liu et al. (2009, 757877) found evidence of oxidative damage that included decreased SOD activity in the male brain and female liver and decreased T-AOC in male and female livers. Overall, oxidative damage was observed in younger offspring and was slightly more evident among males. In a subchronic exposure study, evidence of increased oxidative stress was observed among male C57BL/6 mice dosed once with 0, 2.5, 5, or 10 mg/kg/day PFOS via oral gavage for 30 days {Xing, 2016, 3981506}. Dose-dependent reductions were observed for levels of the antioxidant enzymes SOD, CAT, and glutathione peroxidase (GSH-Px) in the liver; the T-AOC (i.e., free radical scavenging capacity) was also reduced in hepatic tissues, with the lowest capacity observed at the highest dose. Lipid peroxidation reported as MDA levels were significantly increased in hepatic tissues of rats exposed to PFOS. The highest MDA levels were observed in the highest dose group. Results from the Lv et al. (2018, 5080395) subchronic exposure study also showed evidence of increased oxidative stress and decreased mechanisms of defense against oxidative stress following PFOS exposure {Lv, 2018, 5080395}. In an unspecified species of male mice, intragastric

administration of 10 mg/kg/day PFOS for three weeks resulted in significant increases in MDA and hydrogen peroxide production and significant decreases in SOD activity and GSH levels in the liver. Nrf2 protein expression was significantly decreased following PFOS exposure compared to unexposed controls. Additionally, transcriptional levels of *Sod*, *Cat*, and *Ho-1* mRNA were significantly decreased in the liver.

One gene expression compendium study aimed to examine the relationship between activation of xenobiotic receptors, Nrf2, and oxidative stress by comparing the microarray profiles in mouse livers (strain and species not specified) {Rooney, 2019, 6988236}. The study authors compiled gene expression data from 163 chemical exposures found within Illumina's BaseSpace Correlation Engine. Gene expression data for PFOS exposure was obtained from a previously published paper by Rosen, et al., (2010, 1274165). In WT (129S1/SvlmJ) male mice, Nrf2 activation was observed (as seen by increases in gene expression biomarkers) after a 7-day exposure to 10 mg/kg/day PFOS via gavage. In *Pparα*-null mice, this activation was observed at both the 3 and 10 mg/kg/day doses. CAR was similarly activated in these two strains of mice. The authors proposed that CAR activation by chemical exposure (PFOS or otherwise) leads to Nrf2 activation and that oxidative stress may be a mediator.

3.4.1.3.7.3 *In Vitro* Models

Several studies examined oxidative stress endpoints in hepatic primary cells {Khansari, 2017, 3981272; Rosen, 2013, 2919147; Xu, 2019, 5381556; Xu, 2020, 6316207}. Khansari et al. (2017, 3981272) dosed rat hepatocytes with 25 µM PFOS for three hours and demonstrated significantly increased production of ROS, measured with the DCFDA probe, and lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS) content, compared to controls. Additionally, PFOS treatment resulted in increased damage of lysosomal membranes, likely caused by lipid peroxidation and increased levels of ROS. The authors also noted that PFOS treatment resulted in mitochondrial membrane potential collapse; disruptions in mitochondrial membrane potential in itself may result in increased ROS production, which could then create a positive feedback loop of further mitochondrial dysfunction and increased ROS. The authors suggest that these results demonstrate a potential oxidative stress-related mechanism underlying PFOS hepatoxicity.

Rosen et al. (2013, 2919147) assessed oxidative stress-related gene expression changes using TaqMan low density arrays (TLDA) in both mouse and human primary hepatocytes exposed to PFOS ranging from 0-250 µM. PFOS exposure led to increases in the expression of the nitric oxide synthase 2 (*Nos2* or *iNos*) and *Hmox1* genes in mouse primary hepatocytes. In human primary hepatocytes exposed to 100 µM PFOS, *NOS2* expression decreased while *HMOX1* expression increased.

Xu et al. (2019, 5381556) exposed primary hepatocytes from C57Bl/6J male mice to 10, 100, 500, or 1,000 µM PFOS for 24 hours. ROS levels, measured by a CM-H2DCFA fluorescent probe, were significantly increased in cells exposed to the highest level of PFOS. Interestingly, SOD activity was significantly increased in cells exposed to 500 and 1,000 µM PFOS, up to 117% with 1,000 µM, while CAT activity was reduced by 59% in cells at the highest dose level. PFOS exposure also led to alterations in the structure of SOD, with PFOS exposure resulting in an increased percentage of α-helix structures (26.9%) and a decreased percentage of β-sheet structures (21.9%), providing evidence of polypeptide chain shortening. These structural changes

suggest that PFOS interacts directly with SOD. Alterations in the resonance light scattering (RLS) measures further revealed the impact of PFOS exposure on SOD protein structures in that protein aggregations were observed at low doses of PFOS, but the aggregations were destroyed at higher doses of PFOS, leading to increased SOD activity. The authors suggest that this may result from agglomerate dispersion following the destruction of the solvent shell on the surface of SOD at high doses of PFOS or from protein collapse following PFOS binding. Additionally, GSH content was increased by 199% in cells exposed to the highest dose level; the authors suggest that increases in GSH may reflect cellular adaptations to oxidative stress and can lead to detoxification of oxidized GSSG to GSH.

In a third study using primary mouse hepatocytes, Xu et al. (2020, 6316207) exposed cultured cells to 10, 100, 500, or 1,000 μM of PFOS for 24 hours to examine oxidative stress related cell apoptosis. The authors examined the impact of PFOS exposure on endogenous levels of lysozyme (LYZ), an enzyme that inhibits oxidative stress-induced damage, and demonstrated that PFOS exposure impacted LYZ molecular structure, subsequently decreasing activity levels, leading to oxidative stress-induced apoptosis. Decreases in peak intensity at 206 nm during ultraviolet-visible (UV-vis) absorption spectrometry represented an unfolding of the LYZ molecule following exposure to PFOS, which inhibited enzyme activity. At exposure levels of 100 μM and above, LYZ enzyme activity decreased to 761% of control levels. Such an impact on LYZ activity was deemed to be related to the high affinity of PFOS for key central binding sites on the LYZ molecule.

Four additional studies examined oxidative stress endpoints following PFOS exposure in HepG2 cell lines {Wan, 2016, 3981504; Wielsøe, 2015, 2533367; Shan, 2013, 2850950; Florentin, 2011, 2919235}. Two studies reported increases in ROS levels following PFOS exposure {Wan, 2016, 3981504; Wielsøe, 2015, 2533367}, while two studies did not observe statistical differences in ROS levels following 1- or 24-hour PFOS exposures up to 400 μM {Florentin, 2011, 2919235} or following 3-hour PFOS exposures up to 400 μM {Shan, 2013, 2850950}. Wan et al. (2016, 3981504) dosed HepG2 cells with either 0, 10, 20, 30, 40, or 50 μM PFOS for 24 hours or with 50 μM PFOS for 1, 3, 6, 12, or 24 hours. ROS generation, analyzed using DCFH-DA, was increased in a dose-dependent manner in cells dosed with 50 μM across multiple time points, with a peak in levels observed at 12 hours of exposure and a decrease in levels at 24 hours of exposure; ROS production was significantly increased compared to control levels at 24 hours. Significant decreases were observed in GSH and protein expression of total-Nrf2, HO-1, and NQO-1 in a dose- and time-dependent manner. Expression of *miR-155*, a microRNA suspected to play a key role in oxidative stress via the Nrf2 antioxidant pathway, increased nearly 12-fold following 24-hour 50 μM PFOS exposure. When cells were pre-treated with CAT prior to PFOS exposure, ROS production was decreased along with *miR-155* expression. SOD pre-treatment did not lead to significant effects. Wan et al. (2016, 3981504) concluded that *miR-155* plays a key role in the inhibition of the Nrf2 signaling pathway and can be upregulated with PFOS exposure.

Wielsøe et al. (2015, 2533367) incubated HepG2 cells with up to 200μM PFOS to detect changes in ROS, T-AOC, and DNA damage. PFOS exposure significantly increased ROS production, as measured with the carboxy-H2DCFDA probe, as well as DNA damage, as indicated by increased mean percent tail intensity in a comet assay, which is an indicator of DNA strand breaks. Shan et al., 2013 exposed HepG2 cells to 100, 200, 300, or 400 μM PFOS for 3

hours and found an increase in ROS generation with only 100 μM PFOS, though the effect was not statistically significant. Additionally, no changes were observed in the GSH/GSSG ratio.

3.4.1.3.7.4 Conclusions

Results from new studies published since the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} further support the conclusions that implicate PFOS in inducing oxidative stress leading to hepatocytic damage. Evidence of increased oxidative stress in the liver, including increased ROS levels, changes in GSH and GSSG levels, and decreases in T-AOC, were observed following both *in vivo* and *in vitro* exposures to PFOS. PFOS exposure was also associated with increased levels of markers of oxidative damage and decreased activity or levels of protective antioxidants that play a role in the reduction of oxidative damage. Interestingly, PFOS exposure appeared to result in inhibition of the Nrf2 signaling pathway, with evidence of decreased Nrf2 protein levels and reductions of the expression and activity of genes and proteins downstream of this transcription factor. There was also evidence that PFOS can disrupt the structure and subsequent function of crucial enzymes that mitigate ROS production and oxidative damage, SOD and LYZ. While further research is needed to fully understand the mechanisms by which PFOS disrupts oxidative stress responses, it is clear that PFOS induces oxidative stress in hepatic tissues.

3.4.1.4 Evidence Integration

There is *moderate* evidence for an association between PFOS exposure and hepatic effects in humans based on associations with liver biomarkers, especially ALT, in several *medium* confidence studies. Consistent with the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}, the epidemiological data provide consistent evidence of a positive association between PFOS exposure and ALT in adults. However, the associations were not large in magnitude, and it is unclear whether the observed changes are clinically adverse. Evidence for other liver enzymes and in children and adolescents is less consistent. Results for functional measures of liver toxicity, specifically histology results, are mixed. There is some indication of higher risk of liver disease with higher exposure, coherent with the liver enzyme findings, but there is inconsistency for lobular inflammation among the two available studies, which decreases certainty. Among the studies of ALT in adults, two presented correlations across PFAS {Nian, 2019, 5080307; Salihovic, 2018, 5083555}; PFOA and PFOS were moderately correlated in both studies ($r = 0.4–0.5$). Jin et al. (2020, 6315720), which reported positive associations with histology, reported fairly low correlations between PFOS/PFOA ($r = 0.14$), which reduces the concern for confounding in that population. It is not possible to rule out potential confounding across PFAS with this evidence, but there is also no evidence that confounding can explain the observed associations.

In summary, across studies in the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and this updated systematic review, there is generally consistent evidence of a positive association between exposure to PFOS and ALT. However, one source of uncertainty in epidemiology studies of PFAS is confounding across the PFAS as individuals are exposed to a mixture of PFAS and it is difficult to disentangle the effects. This cannot be ruled out in this body of evidence given the attenuation of the association in Lin et al. (2010, 1291111), the only general population study that performed multi-pollutant modeling. The positive associations with ALT are also supported by the recent meta-analysis of 25 studies in adolescents and adults {Costello, 2022, 10285082}. Associations for other hepatic outcomes were less consistent, including for

functional outcomes such as liver disease. Thus, while there is evidence of an association between PFOS and ALT, there is residual uncertainty.

The animal evidence for an association between PFOS exposure and hepatic toxicity is *robust* based on 20 *high* or *medium* confidence studies that show hepatic alterations. However, it is important to distinguish between alterations that may be non-adverse (e.g., hepatocellular hypertrophy alone) and those that indicate functional impairment or lesions {U.S. EPA, 2002, 625713; FDA, 2009, 6987952; EMEA, 2010, 3056796; Hall, 2012, 2718645}. EPA considers responses such as increased relative liver weight and hepatocellular hypertrophy adverse when accompanied by hepatotoxic effects such as necrosis, inflammation, or biologically significant increases in enzymes indicative of liver toxicity {U.S. EPA, 2002, 625713}.

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

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

Mechanistic data can contribute to the understanding toxicity in the context of relevance of data collected from laboratory models in relation to observed human effects and the application of such data in human hazard. There are several studies that have proposed potential underlying mechanisms of the hepatotoxicity observed in rodents exposed to PFOS, some of which have also been tested in human cells *in vitro*. Mechanistic evidence supports a role of nuclear receptors, including the activation of PPARα and CAR and a decrease in HNF4α, in PFOS-induced hepatotoxicity based on data collected *in vivo* in rodents and *in vitro* in both human and rodent models. Findings support a role of these nuclear receptors in steatosis and hepatomegaly observed in rodents in laboratory studies. However, it should be noted that although substantial evidence exists demonstrating expression changes in gene targets of the nuclear receptors PPARα, conflicting results have been reported for activation of the PPARα signaling pathway *in vitro* between human and rodent cells, as well as across studies in different cells/cells lines from the same species. Nonetheless, cells transfected with human PPARα demonstrated that PFOS can increase PPAR activation. Gene expression signatures for CAR and PPAR activation has been observed in mice exposed to PFOS, with CAR activation generally more significant in *PPARα*-null mice, leading authors to conclude that CAR likely plays a subsequent role to PPARα in mediating the adverse hepatic effects of PFOS. PPARα and CAR are known to play important roles in liver homeostasis and have been implicated in liver dysfunction, including steatosis.

Therefore, PFOS exposure may lead to liver toxicity through the activation of multiple nuclear receptors in both rodents and humans.

HNF4α appears to play an important role in hepatotoxic effects related to PFOS exposure. PFOS exposure led to a decrease in the protein expression of HNF4α in mice, which was associated with an increase in relative liver weight. The *in vivo* alterations to HNF4α have been confirmed by *in vitro* studies conducted in primary human hepatocytes and HepaRG cells, in which HNF4α protein and gene expression was decreased. Importantly, increased cell proliferation in the liver is related to reduction in HNF4α, both of which are reported effects of PFOS.

Regarding the cytotoxic potential of PFOS, results from *in vitro* exposure of both human and rodent cells are variable and inconsistent in the concentrations at which PFOS causes cytotoxicity, as well as whether or not PFOS is cytotoxic at any concentration tested *in vitro*. Some studies evaluated mechanisms of the cell death, such as induction of apoptotic pathways, with inconsistent results. *In vivo*, increases and decreases in apoptosis was observed in the livers of mice, with variations related to duration of exposure, type of exposure (dietary or gavage), and whether or not a recovery period was included in the study design. Oxidative stress, alterations to p53 signaling, and mitochondrial damage have been reported *in vivo* in rodent studies as well as *in vitro* in rodent cells; however, additional research is necessary to fully characterize the involvement of such events in alterations to apoptotic signaling. While necrosis was not directly evaluated, two transcriptomic analyses (one in primary human hepatocytes and one in zebrafish) reported that PFOS induced changes in the expression of genes involved in liver necrosis and damage. Increased hepatic cell proliferation has been more consistently reported in *in vivo* and *in vitro* models, and is associated with increased liver weights and steatosis, which have also been observed in rodents exposed to PFOS.

Inflammation and immunomodulation have also been reported in relation to PFOS, and molecular-level alterations in inflammatory and immune response pathways can be linked to inflammation observed in the livers of rodents exposed to PFOS. In rats, PFOS resulted in increased serum TNFα and hepatic *Tnfα* gene expression, indicating an increased pro-inflammatory response, which was accompanied by intense inflammatory infiltrates in the periportal area and an increase in inflammatory foci. Decreased serum TNFα has been observed in humans in relation to PFOS exposure, indicating that alterations to TNFα may have species differences and/or be dependent upon exposure duration and dose. Alterations to inflammatory response pathway genes have been reported in human cells *in vitro* (HepG2 cells), supporting the observation in rodents that PFOS exposure leads to inflammatory response. Although further assessment of human populations and human cell lines is needed to clarify the ability of PFOS to induce inflammatory and immune responses in humans, the currently available evidence suggest PFOS exposure can alter the hepatic immune and inflammatory responses.

3.4.1.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, ***evidence indicates*** that PFOS exposure is likely to cause hepatotoxicity in humans under relevant exposure circumstances (Table 3-5). This conclusion is based primarily on coherent liver effects in animal models following exposure to doses as low as 0.02 mg/kg/day PFOS. The available mechanistic information overall provide support for the biological plausibility of the phenotypic effects observed in exposed animals as well as the activation of relevant molecular

and cellular pathways across human and animal models in support of the human relevance of the animal findings. In human studies, there is generally consistent evidence of a positive association with ALT, at median plasma PFOS levels as low as 0.57 ng/mL. Although a few associations between other liver serum biomarkers and PFOS exposure were identified in *medium* confidence epidemiological studies, there is considerable uncertainty in the results due to inconsistency across studies.

Table 3-5. Evidence Profile Table for PFOS Hepatic Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.1.1)					⊕⊕⊙ ***Evidence Indicates (likely)***
Serum biomarkers of hepatic injury 8 *Medium* confidence studies 3 *Low* confidence studies	In adults, significant increases in ALT were observed in *medium* confidence studies (3/5). Findings for AST and GGT were similar to ALT, indicating increased levels of these enzymes, however, some analyses stratified by sex or weight status (i.e., obesity) were less consistent. Findings for liver enzymes in children were mixed.	• *Medium* confidence studies • *Consistent direction* of effect for ALT • *Coherence* of findings between liver enzyme increases	• *Low* confidence studies • *Inconsistent direction* of effect in children.	⊕⊕⊙ *Moderate* Evidence for hepatic effects is based on increases in ALT in adults. Other supporting evidence includes increases in other liver enzymes such as AST and GGT and increased incidence of liver disease mortality in occupational settings. Minor uncertainties remain regarding mixed liver enzyme findings in children and coherence of liver enzyme and albumin findings.	*Primary basis and cross-stream coherence*: Human data indicated consistent evidence of hepatoxicity as noted by increased serum biomarkers of hepatic injury (primarily ALT) with coherent results for increased incidence of hepatic nonneoplastic lesions, increased liver weight, and elevated serum biomarkers of hepatic injury in animal models. Although a few associations between other serum biomarkers of hepatic injury and PFOS exposure were identified in *medium* confidence epidemiological studies, there is considerable uncertainty in the results due to inconsistency across studies. *Human relevance and other inferences:* The available mechanistic information overall provide
Liver disease or injury 3 *Medium* confidence studies	Findings for markers of liver inflammation were mixed. In adults, one study (1/2) reported decreased odds of lobular inflammation while another study (1/2) reported increased odds of lobular inflammation and non-alcoholic steatosis. Results from the only study in children were imprecise.	• *Medium* confidence studies	• *Limited number of studies* examining the outcome • *Imprecision* of findings		
Serum protein 2 *Medium* confidence studies 1 *Low* confidence study	Three studies in adults reported significantly increased albumin (3/3). For one study, significance varied by glomerular filtration rate	• *Medium* confidence studies • *Consistent* direction of	• *Low* confidence study • *Limited number of studies* examining the outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	status. No studies were conducted in children.	effect for albumin			support for the biological plausibility of the phenotypic effects observed in exposed animals as well as the activation of relevant molecular and cellular pathways across human and animal models in support of the human relevance of the animal findings.
Serum iron 1 *Medium* confidence study	Only one large cross-sectional study examined serum iron concentrations and reported a significant positive association.	• *Medium* confidence study	• *Limited number of studies* examining the outcome		
Evidence from *In Vivo* Animal Toxicological Studies (Section 3.4.1.2)					
Liver histopathology 2 *High* confidence studies 5 *Medium* confidence studies	Histopathological alterations in liver were reported in rodents or non-human primates exposed to PFOS for varying durations (6/7). Hepatocellular hypertrophy was most consistently (5/7) observed across sex, species, and duration of exposure and in a dose-responsive manner. Other observed lesions included: cystic or hepatocyte degeneration (2/7), focal or flake-like necrosis (2/7), steatosis (1/7), centrilobular or cytoplasmic vacuolation (6/7) and inflammatory cellular infiltration into liver tissue (4/7).	• *High* and *medium* confidence studies • *Consistent* direction of effects across study design, sex, and species • *Dose-dependent* response • *Coherence* of findings in other endpoints indicating liver damage (i.e., increased serum biomarkers and liver weight) • *Large magnitude* of effect, with some responses reaching 100% incidence in some dose	• No factors noted	⊕⊕⊕ *Robust* Evidence is based on 20 *high* or *medium* confidence animal toxicological studies indicating increased incidence of hepatic nonneoplastic lesions, increased liver weight, and elevated serum biomarkers of hepatic injury. 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. EPA considers responses such as increased relative liver weight and hepatocellular hypertrophy adverse when	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
		groups (i.e., hypertrophy) or are considered severe (i.e., cell or necrosis and cystic degeneration)		accompanied by hepatotoxic effects such as necrosis and inflammation. 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.	
Liver weight 2 *High* confidence studies 14 *Medium* confidence studies	Liver weights were increased in male and female mice, rats, and non-human primates at higher doses across a variety of study designs including developmental, short-term, subchronic, and chronic (11/14). Liver weight increases in pups exposed *in utero* were also observed (2/5).	• *High* and *medium* confidence studies • *Consistent direction* of effects across study design, sex, and species • *Coherence* of effects with other responses indicating increased liver size (e.g., hepatocellular hypertrophy)	• *Confounding variables* such as decreases in body weights		
Serum biomarkers of hepatic injury 3 *High* confidence studies 7 *Medium* confidence studies	ALT (7/7), AST (4/7), ALP (3/4), and GGT (1/1) levels were increased in male adult rodents. Measurements of ALT (1/5), AST (0/5), and ALP (1/2) in females found little evidence that PFOS exposure increased enzyme levels. Several studies found increased	• *High* and *medium* confidence studies • *Consistent* direction of effects across study design, sex, and species	• *Limited number of studies* examining outcome • *Inconsistent* direction of effects between sex		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	bilirubin (3/3), albumin (2/2), and albumin/globulin ratio (2/2) in male and female animals, with an increase in total protein in females only (1/2), occurring predominantly in high dose groups only. Increased concentrations of bile salts/acids were found in males (2/3) and females (1/2).	• *Dose-dependent* response • *Coherence* of findings with other responses indicating hepatobiliary damage (i.e., histopathological lesions) • *Large magnitude* of effect, with evidence of biologically significant increases (i.e., ≥100% control responses) in serum liver enzymes indicating adversity			

Mechanistic Evidence and Supplemental Information (Section 3.4.1.3)		
Biological Events or Pathways	**Summary of Key Findings, Interpretation, and Limitations**	**Evidence Stream Judgement**
Molecular initiating events – PPARα	**Key findings and interpretation:** Activation of PPARα *in vivo* in rodents and *in vitro* in human and rodent cells. Increased expression of PPARα-target genes *in vitro* in rat and human hepatocytes, and cells transfected with human PPARα. Altered expression of genes involved in lipid metabolism and lipid homeostasis. Gene expression changes related to lipid metabolism were observed in both wild type and PPARα-null mice. **Limitations:**	Overall, studies in rodent and human in vitro models and *in vivo* in rodent studies suggest that PFOS induces hepatic effects, at least in part, through PPARα. The evidence also suggests a role for PPARα-independent

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	Conflicting results have been reported for activation of the PPARα signaling pathway *in vitro* between human and rodent cells.			pathways in the MOA for noncancer liver effects of PFOS, particularly CAR activation and decreased expression of HNF4α.	
Molecular or cellular initiating events – other pathways	**Key findings and interpretation:** Activation of CAR *in vivo* in rodents and *in vitro* in both human and rodent models. Gene expression signatures for CAR activation observed in mice; more significant in *Pparα*-null mice than in wild type mice. Decrease in HNF4α protein expression, and changes in the expression of genes regulated by HNF4α *in vivo* in mice. Decrease in HNF4α gene and protein expression *in vitro* in human hepatocytes. Reduction in HNF4α is associated with increased cell proliferation, which was observed separately in PFOS-exposed animals. Upregulation of PPARγ, CAR/PXR, or LXR/RXR in mice. **Limitations:** Evidence is limited for some receptors, such as PPARγ and LXR/RXR.				

Notes: ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CAR = constitutive androstane receptor; GGT = gamma-glutamyl transpeptidase; HNF4α = hepatocyte nuclear factor 4-alpha; LXR = liver X receptor; PPARα = peroxisome proliferator-activated receptor alpha; MOA = mode of action; PPARγ = peroxisome proliferator-activated receptor gamma; PXR = pregnane X receptor; RXR = retinoid X receptor.

3.4.2 Immune

EPA identified 46 epidemiological and 13 animal toxicological studies that investigated the association between PFOS and immune effects. Of the epidemiological studies, 2 were classified as *high* confidence, 28 as *medium* confidence, 10 as *low* confidence, 5 as *mixed* (5 *medium/low*) confidence, and 1 was considered *uninformative* (Section 3.4.2.1). Of the animal toxicological studies, 1 was classified as *high* confidence, 9 as *medium* confidence, 1 as *low* confidence, and 2 were considered *mixed* (*high/low* and *medium/low*) (Section 3.4.2.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.2.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.2.1.1 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}.

There are 8 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and immune effects. Study quality evaluations for these 8 studies are shown in Figure 3-16.

In the 2016 PFOS HESD, there was consistent evidence of an association between PFOS exposure and immunosuppression in children. Two studies reported decreases in response to one or more vaccines in relation to higher exposure to PFOS in children {Grandjean, 2012, 1248827; Granum, 2013, 1937228}. In one study of adults, no association was observed {Looker, 2014, 2850913}. Antibody responses for diphtheria and tetanus in children (n = 587) were examined at multiple timepoints in a study on a Faroese birth cohort {Grandjean, 2012, 1248827}. Prenatal and age five serum PFOS concentrations were inversely associated with childhood diphtheria antibody response at all measured timepoints, and the association was significant for anti-diphtheria antibody concentrations pre-booster at age five and at age seven, modeled using prenatal and age five serum PFOS concentrations, respectively. The antibody response for tetanus was inversely associated with prenatal and age five serum PFOS concentrations but was only significant for the association between age five serum PFOS concentrations and post-booster anti-tetanus antibody concentrations. Prenatal PFOS exposure was associated with diminished vaccine response in a different birth cohort study {Granum, 2013, 1937228, MoBa}. Decreases in the anti-rubella antibody response were significantly associated with elevated prenatal PFOS concentrations among 3-year-old children. No association was observed for the only study {Looker, 2014, 2850913} in adults, examining influenza vaccine responses in a high-exposure community (C8 Health Project).

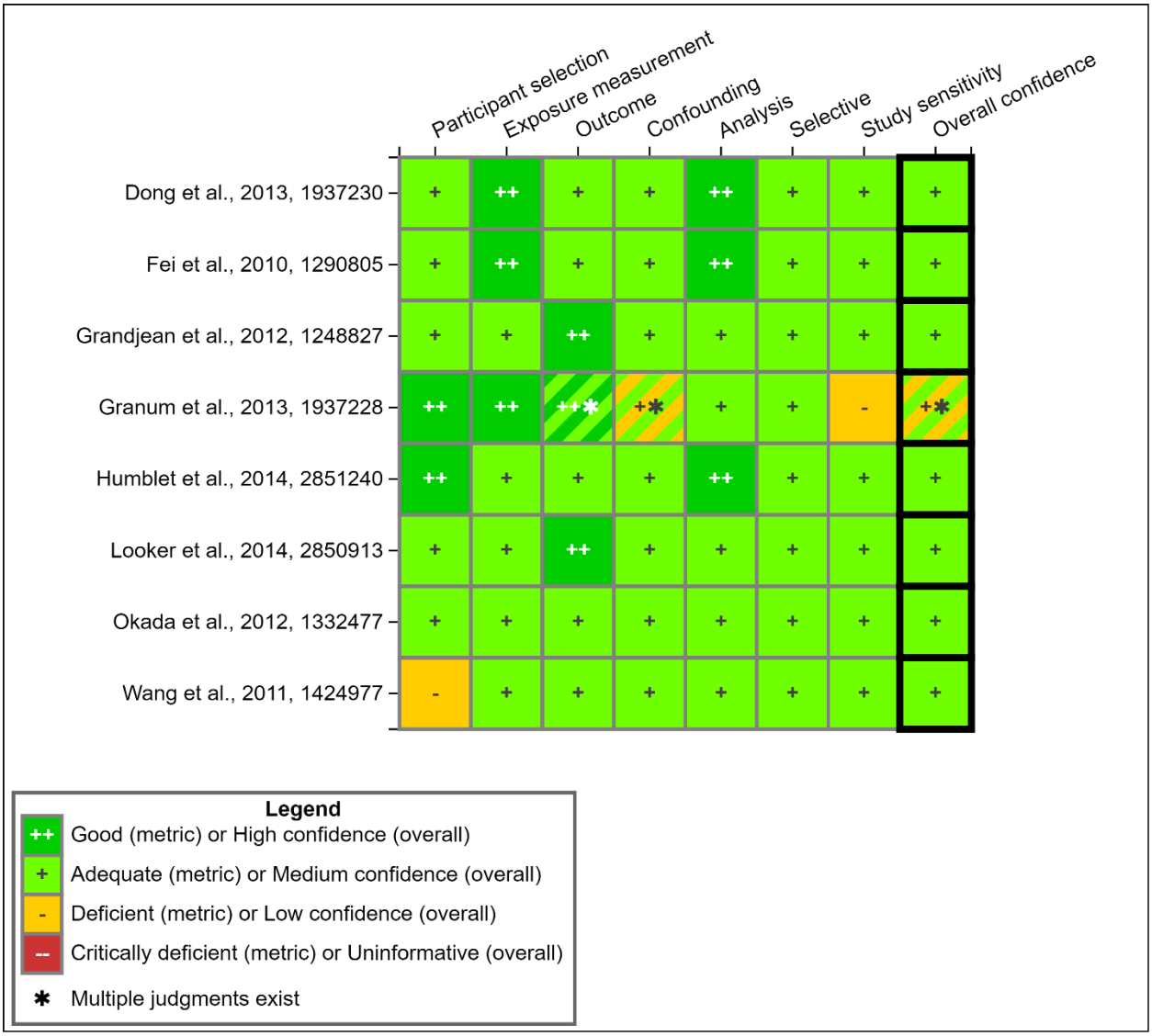


Figure 3-16. Summary of Study Evaluation for Pre-2016 Epidemiology Studies of PFOS and Immune Effects

Interactive figure and additional study details available on HAWC.

There are 27 new studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and immunosuppression effects. Study quality evaluations for these 27 studies are shown in Figure 3-17 and Figure 3-18. One study from the 2016 PFOS HESD {Grandjean, 2012, 1248827} was updated during this period, and the update was included in the systematic review {Grandjean, 2017, 3858518}.

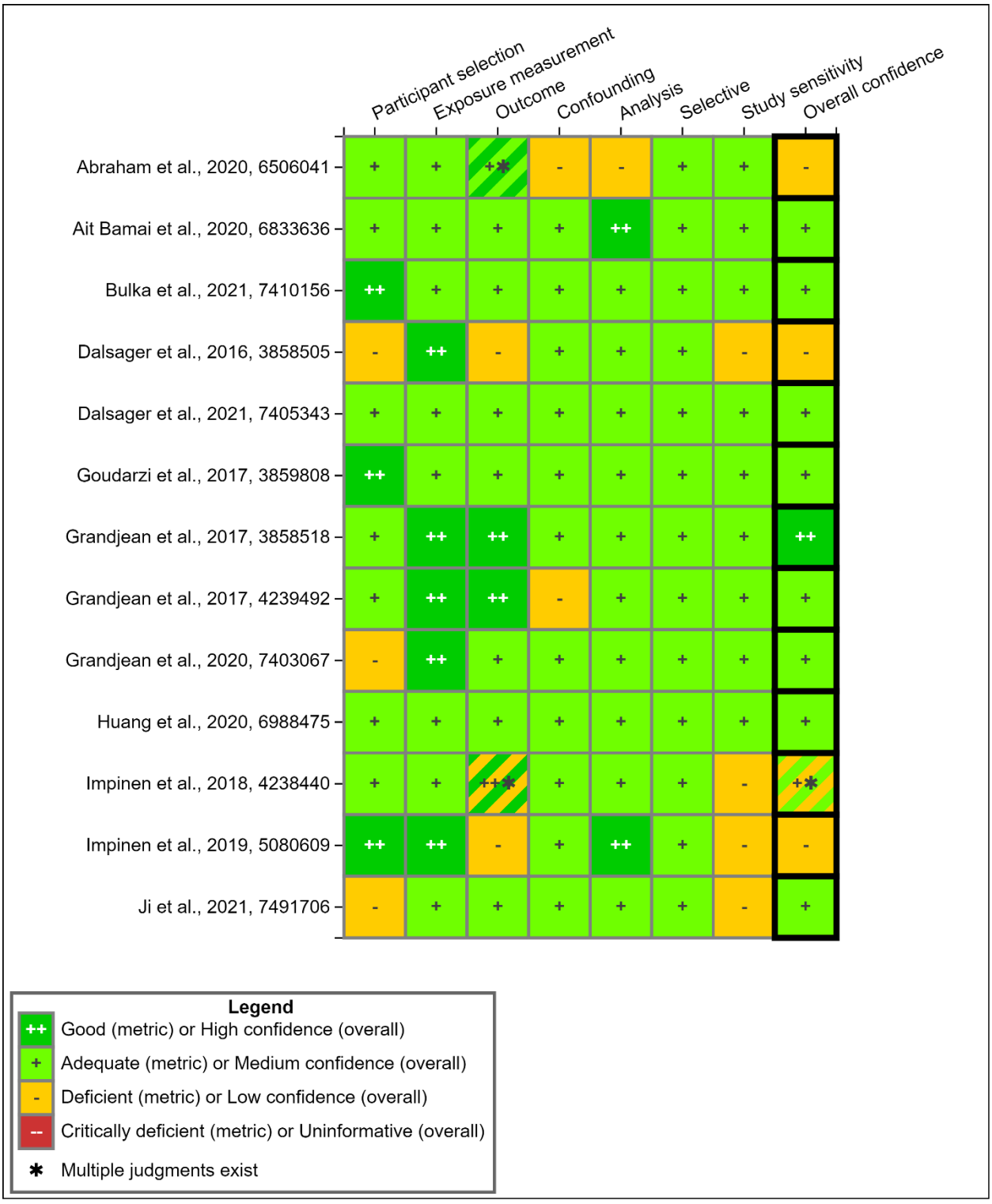


Figure 3-17. Summary of Study Evaluation for Epidemiology Studies of PFOS and Immunosuppression Effects

Interactive figure and additional study details available on HAWC.

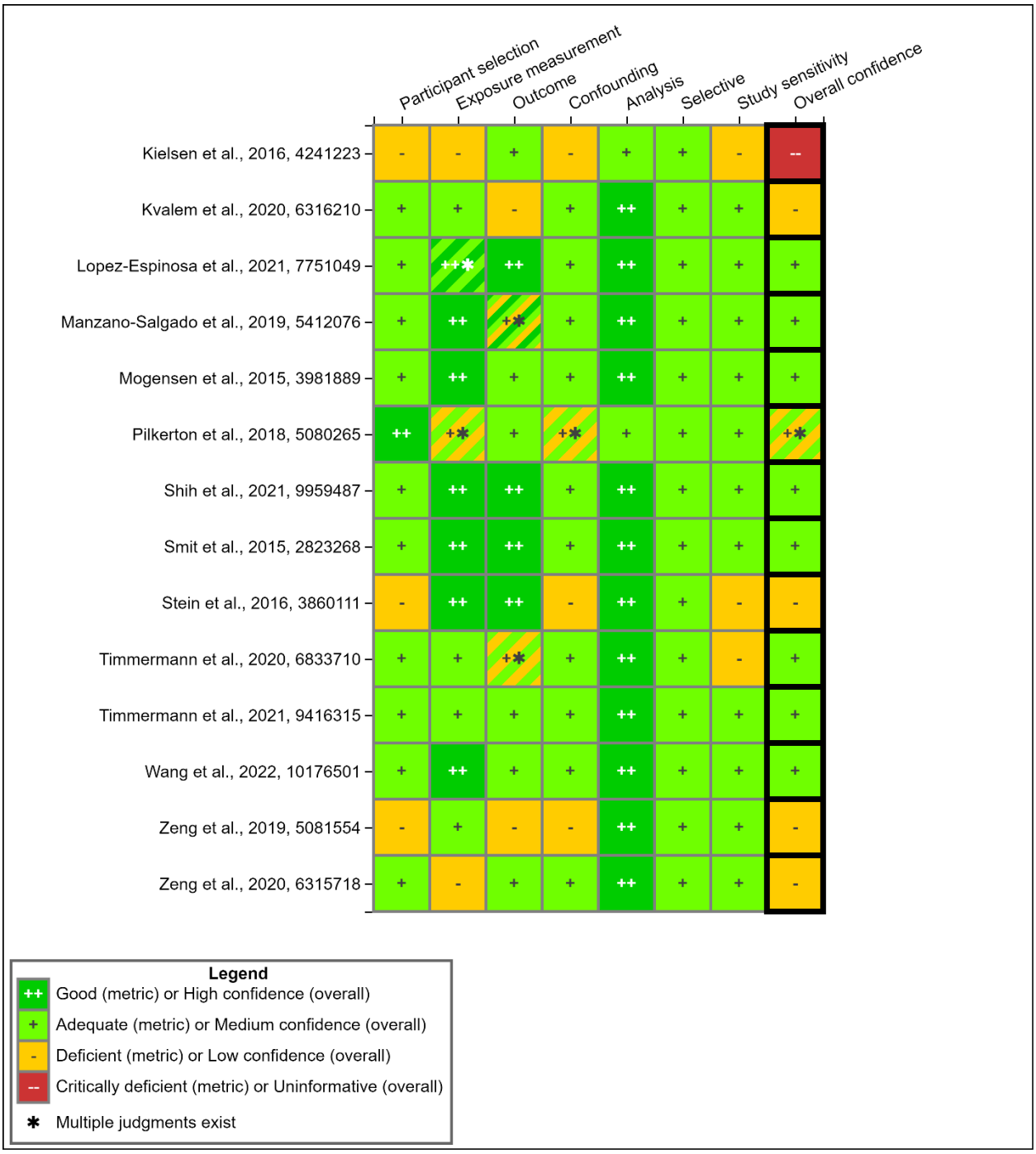


Figure 3-18. Summary of Study Evaluation for Epidemiology Studies of PFOS and Immunosuppression Effects (Continued)

Interactive figure and additional study details available on HAWC.

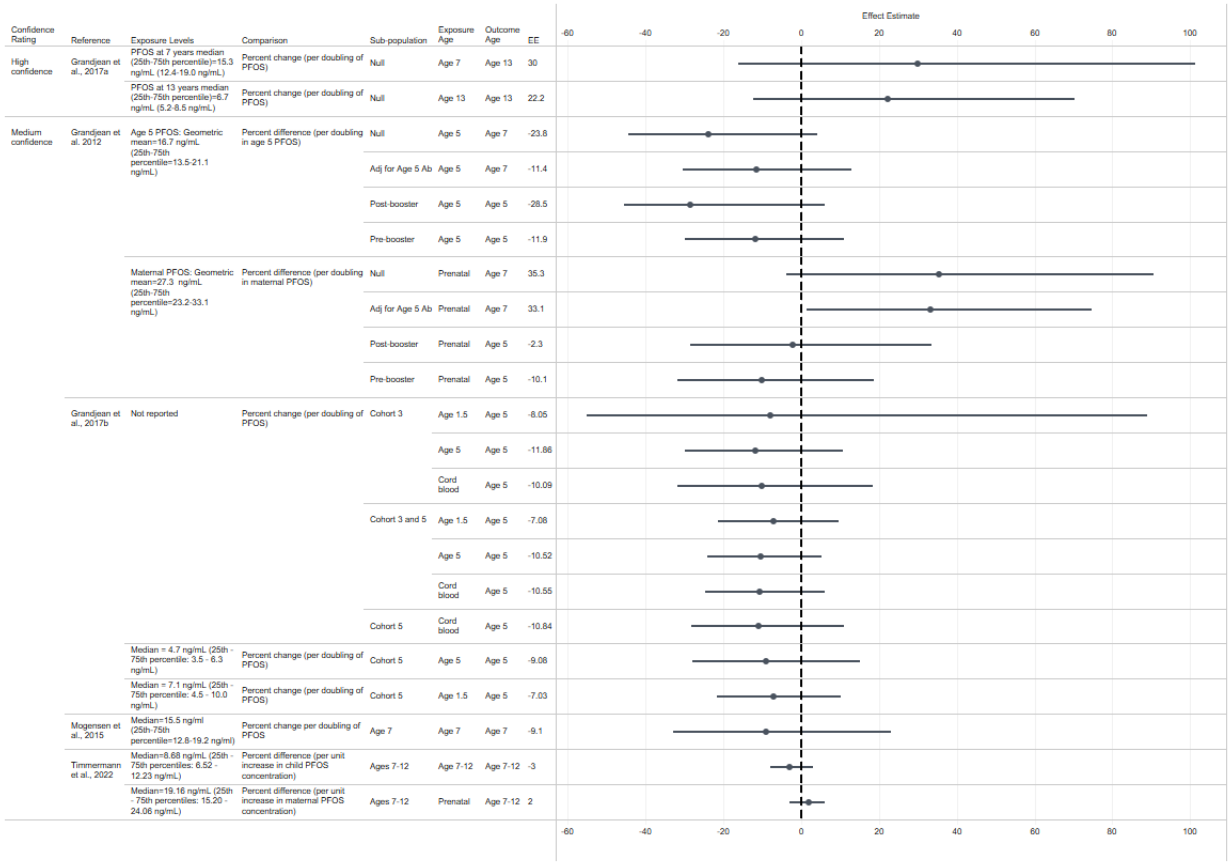
3.4.2.1.1.1 Vaccine Response

Twelve studies (thirteen publications[10]) studied the relationship between antibody response to vaccination and PFOS exposure. Six of these studies investigated antibody response to vaccination in children {Timmermann, 2020, 6833710; Abraham, 2020, 6506041; Grandjean, 2017, 3858518; Mogensen, 2015, 3981889; Grandjean, 2017, 4239492; Timmermann, 2021, 9416315}. In adults, two studies investigated antibody response to diphtheria and tetanus {Kielsen, 2016, 4241223; Shih, 2021, 9959487}, one study investigated hepatitis vaccine response {Shih, 2021, 9959487}, one study investigated adult flu vaccine response {Stein, 2016, 3860111}, and one study measured rubella antibodies in both adolescents (aged 12 and older) and adults {Pilkerton, 2018, 5080265}. In addition, one study {Zeng, 2019, 5081554} measured natural antibody exposure to hand, foot, and mouth disease (HFMD), and one study {Zeng, 2020, 6315718} measured hepatitis b antibodies in adults. Overall, one study was *high* confidence { Grandjean, 2017, 3858518}, five studies were *medium* confidence {Grandjean, 2017, 4239492; Timmermann, 2020, 6833710; Mogensen, 2015, 3981889; Timmermann, 2021, 9416315; Shih, 2021, 9959487}, four were *low* confidence {Stein, 2016, 3860111; Zeng, 2019, 5081554; Zeng, 2020, 6315718; Abraham, 2020, 6506041}, one was *mixed* (*medium/low* confidence) {Pilkerton, 2018, 5080265}, and one was *uninformative*.

Of the studies that measured antibody response to vaccination in children, four studies were cohorts {Timmermann, 2020, 6833710; Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Mogensen, 2015, 3981889}, and two were cross-sectional {Abraham, 2020, 6506041; Timmermann, 2021, 9416315} (maternal serum was available for a subset of participants in Timmermann et al. (2021, 9416315)). These included multiple prospective birth cohorts in the Faroe Islands, one with enrollment in 1997–2000 and subsequent follow-up to age 13 {Grandjean, 2017, 3858518} and one with enrollment in 2007–2009 and follow-up to age 5 {Grandjean, 2017, 4239492} (one additional cohort in the Faroe Islands examined outcomes in adults with enrollment in 1986–1987 and follow-up to age 28 {Shih, 2021, 9959487}). Five of these studies measured antibody response to tetanus vaccination {Abraham, 2020, 6506041; Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Mogensen, 2015, 3981889; Timmermann, 2021, 9416315}; the same studies also measured antibody response to diphtheria vaccination; one study measured antibody response to measles vaccination {Timmermann, 2020, 6833710}, and one study to *Haemophilus influenzae* type b (Hib) vaccination {Abraham, 2020, 6506041}.

The results for this set of studies in children are shown in Table 3-6 and the Appendix (see PFOS Appendix). The Faroe Islands studies {Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Mogensen, 2015, 3981889} observed associations between higher levels of PFOS and lower antibody levels against tetanus and diphtheria in children at 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 the perinatal period and at later time periods from children at age 5, 7, and 13 years (Table 3-6). No biological rationale has been identified as to whether one particular time period or duration of exposure or outcome measurement is more sensitive to an overall immune response to PFOS exposure.

[10] Multiple publications of the same study: the study populations are the same in Grandjean et al. (2017, 3858518) and Mogensen et al. (2015, 3981889).



Confidence Rating	Reference	Exposure Levels	Comparison	Sub-population	Exposure Age	Outcome Age	EE
High confidence	Grandjean et al., 2017a	PFOS at 7 years median (25th-75th percentile)=15.3 ng/mL (12.4-19.0 ng/mL)	Percent change (per doubling of PFOS)	Null	Age 7	Age 13	30
		PFOS at 13 years median (25th-75th percentile)=6.7 ng/mL (5.2-8.5 ng/mL)	Percent change (per doubling of PFOS)	Null	Age 13	Age 13	22.2
Medium confidence	Grandjean et al. 2012	Age 5 PFOS: Geometric mean=16.7 ng/mL (25th-75th percentile=13.5-21.1 ng/mL)	Percent difference (per doubling in age 5 PFOS)	Null	Age 5	Age 7	-23.8
				Adj for Age 5 Ab	Age 5	Age 7	-11.4
				Post-booster	Age 5	Age 5	-28.5
				Pre-booster	Age 5	Age 5	-11.9
		Maternal PFOS: Geometric mean=27.3 ng/mL (25th-75th percentile=23.2-33.1 ng/mL)	Percent difference (per doubling in maternal PFOS)	Null	Prenatal	Age 7	35.3
				Adj for Age 5 Ab	Prenatal	Age 7	33.1
				Post-booster	Prenatal	Age 5	-2.3
				Pre-booster	Prenatal	Age 5	-10.1
	Grandjean et al., 2017b	Not reported	Percent change (per doubling of PFOS)	Cohort 3	Age 1.5	Age 5	-8.05
					Age 5	Age 5	-11.86
					Cord blood	Age 5	-10.09
				Cohort 3 and 5	Age 1.5	Age 5	-7.08
					Age 5	Age 5	-10.52
					Cord blood	Age 5	-10.55
				Cohort 5	Cord blood	Age 5	-10.84
		Median = 4.7 ng/mL (25th - 75th percentile: 3.5 - 6.3 ng/mL)	Percent change (per doubling of PFOS)	Cohort 5	Age 5	Age 5	-9.08
		Median = 7.1 ng/mL (25th - 75th percentile: 4.5 - 10.0 ng/mL)	Percent change (per doubling of PFOS)	Cohort 5	Age 1.5	Age 5	-7.03
	Mogensen et al., 2015	Median=15.5 ng/ml (25th-75th percentile=12.8-19.2 ng/ml)	Percent change per doubling of PFOS	Age 7	Age 7	Age 7	-9.1
	Timmermann et al., 2022	Median=8.68 ng/mL (25th - 75th percentiles: 6.52 - 12.23 ng/mL)	Percent difference (per unit increase in child PFOS concentration)	Ages 7-12	Age 7-12	Age 7-12	-3
		Median=19.16 ng/mL (25th - 75th percentiles: 15.20 - 24.06 ng/mL)	Percent difference (per unit increase in maternal PFOS concentration)	Ages 7-12	Prenatal	Age 7-12	2

Figure 3-19. Overall Tetanus Antibody Levels in Children from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.
Grandjean et al., 2012 was reviewed as a part of the 2016 HESD

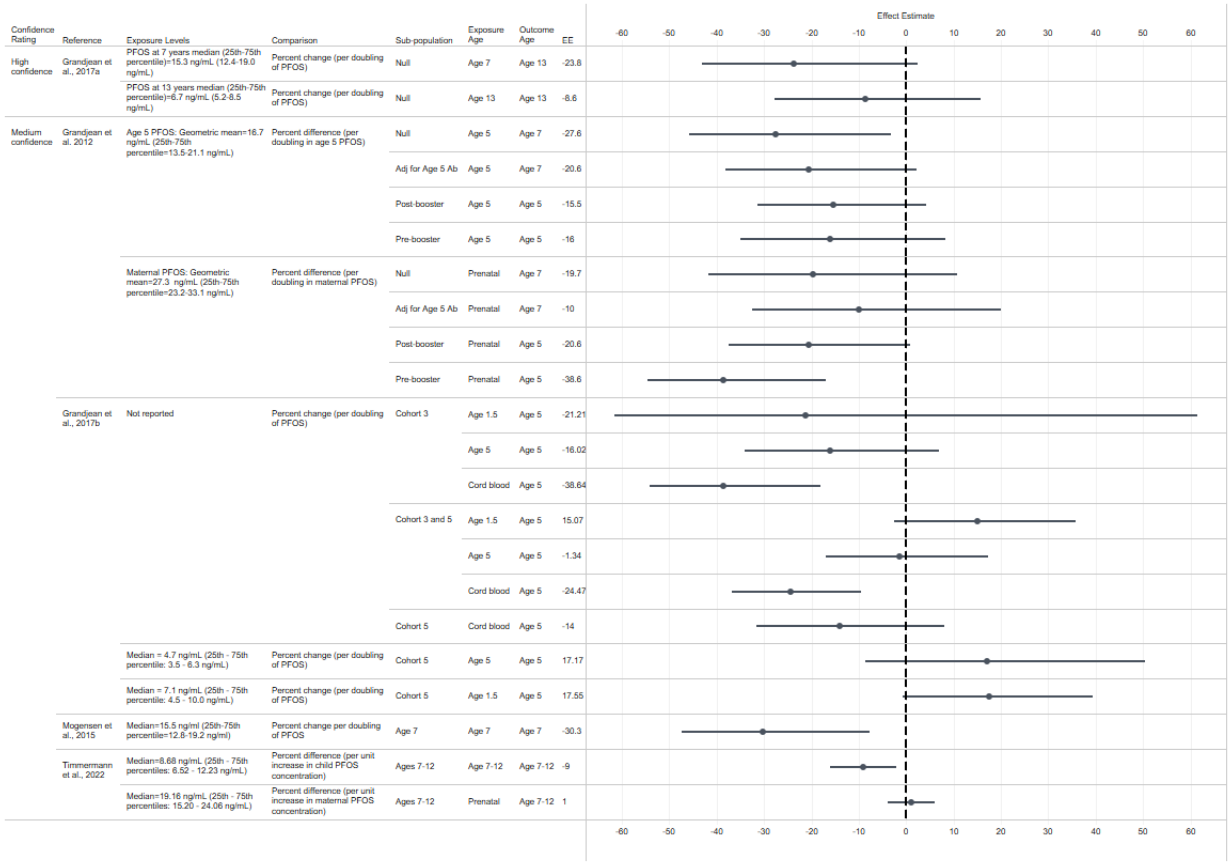


Figure 3-20. Overall Diphtheria Antibody Levels in Children from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.
Grandjean et al., 2012 was reviewed as a part of the 2016 HESD

Figure 3-21. Odds of Being Below the Protective Level Against Diphtheria (Antibody Concentrations < 0.1 IU/mL) from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

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

Table 3-6. Associations between PFOS Exposure and Vaccine Response in Faroe Island Studies

Exposure measurement timing, levels (ng/mL)[a]	Diphtheria Antibody Associations with PFOS by Age at Assessment			Tetanus Antibody Associations with PFOS by Age at Assessment		
	5 years (Pre-Booster) (C3 and/or C5)	**7 years (C3 only)**	**13 years (C3 only)**	**5 years (Pre-Booster) (C3 and/or C5)**	**7 years (C3 only)**	**13 years (C3 only)**
Maternal C3: GM: 27.3 (23.2–33.1)	↓ (C3; age, sex)[b] **BMD/BMDL** (C3&5; sex, birth cohort, log-PFOS)[c]	↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 years)[b]	–	↓ (C3; age, sex)[b] **BMD/BMDL** (C3&5; sex, birth cohort, log-PFOS)[c]	↑↑ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 years)[b]	–
Birth (modeled)	↓↓ (C3; age, sex)[d] ↓↓ (C3&5; age, sex)[d] ↓ (C5; age, sex)[d]	–	–	↓ (C3; age, sex)[d] ↓ (C3&5; age, sex)[d] ↓ (C5; age, sex)[d]	–	–
18 months C3: NR C5: 7.1 (4.5–10.0)	↓ (C3; age, sex)[d] ↑ (C3&5; age, sex)[d] ↑ (C5; age, sex)[d]	–	–	↓ (C3; age, sex)[d] ↓ (C3&5; age, sex)[d] ↓ (C5; age, sex)[d]	–	–
5 years C3: GM: 16.7 (13.5–21.1) C5: 4.7 (3.5–6.3)	↓↓ (C3; age, sex)[b] ↓ (C3; age, sex)[d] ↓ (C3&5; age, sex)[d] ↑ (C5; age, sex)[d]	↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 years)[b] **BMD/BMDL** (C3; sex, age, and booster type at age 5)[e] **BMD/BMDL** (C3; sex, booster type at age 5, log-PFOS)[c]	–	↓ (C3; age, sex)[b] ↓ (C3; age, sex)[d] ↓ (C3&5; age, sex)[d] ↓ (C5; age, sex)[d]	↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 years)[b] **BMD/BMDL** (C3; sex, age, and booster type at age 5)[e] **BMD/BMDL** (C3; sex, booster type at age 5, log-PFOS)[c]	–

Exposure measurement timing, levels (ng/mL)[a]	Diphtheria Antibody Associations with PFOS by Age at Assessment			Tetanus Antibody Associations with PFOS by Age at Assessment		
	5 years (Pre-Booster) (C3 and/or C5)	7 years (C3 only)	13 years (C3 only)	5 years (Pre-Booster) (C3 and/or C5)	7 years (C3 only)	13 years (C3 only)
7 years C3: 15.3 (12.4–19.0)	–	↓↓ (C3; age, sex, booster type)[f] ↓ (C3; sex, age at antibody assessment, booster type at age 5)[g]	↓↓ (C3; sex, age at antibody assessment, booster type at age 5)[g]	–	↓ (C3; age, sex, booster type)[f] ↑ (C3; sex, age at antibody assessment, booster type at age 5)[g]	↑ (C3; sex, age at antibody assessment, booster type at age 5)[g]
13 years C3: 6.7 (5.2–8.5)	–	–	↓ (C3; sex, age at antibody assessment, booster type at age 5)[g]	–	–	↑ (C3; sex, age at antibody assessment, booster type at age 5)[g]

Notes: C3 = cohort 3, born 1997–2000; C5 = cohort 5, born 2007–2009; GM = geometric mean; NR = not reported.
Arrows indicate direction of association with PFOS levels; double arrows indicate statistical significance ($p < 0.05$) where reported. Arrows are followed by parenthetical information denoting the cohort(s) studied and confounders (factors the models presented adjusted for).
[a] Exposure levels reported from serum as median (25^{th}–75^{th} percentile) unless otherwise noted.
[b] Grandjean et al. (2012, 1248827); *medium* confidence.
[c] Budtz-Jørgensen and Grandjean (2018, 5083631); *medium* confidence.
[d] Grandjean et al. (2017, 4239492); *medium* confidence.
[e] Grandjean and Budtz-Jørgensen (2013, 1937222); *medium* confidence.
[f] Mogensen et al. (2015, 3981889); *medium* confidence.
[g] Grandjean et al. (2017, 3858518); *medium* confidence.

The cross-sectional study of these antibodies in Greenlandic children {Timmermann, 2021, 9416315} reported results that differed in direction of association based on the covariate set selected. The exposure measurement in these analyses may not have represented an etiologically relevant window; cross-sectional analyses in the Faroe Islands studies at similar ages also found weaker associations than analyses for some other exposure windows. However, a subset of the study population did have maternal samples available, and those results were null. On the other hand, this study was the only one to examine the odds ratio for not being protected against diphtheria (antibody concentrations, which has clear clinical significance, and they reported elevated odds of not being protected (based on antibody concentrations < 0.1 IU/mL, OR (95% CI) per unit increase in exposure: 1.14 (1.04, 1.26)). Looking at other vaccines, Timmermann et al. (2020, 6833710) also observed inverse associations between elevated levels of PFOS and lower adjusted antibody levels against measles (statistically significant only in group with fewer measles vaccinations). Lastly, the low confidence cross-sectional study at age one, Abraham et al. (2020, 6506041), did not observe associations between adjusted tetanus, Hib, and diphtheria antibody levels and PFOS concentrations.

Of the three studies that measured vaccine response in adults or adolescents, two were cohorts {Stein, 2016, 3860111; Shih, 2021, 9959487}, and one was a cross-sectional analysis {Pilkerton, 2018, 5080265}. Shih et al. (2021, 9559487) measured exposure in cord blood and at multiple points through childhood to early adulthood, with outcome measurement at age 28 years; this study was *medium* confidence. Stein et al. (2016, 3860111) utilized a convenience sampling to recruit participants, had low seroconversion rates, and was at high risk of residual confounding, so was *low* confidence. The adult population in Pilkerton et al. (2018, 5080265) suffered from potential exposure misclassification due to concurrent exposure and outcome measurements and was also *low* confidence, but this was less of a concern for the adolescent participants so this sub-population was rated as *medium* confidence for adolescence antibody response to vaccinations. Shih et al. (2021, 9959487) reported inconsistent direction of associations across exposure windows and vaccines (diphtheria, tetanus, Hepatitis A, Hepatitis B). Results also differed by sex, but without a consistent direction (i.e., stronger associations were sometimes observed in women and sometimes men). Similar to the results in 13-year-olds in the other Faroe Island cohorts, this may indicate that by age 28, the effect of developmental exposure is less relevant. Neither of the other studies reported associations with immunosuppression.

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

In a C8 Health project study, Lopez- Espinoza et al. (2021, 7751049) measured serum PFAS and white blood cell types in 42,782 (2005–2006) and 526 (2010) adults from an area with PFOA drinking water contamination in the Mid-Ohio Valley (USA). Generally positive monotonic associations between total lymphocytes and PFOS were found in both surveys (difference range: 1.95–3.39% for count and 0.61–0.77 for percentage, per PFOS IQR increment). Significant decreasing associations were observed neutrophils across the surveys and total white blood cell count percent difference in the 2005–2006 survey. Findings were inconsistent for lymphocyte subtypes.

3.4.2.1.1.2 Infectious Disease

Overall, ten studies (11 publications[11]) measured associations between PFOS exposure and infectious diseases (or disease symptoms) in children with follow-ups between one and 16 years. Infectious diseases measured included: common cold, lower respiratory tract infections, respiratory syncytial virus (RSV), otitis media, pneumonia, chickenpox, varicella, bronchitis, bronchiolitis, ear infections, gastric flu, urinary tract infections, and streptococcus. Of the studies measuring associations between infectious disease and PFOS exposure, eight (nine publications) were cohorts {Ait Bamai, 2020, 6833636; Dalsager, 2016, 3858505; Dalsager, 2021, 7405343; Kvalem, 2020, 6316210; Manzano-Salgado, 2019, 5412076; Goudarzi, 2017, 3859808; Impinen, 2019, 5080609; Wang, 2022, 10176501; Huang, 2020, 6988475}, one was a case control study nested in a cohort {Impinen, 2018, 4238440}, and one was a cross-sectional study {Abraham, 2020, 6506041}. Five studies measured PFOS concentrations from mothers during pregnancy {Ait Bamai, 2020, 6833636; Dalsager, 2016, 3858505; Manzano-Salgado, 2019, 5412076; Goudarzi, 2017, 3859808; Impinen, 2019, 5080609}. Impinen et al. (2018, 4238440) measured PFOS concentrations from cord blood at delivery. Two studies measured PFOS concentrations in children's serum at age one year {Abraham, 2020, 6506041} and at age 10 years {Kvalem, 2020, 6316210}.

Several of the studies measured infectious disease incidences as parental self-report, which may have led to outcome misclassification {Kvalem, 2020, 6316210; Abraham, 2020, 6506041; Impinen, 2018, 4238440; Impinen, 2019, 5080609}. Four studies measured infections as the doctor-diagnosed incidence of disease over a particular period {Goudarzi, 2017, 3859808; Manzano-Salgado, 2019, 5412076; Ait Bamai, 2020, 6833636; Huang, 2020, 6988475}, and Wang et al. (2022, 10176501) used a combination of parental report and medical records. One study used hospitalizations as an outcome, with events identified based on medical records {Dalsager, 2021, 7405343}. Overall, seven studies were *medium* confidence {Abraham, 2020, 6506041; Ait Bamai, 2020, 6833636; Goudarzi, 2017, 3859808; Manzano-Salgado, 2019, 5412076; Dalsager, 2021, 7405343; Wang, 2022, 10176501; Huang, 2020; 6988475} and four were *low* confidence {Dalsager, 2016, 3858505; Impinen, 2018, 4238440; Impinen, 2019, 5080609; Kvalem, 2020, 6316210}.

Increased incidence of some infectious diseases in relation to PFOS exposure was observed, although results were not consistent across studies. Results from these studies are available in the Appendix (see PFOS Appendix). The most commonly examined type of infections was respiratory, including pneumonia/bronchitis, upper and lower respiratory tract, throat infections,

[11] Multiple publications of the same study: both Dalsager et al. (2016, 3858505) and Dalsager et al. (2021, 7405343) use data from the Odense cohort in Denmark and thus have overlapping, though not identical populations. They received different ratings due to outcome ascertainment methods.

and common colds. Dalsager et al. (2021, 7405343), a *medium* confidence study, reported higher rates of hospitalization for upper and lower respiratory tract infections with higher PFOS exposure (statistically significant for lower respiratory tract). Among studies that examined incidence, two studies (one *medium* and one *low* confidence) examining pneumonia/bronchitis observed statistically significant associations between elevated PFOS concentration and increased risk of developing pneumonia in 0- to 3-year-old children {Impinen, 2019, 5080609} and 7-year-old children {Ait Bamai, 2020, 6833636}; however, two other *medium* confidence studies did not report an increase in infections {Abraham, 2020, 6506041; Wang, 2022, 10176501}. Huang et al. (2020, 6988475) examined recurrent respiratory infections and found a positive association with recurrent respiratory infections but not total infections. Two *low* and one *medium* confidence studies found positive associations with lower respiratory infection {Kvalem, 2020, 6316210; Impinen, 2018, 4238440; Dalsager, 2021, 7405343}, while another *medium* confidence study reported no association {Manzano-Salgado, 2019, 5412076}. There were also non statistically significant positive associations seen for PFOS in relation to chickenpox {Ait Bamai, 2020, 6833636}, common cold {Wang, 2022, 10176501}, and cough {Dalsager, 2016, 3858505}, but statistically significant inverse associations were observed for RSV {Ait Bamai, 2020, 6833636} and common cold {Impinen, 2018, 4238440}. Outside of respiratory infections, two *medium* confidence studies examined total infectious diseases. Dalsager et al. (2021, 7405343) reported higher rates of hospitalization for any infections with higher PFOS exposure (not statistically significant), while {Goudarzi, 2017, 3859808} reported higher odds of total infectious diseases. Results for other infection types, including gastrointestinal, generally did not indicate a positive association.

In addition to the studies in children, three studies examined infectious disease in adults, {Ji, 2021, 7491706; Grandjean, 2020, 7403067; Bulka, 2021, 7410156}. Results from these studies are available in the Appendix (see PFOS Appendix). All three studies were *medium* confidence. Ji et al. (2021, 7491706) was a case-control study of COVID-19 infection. They reported higher odds of infection with higher exposure (OR (95% CI) per log-2 SD increase in PFOS: 1.94 (1.39, 2.96)). In contrast, a cross-sectional study examining severity of COVID-19 illness in Denmark using biobank samples and national registry data Grandjean et al. (2020, 7403067) reported no association between PFOS exposure and increased COVID-19 severity. Bulka et al. (2021, 7410156) used NHANES data from 1999–2016 in adolescents and adults and examined immunoglobulin G (IgG) antibody levels to several persistent infections, including cytomegalovirus, Epstein Barr virus, hepatitis C and E, herpes simplex 1 and 2, human immunodeficiency virus (HIV), Toxoplasma gondii and Toxocara species. High levels of these antibodies were interpreted as presence of a persistent infection. They found higher prevalence of Herpes simplex viruses 1 and 2, Toxoplasma gondii and Toxocara species and total pathogen burden with higher PFOS exposure in adults (not statistically significant for HSV-2 and Toxoplasma gondii) but no association with other individual pathogens.

3.4.2.1.2 Immune Hypersensitivity

Another major category of immune response is the evaluation of sensitization-related or allergic responses resulting from exaggerated immune reactions (e.g., allergies or allergic asthma) to foreign agents {IPCS, 2012, 1249755}. A chemical may be either a direct sensitizer (i.e., promote a specific immunoglobulin E (IgE)-mediated immune response to the chemical itself) or may promote or exacerbate a hypersensitivity-related outcome without evoking a direct response. For example, chemical exposure could promote a physiological response resulting in a

propensity for sensitization to other allergens (pet fur, dust, pollen etc.). Hypersensitivity responses occur in two phases. The first phase, sensitization, is without symptoms, and it is during this step that a specific interaction is developed with the sensitizing agent so that the immune system is prepared to react to the next exposure. Once an individual or animal has been sensitized, contact with that same (or, in some cases, a similar) agent leads to the second phase, elicitation, and symptoms of allergic disease. Although these responses are mediated by circulating factors such as T cells, IgE, and inflammatory cytokines, there are many health effects associated with hypersensitivity and allergic response. Functional measures of sensitivity and allergic response consist of health effects such as allergies or asthma and skin prick tests.

In the 2016 PFOS HESD, one of two studies reported higher odds of asthma with higher PFOS exposure in children. A case-control study {Dong, 2013, 1937230} of children in Taiwan reported an increased odds of asthma with increasing childhood PFOS exposure. The magnitude of association was particularly large comparing each of the highest quartiles of exposure to the lowest. In cross-sectional analyses of asthmatic children, the study authors reported monotonic increases by quartile of exposure for IgE in serum, absolute eosinophil counts, eosinophilic cationic protein, and asthma severity score. No association for current or ever asthma was observed among NHANES (1999–2000, 2003–2008) adolescents {Humblet, 2014, 2851240}.

There are 23 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and immune hypersensitivity (i.e., asthma, allergy, and eczema) effects. Study quality evaluations for these 23 studies are shown in Figure 3-22.

Figure 3-22. Summary of Study Evaluation for Epidemiology Studies of PFOS and Immune Hypersensitivity Effects

Interactive figure and additional study details available on HAWC.

Thirteen studies (fifteen publications)[12] examined asthma (or asthma symptoms) and PFOS exposure. Nine of these studies were cohorts {Averina, 2019, 5080647; Beck, 2019, 5922599; Kvalem, 2020, 6316210; Manzano-Salgado, 2019, 5412076; Zeng, 2019, 5412431; Impinen, 2019, 5080609; Smit, 2015, 2823268; Timmermann, 2017, 3858497; Workman, 2019, 5387046}; three studies (five publications) were case-control investigations {Zhou, 2016, 3981296; Zhou, 2017, 3858488; Zhu, 2016, 3360105}, including one nested case-control, {Gaylord, 2019, 5080201; Impinen, 2018, 4238440}; and one was a cross-sectional analysis {Jackson-Browne, 2020, 6833598}. Seven studies measured the prevalence of "current" asthma for at least one time point {Averina, 2019, 5080647; Beck, 2019, 5922599; Manzano-Salgado, 2019, 5412076; Kvalem, 2020, 6316210; Impinen, 2018, 4238440; Impinen, 2019, 5080609; Zeng, 2019, 5412431}. Eight studies measured "ever" asthma for at least one time point {Averina, 2019, 5080647; Manzano-Salgado, 2019, 5412076; Jackson-Browne, 2020, 6833598; Gaylord, 2019, 5080201; Impinen, 2018, 4238440; Impinen, 2019, 5080609; Smit, 2015, 2823268; Timmermann, 2017, 3858497}. Incident or recurrent wheeze was examined in one study {Workman, 2019, 5387046}. Overall, nine studies were rated *medium* confidence, and six studies were *low* confidence for asthma (Figure 3-22). 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 Genetic and Biomarkers study for Childhood Asthma (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 (see PFOS Appendix). Several studies observed positive associations with ORs greater than 1.2 between PFOS concentration levels and increased "current" or "ever" asthma {Beck, 2019, 5922599; Timmermann, 2017, 3858497; Jackson-Browne, 2020, 6833598; Zeng, 2019, 5412431; Impinen, 2018, 4238440; Averina, 2019, 5080647}, but often only within population subgroups. Averina et al. (2019, 5080647) observed statistically significant increased odds of self-reported doctor diagnosed asthma among adolescents in their first year of high school. Jackson-Browne et al. (2020, 6833598) reported statistically significant increased odds of "ever" asthma from increased PFOS concentrations in children aged 3 to 5 years. No association was observed at ages 6–11 years, and the overall association was small (OR: 1.1). Beck et al. (2019, 5922599) observed increased odds of self-reported asthma per PFOS increase in boys ($p > 0.05$), but this was not observed in girls. For doctor diagnosed asthma in the same study, an inverse association ($p > 0.05$) was observed in boys and a positive association ($p > 0.05$) was observed in girls. Zeng et al. (2019, 5412431) observed a positive association in boys and an inverse association in girls (both $p > 0.05$). Impinen et al. (2018, 4238440) reported higher odds of ever asthma. The *low* confidence study, Timmermann et al. (2017, 3858497), observed positive associations ($p > 0.05$) between increased asthma odds and elevated PFOS concentrations in small subset of children aged 5 and 13 who did not receive their measles, mumps, and rubella (MMR) vaccination before age 5. However, in

[12] 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.

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

In addition to the studies of asthma in children, one *medium* confidence study {Xu, 2020, 6988472} using data from NHANES examined fractional exhaled nitric oxide (FeNO), a measure of airway inflammation, in adults. Among participants without current asthma, this study found higher FeNO levels with higher PFOS exposure, indicating greater inflammation (percent change (95% CI) for tertiles vs. T1, T2: 1.80 (−1.53, 5.25); T3: 5.02 (1.40, 8.77)).

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

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

and self-reported food allergies and reported statistically significant positive associations with self-reported food allergies in NHANES 2007–2010 but not in in NHANES 2005–2006.

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

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

One *medium* confidence nested case-control study examined chronic spontaneous urticaria {Shen, 2022, 10176753}. They found no association between PFOS exposure and case status.

3.4.2.1.3 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 PFOS HESD did not identify epidemiological evidence examining the association between PFOS exposure and autoimmune conditions. There are 4 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and autoimmune disease effects. Study quality evaluations for these 4 studies are shown in Figure 3-23.

Four case-control studies examined PFOS exposure and autoimmune diseases (Figure 3-23). Two studies examined MS {Ammitzbøll, 2019, 5080379} and ulcerative colitis {Steenland, 2018, 5079806} in adults, and two studies examined celiac disease in children {Sinisalu, 2020, 7211554} and young adults {Gaylord, 2020, 6833754}. PFOS was measured in blood components (i.e., blood, plasma, or serum) for all studies (see PFOS Appendix). One study was *medium* confidence {Gaylord, 2020, 6833754} with minimal deficiencies, and three studies were considered *low* confidence {Ammitzbøll, 2019, 5080379; Steenland, 2018, 5079806; Sinisalu, 2020, 7211554}. Information on participant selection, particularly control selection, was not

reported in Ammitzbøll et al. (2019, 5080379). Additionally, PFOS was evaluated as a dependent rather than independent variable, making no informative determinations about associations between PFOS exposure and risk of MS, and contributed to a *low* confidence rating. Steenland et al. (2018, 5079806) examined exposure concentrations one to two years after diagnosis of celiac disease, resulting in some concern for reverse causation. Additionally, there was potential for residual confounding by SES which was not considered in the analysis. These factors together contributed to a *low* confidence rating.

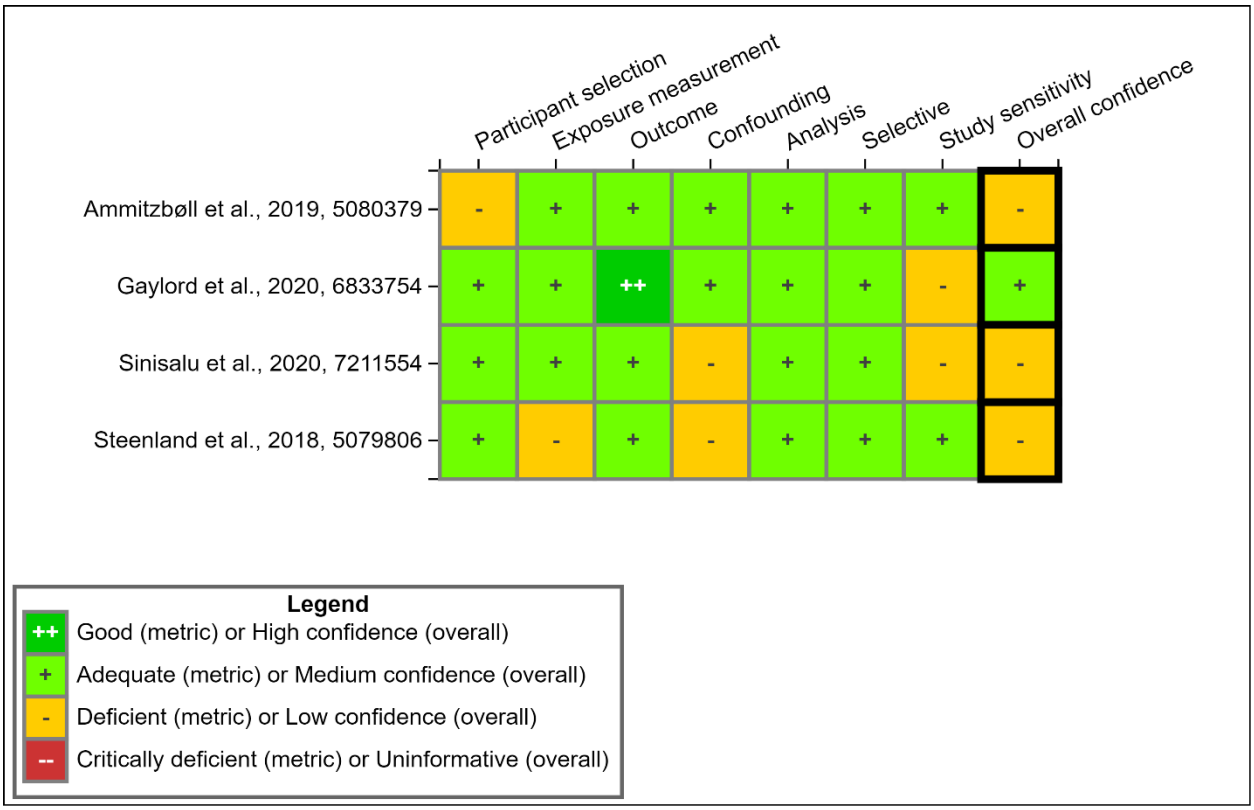


Figure 3-23. Summary of Study Evaluation for Epidemiology Studies of PFOS and Autoimmune Effects

Interactive figure and additional study details available on HAWC.

Ammitzbøll et al. (2019, 5080379) observed lower PFOS concentrations among healthy controls compared to those with MS. Serum PFOS concentrations were 17% lower (95% CI: −27%, −6%; p = 0.004) in healthy controls compared to cases of relapsing remitting MS and clinically isolated MS. Restricting the analysis to men, serum PFOS levels were 28% lower (95% CI: −32%, −3%; p = 0.023) in healthy controls compared to cases. The result was similar among women but did not reach significance (p = 0.093).

In children and young adults, the odds of celiac disease were elevated but not significantly {Gaylord, 2020, 6833754}. However, the effect was much stronger in females only (OR: 12.8; 95% CI: 1.17, 141; p < 0.05). A marginally significant (p = 0.06) decrease in serum PFOS was

observed among adult cases of ulcerative colitis compared to healthy controls {Steenland, 2018, 5079806}.

In the prospective observational Finnish Diabetes Prediction and Prevention (DIPP) study in which children genetically at risk to develop type 1 diabetes (T1D) and celiac disease (CD) were followed from birth, with blood samples taken at birth and 3 months of age {Sinisalu, 2020, 7211554}, there was no significant difference in the levels of PFOS exposure in those children that later developed CD, which may be due to the small sample size, but age at diagnosis of CD was strongly associated with the PFOS exposure.

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

3.4.2.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 3 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and 10 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and hepatic effects. Study quality evaluations for these 13 studies are shown in Figure 3-24.

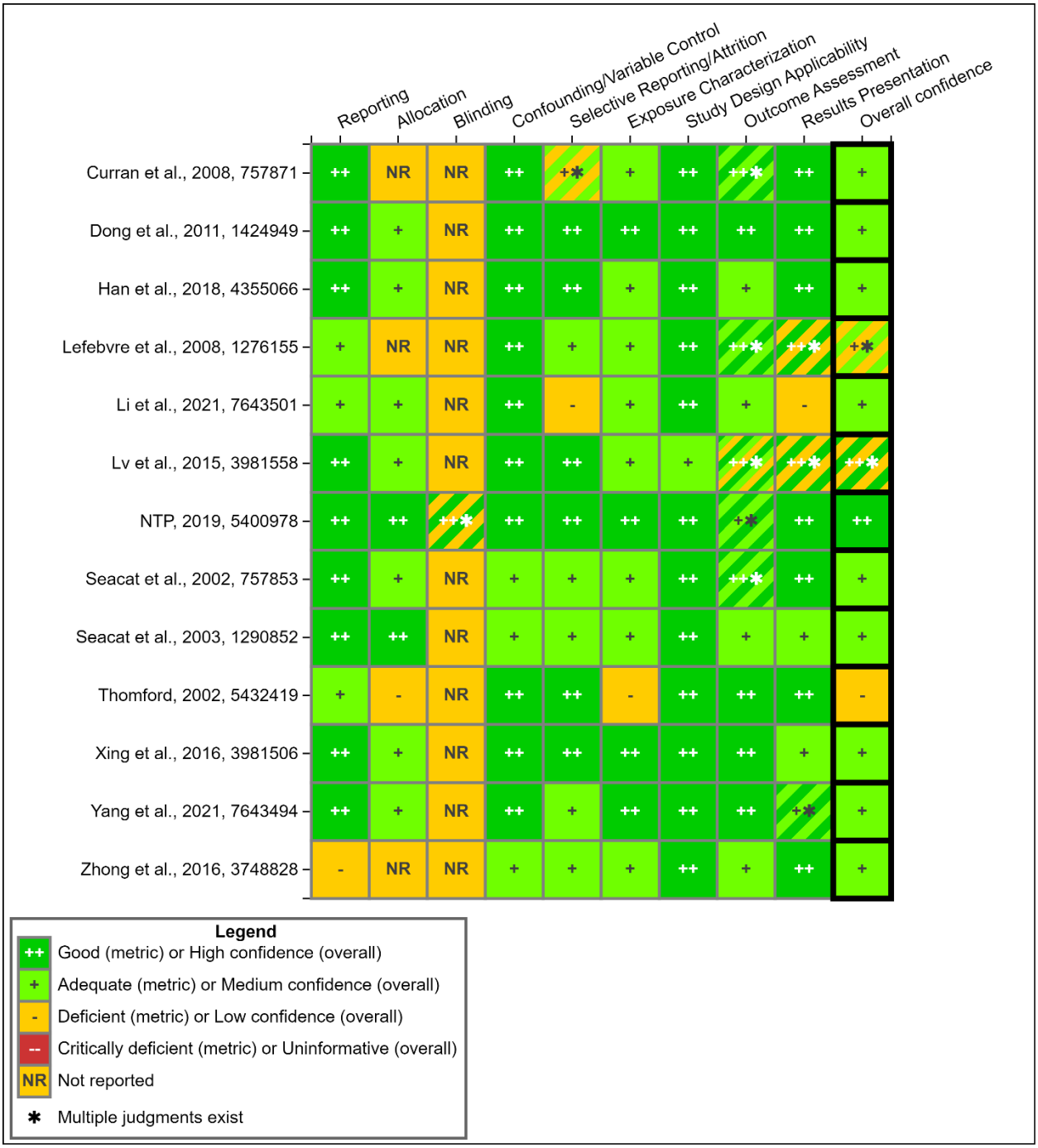


Figure 3-24. Summary of Study Evaluation for Toxicology Studies of PFOS and Immune Effects[a]

Interactive figure and additional study details available on HAWC.
[a] Lefebvre et al. (2008, 1276155) reported on the same animals as Curran et al. (2008, 757871).

The immune system could be a target of PFOS toxicity as effects have been observed across animal toxicological studies of varying durations of oral exposure to PFOS. Effects include

changes in spleen and/or thymus weights, extramedullary hematopoiesis, perturbations in activity level or composition of various immune cell populations, and diminished ability to generate an immune response. Studies indicate that PFOS exposure may result in dose- and sex-specific immunomodulatory effects.

3.4.2.2.1 Organ Weight

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

3.4.2.2.1.1 Spleen

Two separate 28-day studies reported absolute and relative spleen weights in male and female rats exposed to PFOS. Lefebvre et al. (2008, 1276155) observed reduced absolute spleen weights in male rats of the highest exposure group in Sprague-Dawley rats given PFOS in diet (0.14–6.34 mg/kg/day in males and 0.15–7.58 mg/kg/day in females). When expressed as percent body weight, these changes were not significant and were within 5% of control for any given exposed group. In contrast, absolute spleen weights were not affected by PFOS exposure in females, but relative spleen weights were significantly higher (18% higher than controls) in the highest exposure group. The increased relative spleen weights in females may be explained by lower body weights of the two highest exposure groups. Another 28-day study by NTP (2019, 5400978) administered PFOS (0.312, 0.625, 1.25, 2.5, or 5 mg/kg/day) to Sprague-Dawley rats for 28 days and observed dose dependent reductions in absolute spleen weights at 1.25 mg/kg/day and higher in males only; no effects were observed in females. Spleen weights relative to body weight were not significantly reduced in either sex. While body weights were not significantly different throughout treatment, the high-dose group tended to have lower body weight with a significant, but < 10%, difference from the control. Therefore, differences in body weight cannot explain the decreased absolute weight.

In four separate studies, male C57BL/6 mice were administered 5, 20, or 40 mg/kg/day PFOS for 7 days {Zheng, 2009, 1429960}, fed chow with 0.001, 0.005, or 0.02% PFOS (equivalent to ~40 mg/kg/day) for 10 days {Qazi, 2009, 1937260}, 0.008–2.083 mg/kg/day PFOS for 60 days {Dong, 2009, 1424951}, or administered 0.008–0.833 mg/kg/day PFOS for 60 days via gavage{Dong, 2011, 1424949}. Decreased absolute and relative splenic weights tended to be observed only at the highest doses for each study. Female mice were not assessed. These findings are complimented by Xing et al. (2016, 3981506), where a reduction in relative spleen weight was observed in male C57BL/6J mice following exposure to 10 mg/kg/day PFOS for 30 days via gavage. No effects were observed at other doses (2.5 and 5 mg/kg/day) {Xing, 2016, 3981506}.

In a developmental study, spleens were weighed in 4- and 8-week-old offspring of pregnant C57BL/6 mice given 0, 0.1, 1, or 5 mg/kg/day PFOS from GD 1–17 via gavage. Relative spleen weights were reduced in male pups from the 5 mg/kg/day exposure group at four-weeks. No significant effects were observed in lower dose groups, at the 8-week time point, or in females {Zhong, 2016, 3748828}.

In three separate mouse studies, spleen weights were not significantly altered following short-term exposure to PFOS, including a study of male and female B6C3F1 mice administered 0.00017–0.166 mg/kg/day PFOS for 28 days {Peden-Adams, 2008, 1424797}, male C57BL/6

mice exposed to 0.25 or 2.5 mg/kg/day PFOS for 28 days {Yang, 2021, 7643494}, and male C57BL/6 (H-2^b) mice administered 0.005% PFOS in the diet for 10 days {Qazi, 2010, 1276154}. Similarly, relative spleen weight in male BALB/c mice was not affected at the end of a three-week exposure to 2.5–5 mg/kg/day PFOS {Lv, 2015, 3981558}. Although Qazi et al. (2010, 1276154), observed that relative spleen weight was slightly reduced in C57BL/6 mice following 10-day exposure to 0.005% PFOS, the effects did not reach significance.

3.4.2.2.1.2 Thymus

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

The aforementioned 28-day studies by NTP (2019, 5400978) and Lefebvre et al. (2008, 1276155) reported reductions in absolute and/or relative thymus weights in male Sprague-Dawley rats administered oral PFOS, at the highest doses of 5–7.58 mg/kg/day (Figure 3-25). Reductions in absolute thymus weight were also observed in females of the highest dose in Lefebvre et al. (2008, 1276155). In contrast, females in the NTP study exhibited reduced absolute thymus weights at doses as low as 1.25 mg/kg/day, suggesting a higher sensitivity in females {NTP, 2019, 5400978} (Figure 3-25).

Similarly, reduced thymic weights were observed in male C57BL/6 mice administered 20 or 40 mg/kg/day PFOS via gavage for 7 days {Zheng, 2009, 1429960}, 0.02% PFOS for 10 days in diet {Qazi, 2009, 1937260}, or 0.417–2.083 mg/kg/day PFOS for 60 days {Dong, 2009, 1424951}. A follow up from the latter study {Dong, 2009, 1424951} by Dong et al. (2011 1424949) also exposed adult male C57BL/6 to 0.008–0.833 mg/kg/day PFOS for 60 days via gavage, but reductions in relative thymus weight were only observed in the highest dose. Female mice were not assessed in these studies. Yang et al. (2021, 7643494) exposed male C57BL/6 mice to 0.25 or 2.5 mg/kg/day PFOS for 28 days and observed an 18% and 24%, respectively, reduction in relative thymus weight although these changes were not statistically significant.

In a developmental exposure study, the thymus was weighed in 4- and 8-week-old offspring of pregnant C57BL/6 mice given 0, 0.1, 1, or 5 mg/kg/day PFOS from GD 1–GD 17 via gavage. In male pups from the 5 mg/kg/day exposure group, relative thymus weights were reduced at 4 and 8 weeks of age. However, no effects were observed in lower dose groups or in females {Zhong, 2016, 3748828} (Figure 3-25).

In contrast to the several studies that reported reductions in thymus weight, Qazi et al. (2010, 1276154) and Peden-Adams et al. (2008, 1424797) did not observe any changes in thymus weight. Qazi et al. (2010, 1276154) exposed male C57BL/6 (H-2^b) mice to 0.005% PFOS in the diet for 10 days, while Peden-Adams et al. (2008, 1424797) exposed male and female B6C3F1 mice to 0.00017–0.166 mg/kg/day PFOS for 28 days. The contrasting results of the 28-day study by Peden-Adams et al. (2008, 1424797) and NTP (2019, 5400978) may underscore species differences, however, the dose levels used in the mouse study were generally below the LOEL of the NTP study (5 mg/kg/day).

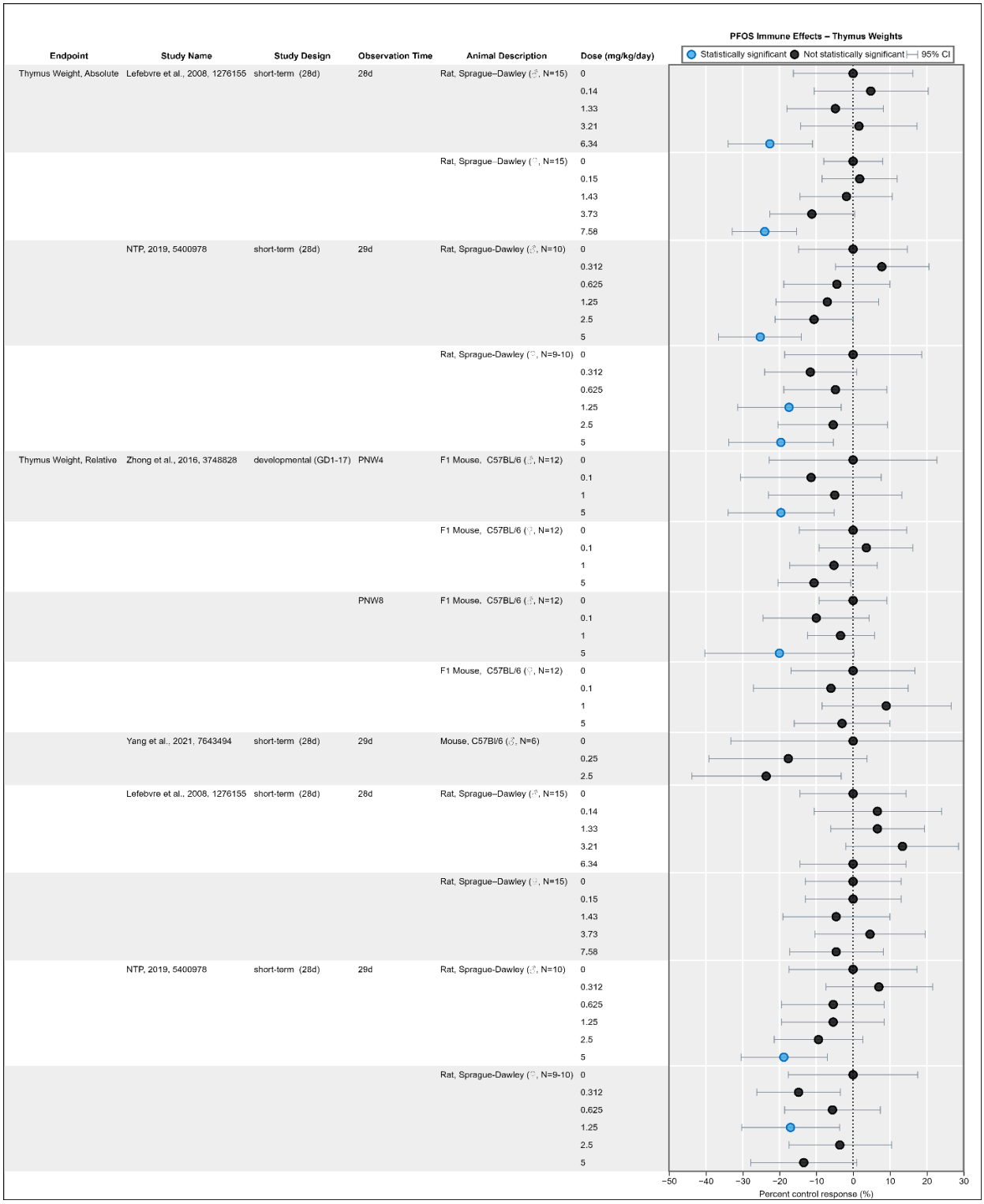


Figure 3-25. Percent Change in Thymus Weights Relative to Controls in Rodents Following Exposure to PFOS

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

3.4.2.2.2 Histopathology

Histopathology of the spleen, thymus, and/or lymph nodes has been evaluated following oral exposure to PFOS across studies of varying durations in rodents (Figure 3-26). In general, short-term and subchronic studies have observed histopathology such as extramedullary hematopoiesis {NTP 2019, 5400978}, bone marrow hypocellularity {NTP, 2019, 5400978}, and other aberrations in the immune organs {Qazi, 2009, 1937260; Lv, 2015, 3981558}.

One study included in the 2016 HESD {U.S. EPA, 2016, 3603365} by Qazi et al. (2009, 1937260) described perturbations in the thymus of male C57BL/6 (H-2^b) mice exposed to 0.02% (equivalent to ~40 mg/kg/day) PFOS in feed for 10 days; the thymic cortex was smaller and devoid of cells and the cortical/medullary junction was indistinguishable. These observations may coincide with the reduction in thymus weight described above {Qazi, 2009, 1937260; NTP, 2019, 5400978}. However, the 28-day study in rats by NTP did not observe histopathologic effects in the thymus of males or females following exposure to 0.312–5 mg/kg/day PFOS {NTP, 2019, 5400978}, and this finding was complemented by a chronic non-human primate study by Seacat et al. (2002 757853), which also found no effects in the thymus of males or females following PFOS exposure (0, 0.03, or 0.15 mg/kg/day).

In spleens of male BALB/c mice, no significant increases in non-neoplastic lesions were observed following exposure to 2.5, 5, or 10 mg/kg/day PFOS for three weeks, though quantitative results were not reported {Lv, 2015, 3981558}. However, the authors {Lv, 2015, 3981558} state that alterations in spleen architecture were observed at the end of the exposure in the 5 and 10 mg/kg/day groups. Moreover, splenic sinusoids, which drain into pulp veins, were dilated and hyperemic. Peripheral splenic pulp structure and splenic cords (also known as red pulp cords or cords of Billroth) were destroyed, the marginal zone disappeared, and megakaryocytes (myeloid cell precursors) were abundant.

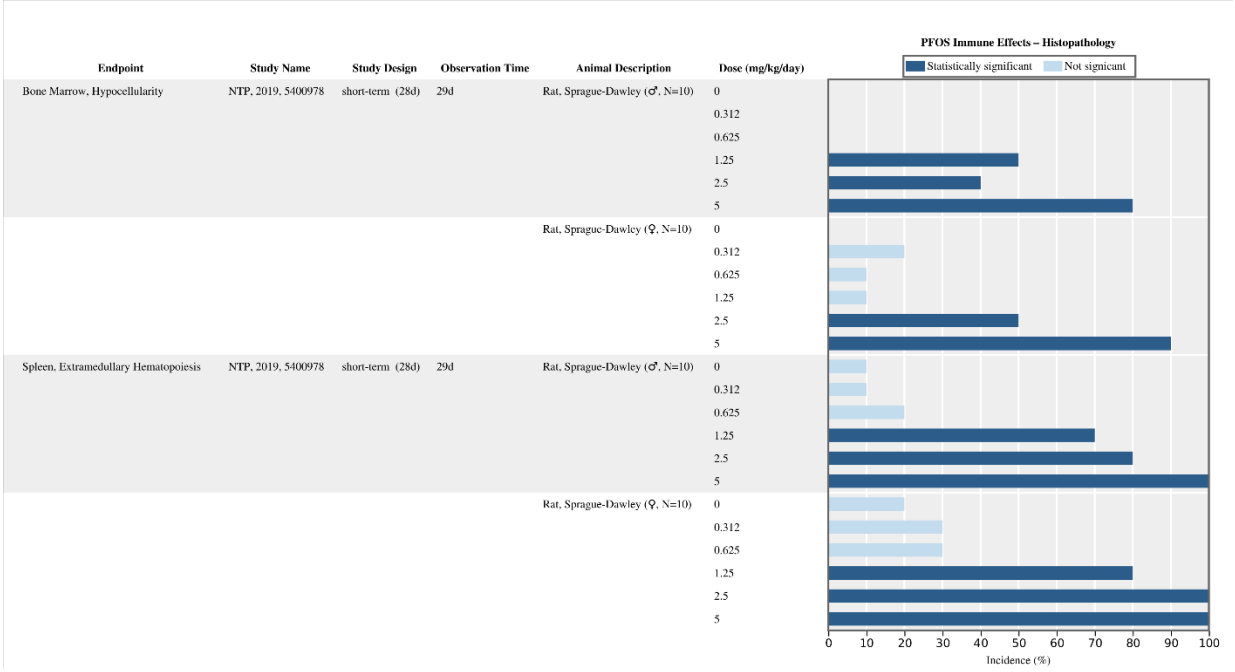


Figure 3-26. Incidences of Immune Cell Histopathology in Rodents Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

Xing et al. (2016, 3981506) examined spleens of male C57BL/6J mice for histopathology; no distinguishable morphological differences were observed between any exposure group (2.5, 5, or 10 mg/kg/day for 30 days) and control. Similarly, Li et al (2021, 7643501) reported that there were no significant lesions observed in the spleen among female BALB/c mice exposed via gavage to 0.1 or 1 mg/kg/day PFOS for 60 days.

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

3.4.2.2.3 Circulating Immune Cells

Effects of PFOS exposure on circulating immune cells have been reported in rodents and non-human primates. Alterations in neutrophil and white blood cell (WBC) populations in the circulation have been observed in rodents, but the directionality of the effect is often inconsistent, possibly reflecting differences in the timing of exposure.

Qazi et al. (2009, 1937259) performed a study to see if exposure to PFOS influenced circulating immune cells. Male C57BL/6 mice were fed chow containing 0.02% PFOS for 10 consecutive days, after which levels of WBCs were evaluated in blood collected from retroorbital puncture. The absolute WBC count was significantly reduced and was mainly a reflection of decreased lymphocytes, as no change in neutrophils was seen. A significant reduction of the relative proportion and absolute number of macrophages in the bone marrow was also reported {Qazi, 2009, 1937259}. In a study by Seacat et al. (2003, 1290852), male and female Sprague-Dawley rats were exposed to 0, 0.5, 2, 5, or 20 ppm PFOS for 14 weeks and WBC counts were determined. The only statistically significant change was an increase in neutrophils in the

20 ppm exposure group (1.33 mg/kg/day dose equivalent) in the males only. No effects were observed at lower exposure groups (0.5, 2.0, 5.0 ppm) nor in females {Seacat, 2003, 1290852}. A shorter (28-day) study in male and female Sprague-Dawley rats exposed to 0.14–7.58 mg/kg/day PFOS did not observe any statistically significant effects on circulating white blood cell populations {Lefebvre, 2008 1276155}. The authors examined a myriad of circulating immune cell endpoints, including WBC, total lymphocytes, as well as the number and percentages of CD3+ (all T cells), CD3+/CD8+ (Cytotoxic T cells), CD3+/CD4+ (Helper T cells), CD45RA+ (B-cells). Although not significant, Helper T cell counts in males and females were elevated from control by 35% or 42%, respectively, which coincided with a 29% or 41% increase in total T cell counts, suggesting that there may be a specific effect of PFOS on helper T cell populations. Similarly, Yang et al. (2021, 7643494) found that exposure of male C57BL/6 mice to 2.5 mg/kg/day PFOS for 28 days did not significantly alter WBC counts, nor percent or number of neutrophils, total lymphocytes, eosinophils, monocytes, and basophils in the serum.

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

3.4.2.2.4 Natural Killer Cell Activity

The available data on the effect of PFOS exposure on natural killer (NK) cell activity indicate that there may be different effects in NK cell activity based on dose, but there are too few studies to make any determination and no single study assesses the continuum of doses to see if there is an opposing effect at different areas of the dose response curve. Oral administration of 0.00017–0.166 mg/kg/day PFOS to male and female B6C3F1 mice for 28 days resulted in increased NK cell activity in males only exposed to 0.017, 0.033, and 0.166 mg/kg/day {Peden-Adams, 2008, 1424797}. Male C57BL/6 mice exposed to 0.083 mg/kg/day PFOS daily for 60 days displayed significantly increased NK cell activity by 38%, but treatment with 0.833 and 2.083 mg/kg/day resulted in decreased NK cell activity {Dong, 2009, 1424951}. Female mice were not assessed in this study. In another assessment of male C57BL/6 mice administered 0–40 mg/kg/day for 7 days, NK cell activity was reduced following exposure to 20 and 40 mg/kg/day {Zheng, 2009, 1429960}. Similarly, Zhong et al. (2016, 3748828) reported that NK cell activity was decreased in 4-week-old male offspring from the 5 mg/kg/day group and also reduced in 8-week-old offspring from the 1 or 5 mg/kg/day group. The latter result was recapitulated in the study by Keil et al. (2008, 1332422) where the female C57BL/6 mice were mated with C3H to derive B6C3F1 offspring. Female offspring from both studies were less sensitive to the PFOS-induced reduction in NK cell activity {Keil, 2008, 1332422; Zhong, 2016, 3748828} as indicated by the lack of statistically significant changes in females exposed to 1 mg/kg/day in each study. Moreover, at 8 weeks, NK cell activity was suppressed by 42.5% and 32.1% in males at the 1 and 5 mg/kg/day treatments, respectively, and was suppressed by 35.1% in females at the 5 mg/kg/day treatment {Keil, 2008, 1332422}. These studies indicate that male mice may be more susceptible to PFOS-induced altered NK cell activity, and that NK cell activity can be increased or decreased following low or high PFOS exposure, respectively (Table 3-7).

Table 3-7. Associations Between PFOS Exposure and Natural Killer Cell Activity in Mice

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

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

3.4.2.2.5 Spleen Cellularity

Splenocyte sub-classes were quantified in several rodent studies (Figure 3-27). Splenic T cell immunophenotypes were slightly affected in male and female B6C3F1 mice exposed to oral administration of 0.00017–0.166 mg/kg/day PFOS for 28 days {Peden-Adams, 2008, 1424797}. In males, $CD4^-/CD8^+$ and $CD4^-/CD8^-$ cells were increased, whereas numbers of $CD4^+/CD8^-$ and $CD4^+/CD8^+$ cells were decreased beginning at 0.0033 mg/kg/day. In females, splenic $CD4^-/CD8^+$ and $CD4^+$/CD8- cells were decreased beginning at 0.0033 mg/kg/day. Significantly decreased splenocyte populations were also observed in male C57BL/6 mice exposed to 0.02% PFOS for 10 days {Qazi, 2009, 1937260}, 20 or 40 mg/kg/day PFOS for 7 days {Zheng, 2009, 1429960}, and 0.417–2.083 mg/kg/day for 60 days {Dong, 2009, 1424951}. Female mice were not evaluated in these studies.

Altered splenic cellular composition was observed in a study by Lv et al. (2015, 3981558) where male BALB/c mice were exposed to 0, 2.5, 5, or 10 mg/kg/day PFOS for 3 weeks {Lv, 2015, 3981558}, and spleens harvested for lymphocyte counting and phenotyping. Fluctuations in lymphocyte counts and T cell proliferation were apparent at the 3-week timepoint. A dose-dependent increase in the number of splenic T cells ($CD3^+$) relative to controls was observed at the end of 3 weeks, reaching significance in the 2.5 and 10 mg/kg/day exposure groups. This coincided with a non-significant increase in T-helper ($CD3^+CD4^+$) and T-cytotoxic ($CD3^+CD8^+$) lymphocytes in the 5 and 10 mg/kg/day groups, all relative to controls. The percentages of T-

helper ($CD3^+CD4^+$) and T-cytotoxic ($CD3^+CD8^+$) lymphocytes were increased in the 10 mg/kg/day groups {Lv, 2015, 3981558}.

Further effects of PFOS on immune cell composition in the spleen have also been reported following developmental exposure by Keil et al. (2008, 1332422) and Zhong et al. (2016, 3748828). Zhong et al. (2016, 3748828) exposed pregnant female C57BL/6 mice to 0.1–5 mg/kg/day PFOS from GD 1–GD 17, and then quantified various immune cell populations in male and female pups. Decreased splenic cell sub-populations ($CD4^+$ and $CD8^+$ cell counts) were observed in the 4-week-old male pups from the 5 mg/kg/day exposure group. At 8-weeks, reductions in $CD8^+$ cells in the spleen were observed in the 5 mg/kg/day exposure group {Zhong, 2016, 3748828}.

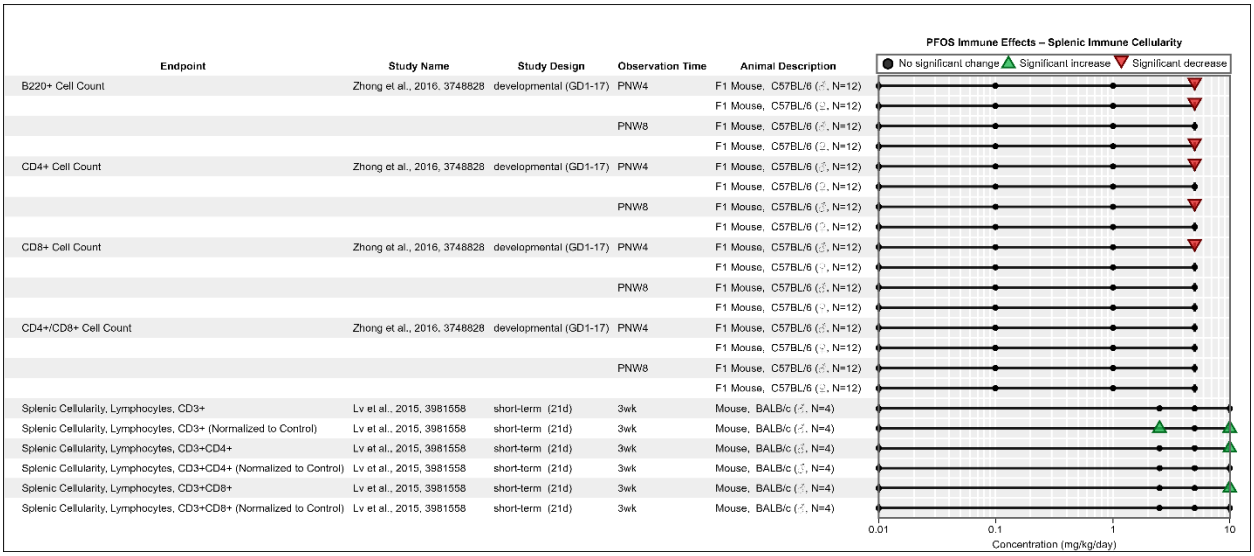


Figure 3-27. Splenocyte Cellularity in Rodents Following Exposure to PFOS (logarithmic scale)[a]

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; PNW = postnatal week; F_1 = first generation.
[a] Zhong et al., 2016 reported data on both splenic and thymic lymphocyte populations for the same experimental animals. Results are shown in separate figures.

3.4.2.2.6 Thymus Cellularity

Thymus cell populations were less sensitive to the effects of PFOS compared to the effects observed in the spleen, as determined by the dose where the change occurred and the number of endpoints that changed following PFOS exposure (Figure 3-28). Indeed, while all splenic T cell CD4/CD8 subpopulations were altered in one study of male B6C3F1 mice beginning at 0.1 mg/kg/day exposures, none of the thymic T cell subpopulations were affected. Furthermore, the effects appeared to also have a female-bias; although thymic $CD4^-/CD8^+$ cells were increased in female B6C3F1 mice exposed to 0.033 or 0.166 mg/kg/day, no effects were observed in males {Peden-Adams, 2008, 1424797}. In contrast, significantly decreased thymocyte populations were observed in male C57BL/6 mice exposed to 0.02% PFOS for 10 days {Qazi, 2009, 1937260}, 20 or 40 mg/kg/day PFOS for 7 days {Zheng, 2009, 1429960}, and 0.417–

2.083 mg/kg/day for 60 days {Dong, 2009, 1424951}. Female mice were not evaluated in these studies.

Effects of PFOS on immune cell composition in the thymus have also been reported following developmental exposure. Pregnant female C57BL/6 mice were dosed with 0.1–5 mg/kg/day PFOS from GD 1–GD 17, and immune cell populations were quantified in male and female pups at 4 and 8 weeks after birth. Decreased thymic lymphocyte sub-populations ($CD4^+$, and $CD4^-/CD8^-$ cell counts) and decreased thymic cellularity were observed in the 4-week-old male pups from the 5 mg/kg/day exposure group, and no effects were observed in females {Zhong, 2016, 3748828}. At 8-weeks, no effects were observed in females and reductions in thymic $CD4^+$ cells were observed in males from the 5 mg/kg/day exposure group. These findings were complimented by Keil et al. (2008, 1332422), who observed a reduction in $CD3^+$ and $CD4^+$ thymocytes in 8-week C57BL/6N male mice following exposure to 0.1–5 mg/kg/day from GD 1–GD 17 {Keil, 2008, 1332422}.

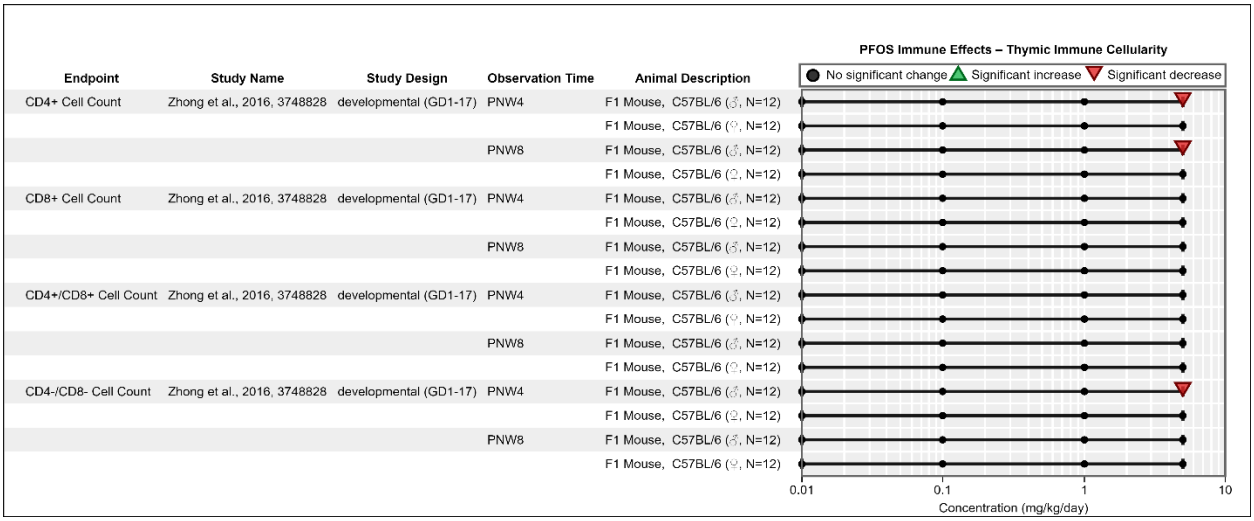


Figure 3-28. Thymocyte Cellularity in Rodents Following Exposure to PFOS (logarithmic scale)

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; PNW = postnatal week; F_1 = first generation.
[a] Zhong et al., 2016 reported data on both splenic and thymic lymphocyte populations for the same experimental animals. Results are shown in separate figures.

3.4.2.2.7 Ability to Generate an Immune Response

Many studies have investigated the effect of PFOS on the ability of rodents to generate an immune response to various antigens. Several mouse studies of varying durations and exposure levels have provided consistent evidence that PFOS can reduce the immune response as determined by reductions in sheep red blood cell-specific immunoglobulin M (IgM) production. Two rodent studies {Lee, 2018 5085013; Yang, 2021 7643494} provide consistent evidence that PFOS can exacerbate the allergic immune response.

Several animal toxicological studies have found evidence indicative of immunosuppression, including reduced IgM titers. Peden-Adams et al. (2008, 1424797) found that the sheep red

blood cell (SRBC) plaque-forming cell (PFC) response, which measures IgM-producing cells, was reduced in male and female B6C3F1 mice administered 0.0017–0.166 mg/kg/day PFOS for 28 days. The response was suppressed at lower PFOS doses in male mice (effect first observed at 0.0017 mg/kg/day) than female mice (effect first observed at 0.017 mg/kg). Because IgM suppression can result from effects on both T and B cells, antibody production was also measured in response to a bacteria-like challenge, trinitrophenyl (TNP)-lipopolysaccharide (LPS), which would induce a T-independent response. Following the TNP-LPS challenge, a decrease in IgM titers was observed in female B6C3F1 mice that had been exposed to 0.334 mg/kg/day PFOS for 21 days. Male animals were not assessed in this study {Peden-Adams, 2008, 1424797}. Similarly, Dong et al. (2009, 1424951) observed a dose-dependent reduction in the SRBC-specific IgM PFC response in male C57BL/6 mice exposed to PFOS daily for 60 days. These results are consistent with a similar study by the same authors in 2011, including a dose-dependent reduction in IgM levels in serum {Dong, 2011 1424949}. The authors also examined the delayed-type hypersensitivity response (DTH) to SRBC. Although IgM levels were reduced in groups exposed to 0.0833 mg/kg/day PFOS or higher, IgG, IgG1, and IgE levels were elevated only in the highest exposure group (0.833 mg/kg/day), and no change was observed in IgG2a levels {Dong, 2011 1424949}. To further assess the DTH response, footpad thickness was measured using digital calipers on the foot used to sensitize the mice to SRBC relative to the non-sensitized foot; no significant increase in footpad swelling was observed. Female mice were not assessed in either of these studies. The DTH response was also assessed by Lefebvre et al. (2008, 1276155) in male and female rats sensitized with the T-dependent antigen, keyhole limpet hemocyanin (KLH), during a 28-day exposure to 0.14–7.58mg/kg/day PFOS (on days 14 and 21) and challenged at the end of study with KLH. There were no significant changes in anti-KLH IgG titers in males or females compared to control, and there were no changes in footpad swelling. Zheng et al. (2009, 1429960) also found that the PFC response to a SRBC challenge was suppressed in male C57BL/6 mice given 5, 20, or 40 mg/kg/day PFOS for 7 days. These rodent studies provide evidence of a PFOS-induced suppression of the immune response to a SRBC challenge that may be more sensitive in male mice (Table 3-8).

Table 3-8. Associations Between PFOS Exposure and Immune Response in Mice

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
Peden-Adams et al. (2008, 1424797)[a]	28 days	0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, 0.166	M	↓ 0.0017–0.166 mg/kg/day
			F	↓ 0.017–0.166 mg/kg/day
Lefebvre et al. (2008, 1276155)[b]	28 days	0, 0.14, 1.33, 3.21, 6.34 (males) or 0, 0.15, 1.43, 3.73, 7.58 (females)	M	n.s.
			F	n.s.
Dong et al. (2009, 1424951)[a]	60 days	0, 0.008, 0.083, 0.417, 0.833, 2.083	M	↓ 0.083–2.083
Dong et al. (2011, 1424949)[a]	60 days	0, 0.008, 0.0167, 0.083, 0.417, 0.833	M	↓ 0.083–0.833
Zheng et al. (2009, 1429960)[a]	7 days	0, 5, 20, 40	M	↓ 5–40 mg/kg/day
Zhong et al. (2016, 3748828)[a]	GD 1–17 4-week assessment	0, 0.1, 1, 5	M	↓ 1–5 mg/kg/day

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
			F	↓ 5 mg/kg/day
	GD 1–17 8-week assessment	0, 0.1, 1, 5	M	n.s.
			F	n.s.
Keil et al. (2008, 1332422)[a]	GD 1–17 8-week assessment	0, 0.1, 1, 5	M	↓ 5 mg/kg/day
			F	n.s.

Notes: F = female; M = male; n.s = nonsignificant.
[a] Sheep red blood cell-specific IgM production.
[b]Keyhole limpet hemocyanin-specific IgG production.

Similar observations were reported in two developmental PFOS exposure studies. Keil et al. (2008, 1332422) and Zhong et al. (2016, 3748828), each exposed pregnant female C57BL/6 mice to 0.1–5 mg/kg/day PFOS from GD 1–GD 17 and then tested the immune responses in offspring at 4 and 8 weeks of age. Four days before sacrifice, mice were injected with SRBC to induce an immune response. In males from the 5 mg/kg/day exposure group, the primary IgM response to SRBC was significantly suppressed by 53% at 8-weeks. In females, the primary IgM response was not altered Keil et al. (2008, 1332422). Similarly, Zhong et al. (2016, 3748828) observed that SRBC-specific IgM production by B-lymphocytes in the spleens of 4-week old mouse pups exposed to 1 or 5 mg/kg/day PFOS *in utero* was reduced by 15% or 28%, respectively. In females, the SRBC-specific IgM response was significantly suppressed by 24% in the 5 mg/kg/day group only. However, no significant changes were observed at 8 weeks.

Alterations in the serum levels of globulin can be associated with decreases in antibody production {FDA, 2002, 88170}. Two 28-day studies {NTP, 2019, 5400978; Curran, 2008, 757871} in male and female Sprague-Dawley rats reported effects on serum globulin levels. In the first study, rats were orally administered 0.312–5 mg/kg/day PFOS. Male rats exhibited significantly decreased globulin while globulin in females did not significantly differ from control values {NTP, 2019, 5400978}. These findings are complemented by a study by Curran et al. {2008, 757871}, in which male and female rats fed diets containing 2–100 mg/kg PFOS (equivalent to 0.14–6.34 mg/kg/day in males and 0.15–7.58 mg/kg/day in females) for 28 days. In male rats, serum albumin/globulin ratios were elevated in the highest exposure group in conjunction with a significant dose-related negative trend in globulin levels. In female rats, no changes were observed in albumin/globulin ratio or globulin levels. In a separate study {Lefebvre, 2008, 1276155} the same authors also reported total levels of IgM, IgG, IgG1, IgG2a, IgG2b, and IgG2c in serum of male and female rats exposed to 0, 2, 20, 50, or 100 mg/kg/day PFOS for 28 days. In males, significant reductions in IgG1 levels were observed at the two lowest doses and a significant positive trend was observed for trend for IgG, IgG2a, and IgG2c. In females, both IgM and IgG2c levels were significantly elevated in the highest dose group.

Two studies by Lee et al. (2018, 5085013) and Yang et al. (2021, 7643494) found evidence that PFOS exposure can exacerbate an allergic immune response in mice. Lee et al. sensitized male ICR mice with ovalbumin (OVA) on day 0 and day 7 and exposed them to 50–150 mg/kg/day PFOS on study day 9, 11, and 13. Serum histamine, TNF-α, IgE, and IgG levels were increased

following exposure, suggesting that PFOS exacerbates mast cell-mediated allergic inflammation. These findings are complemented by studies in male C57BL/6 mice by Yang et al. (2021, 7643494). In that study, mice were exposed to PFOS for 28 days via gavage, sensitized to OVA and adjuvant via subcutaneous injection on days 4 and 11, and challenged with an aerosol of 1% OVA on days 26 to 28. In the serum, exposure to OVA alone or to OVA + PFOS did not lead to elevations in WBC counts, nor percent or number of neutrophils, total lymphocytes, eosinophils, monocytes, and basophils. Serum IgE levels and anti-OVA IgE antibodies were elevated in groups exposed to 0.25 or 2.5 mg/kg/day PFOS + OVA compared to OVA alone or untreated controls. Mice exposed to 0.25 or 2.5 mg/kg/day PFOS alone showed a low level of serum IgE, similar to the control group.

3.4.2.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse immune outcomes is discussed in Sections 3.1.1.6, 3.3.2, 3.3.4, and 3.3.6 of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 24 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to immune effects. A summary of these studies is shown in Figure 3-29.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Big Data, Non-Targeted Analysis	1	0	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	6	0	9	13
Cell Signaling Or Signal Transduction	2	0	4	6
Extracellular Matrix Or Molecules	0	0	2	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	1	2
Hormone Function	1	0	0	1
Inflammation And Immune Response	6	5	12	19
Oxidative Stress	1	0	3	4
Other	1	0	0	1
Grand Total	8	5	15	24

Figure 3-29. Summary of Mechanistic Studies of PFOS and Immune Effects

Interactive figure and additional study details available on Tableau.

3.4.2.3.1 Mechanistic Evidence for PFOS-mediated Effects on the Immune System

Since the 2016 HESD advisory was released, 26 studies were identified that inform the mechanism by which PFOS may alter or perturb immune system function or immune system development and physiology. Recent studies provide mechanistic insights into PFOS effects on immune system development and physiology (5 studies), adaptive immune responses (6 studies), innate immune responses (4 studies), intrinsic cellular defense (1 study), and disruption of inflammatory responses (9 studies). Mechanistic pathways associated with the immune system identified in the recent PFOS literature included inflammation, immune responses, cell viability, cell signaling, oxidative stress, and hormone function.

3.4.2.3.1.1 Mechanistic Evidence for PFOS-mediated Effects on Immune System Development and Physiology

Alterations in immune and allergic responses in exposed children may suggest PFOS-mediated effects in immune system development. In addition, changes in white blood cell count {Oulhote, 2017, 3748921} and alterations in gene expression related to immune and inflammation responses in human cord blood {Pennings, 2016, 3352001} present potential mechanisms of immunotoxicity in children. In animals, PFOS-related health effects related to immune system development and physiology are described in Sections 3.4.2.2.1 to 3.4.2.2.7. Briefly, effects in mice and rats included reduced spleen and thymus weights, alterations in spleen and thymus morphology, and changes in the cellularity and immunophenotypes of lymphocytes. Effects varied by sex and strain.

Three mechanistic studies in mice suggest that changes in immune physiology and development following exposure to PFOS can be sex-dependent. Zhong et al. (2016, 3748828) demonstrated sex-specific impacts of PFOS on immune organ development and physiology in C57BL/6 mice exposed during development. Pups were evaluated after maternal oral exposure to PFOS (0.1, 1.0, or 5.0 mg PFOS/kg/day) from gestational day (GD) 1–17. Sex-dependent alterations in spleen and thymus organ weights, cellularity, and cellular immunophenotypes are discussed in Section 3.4.2.2. These may be linked to sex hormones during development as there was a significant interaction between sex and PFOS concentrations for serum testosterone at 4 and 8 weeks of age, and estradiol at 4 weeks of age. The authors suggest that sex-dependent differences in PFOS excretion, the endocrine-disrupting properties of PFOS, or male or female sex hormone-differences may influence the sex-specific impact on spleen and thymus physiology.

Lv et al. (2015, 3981558) reported disrupted splenic architecture and reduced absolute numbers (albeit increased percentages) of T helper (CD3+CD4+) and cytotoxic T (CD3+CD8+) cells in the spleen of male BALB/c mice administered 10 mg/kg/day PFOS via gastric gavage for 3 weeks followed by a 1-week recovery. Gene expression profiling identified differential regulation of genes involved in mitogen-activated protein kinase (MAPK) signal transduction pathways and in cellular responses to oxidative stress. The effects on gene expression paralleled a dose-dependent increase in intracellular free calcium ([Ca^{2+}], which plays an important role in immune cell proliferation in response to foreign antigens) concentration in splenocytes of exposed animals, suggesting that activation of MAPK signaling pathway and/or oxidative stress genes in response to PFOS may alter splenic architecture via induction of apoptosis in lymphocytes.

Qazi et al. (2012, 1937236) also observed decreased spleen and thymus weights and cellularity as well as reduced numbers of myeloid, pro/pre-B, and immature B cells in bone marrow (BM). In male C57BL/6 (H-2b) mice fed diets containing PFOS compounds (0.001–0.02%, w/w) for 10 days, atrophy of the thymus and spleen as well as hypocellularity of BM was observed at the higher dose of 0.02%. PFOS exposure caused reduced feed consumption and atrophy of the thymus and spleen and hypocellularity of bone marrow cells. Histopathological and flow cytometric analysis of BM showed significant reductions in the total numbers of bone marrow cells as well as the numbers of pro/pre-B (CD19 + CD138 + IgM+) and immature B (CD19+ CD138+ IgM+) cells. Myeloid (Gr1+ CD11b+) cells and B-lymphoid (CD19+) cells were also reduced in mice administered the high dose of PFOS. After 10 days of withdrawal of PFOS from

feed, the effects in bone marrow partially or completely reversed. Interestingly, food restriction alone in the absence of PFOS exposure also led to reduced cell numbers in the thymus and spleen and resulted in reductions of the total numbers of B-lymphoid cells, pro/pre-B, and immature B cells. These findings indicate that immunotoxicity of PFOS may, at least in part, be a consequence of reduced food consumption. Additionally, perturbation of the bone marrow may contribute to reduced numbers of splenic B cells, atrophy of the spleen, and impaired humoral immune responses caused by exposure to PFOS.

3.4.2.3.2 Mechanistic Evidence for PFOS-mediated Effects on Adaptive Immune Responses

3.4.2.3.2.1 Mechanistic data informing suppression of immune responses to vaccines and infectious diseases

The effects of prenatal, childhood, or adult PFOS exposure on responses to vaccines and infectious diseases are described in Section 3.4.2.1. Briefly, studies observed an inverse association between PFOS exposure and vaccine-induced antibody levels to tetanus and to pathogens including human foot and mouth disease (HFMD) and hepatitis B infection. Other studies identified associations between PFOS exposure and increased incidence of infections including those caused by pneumonia and chickenpox, though PFOS was associated with a decrease in the incidence of respiratory syncytial virus (RSV), common cold, ear infection, and urinary tract infection. Six new mechanistic studies were identified that inform PFOS-mediated effects on adaptive immunity (3 in humans and 3 in mice). One mechanistic study directly evaluated PFOS-mediated effects on adaptive immune responses specific to vaccines and infectious disease {Pennings, 2016, 3352001}, and 5 mechanistic studies evaluated non-allergic adaptive immune responses.

As described in Section 3.4.2.1.1, in children exposed to PFOS *in utero*, Granum et al. (2013, 1937228) previously reported an inverse association between maternal serum concentrations of PFOS and anti-rubella antibody levels in serum of 3-year-old children, as well as an increased incidence of the common cold, using samples and data from the Norwegian BraMat cohort. In a follow-up study of early life immunosuppression again using Norwegian BraMat cohort data, Pennings et al. (2016, 3352001) conducted a whole genome transcriptomic microarray analysis of neonatal cord blood samples and compared the results to maternal levels of PFOS (as well as PFOA, perfluorononanoic acid (PFNA), and perfluorohexane sulfonate (PFHxS)) in the blood. Dose–response relationships between PFOS and expression of individual genes, rubella antibody levels, and episodes of the common cold were analyzed. Expression of 636 genes was positively associated with PFOS exposure, and 671 were negatively correlated. A set of 27 genes were correlated between all four of the PFAS evaluated and the number of common cold episodes. Of these, three genes were related to immunological and/or hematopoietic functions, including peroxisome proliferator activated receptor delta (PPARD), SHC adaptor protein 4 (SHC4), and cytokine like 1 (CYTL1), expressed in CD34+ in bone marrow and cord blood mononuclear cells. Of the six genes related to development and/or morphogenesis, two overlapped with immune and hematopoietic functions (PPARD and CYTL1). Interestingly, another gene associated with development and morphogenesis, sphingosine-1-phosphate lyase 1 (SGPL1), has been recently associated with immune responses to viral infections including inhibition of influenza virus replication by promoting antiviral type I interferon innate immune responses {Wolf, 2019, 10259528}. A set of 26 genes overlapped between PFAS and rubella titers,

including two genes also identified in pathway analysis as relevant to regulation of T cell activation (interleukin 27 [IL27] and the adenosine A2a receptor [ADORA2A]). Only one gene (CYTL1) was in common between the sets of genes that overlapped with PFAS exposure and common cold episodes, and PFAS exposure and rubella titers. However, a clear understanding of the function of CYTL1 in hematopoiesis and immune function is lacking. While the correlation between gene expression changes and changes in protein expression or function in cord blood was not investigated in this study, these represent potential candidate genes that mediate the mechanism(s) of early childhood immunotoxicity associated with prenatal exposure to PFOS and other PFAS chemicals.

Lv et al. (2015, 3981558) examined T cells in male BALB/c mice administered 10 mg/kg/day PFOS via gavage for 3 weeks followed by 1-week recovery. Gene expression profiling in spleens was performed using GeneChip® Mouse Genome 430 2.0 Array (Affymetrix Inc., Santa Clara, CA, USA) and quantitative real time PCR (qRT-PCR). The authors identified 1,327 differentially expressed genes (4% of all analyzed genes) in response to PFOS exposure. Biological processes associated with differentially expressed genes included cell cycle, DNA metabolism, mitosis, and DNA replication. Pathway analysis identified significantly upregulated pathways related to the T cell receptor (TCR) and to immune signaling (primary immunodeficiency signaling, inducible co-stimulator [iCOS] – iCOS ligand [iCOSL] signaling in T helper cells, OX40 signaling pathway, and calcium-induced T lymphocyte apoptosis). However, the transducer of ErbB-2.1 (TOB) T cell signaling pathway was significantly downregulated, as were genes associated with nuclear factor erythroid derived 2 like 2 (Nrf2)-mediated oxidative stress response (such as GSTM3 and MGST3). During the recovery period following four weeks of PFOS exposure, immunoblotting confirmed a dose-dependent upregulation of protein levels in spleens for several genes involved in TCR signaling and calcium signaling, including thymocyte selection associated (THEMIS), the CD3 gamma subunit of T-cell receptor complex (CD3G), and calcium/calmodulin dependent protein kinase IV (CAMK4). Additionally, in splenocytes of exposed animals, [Ca2+]i increased in a concentration-dependent manner, and T-cell proliferation in response to Concanavalin A (Con A) stimulation was inhibited by PFOS. The authors suggest that activation of MAPK signaling pathway and/or oxidative stress genes in response to PFOS may alter splenic architecture via induction of apoptosis in lymphocytes. These findings also suggest that altered expression of cell cycle genes, upregulation of genes involved in TCR signaling, and altered calcium homeostasis impact T cell function through inhibition of T cell proliferation and induction of T cell anergy (intrinsic functional inactivation of lymphocytes following an antigen encounter).

Li et al. (2020, 6833655) used an integrative ‘omics approach to evaluate perturbations in the transcriptome and lipidome in human lymphocytes that may impact adaptive immune responses to vaccines or infectious diseases. Lymphocytes were isolated from human donors and cultured before treatment with 50 mM PFOS for 72 hours. PFOS treatment led to a significant induction of the cytokines IL-1, IL-4, IL-6, and IL-8 cytokines relative to controls, as measured by ELISA. Subsequent deep sequencing of RNA for PFOS-treated lymphocytes revealed that numerous differentially expressed genes were related to lymphocyte function and biological processes related to immunity, including immune responses, innate immune responses, and inflammatory responses. Enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database linked PFOS treatment to stimulation of cytokine-cytokine receptor interactions, extracellular matrix (ECM)-receptor interactions, the PI3K-Akt signaling pathway, the

peroxisome proliferator-activated receptor (PPAR) signaling pathway, cholesterol metabolism, and phagosome and lysosome regulation at the gene expression level. The analysis identified differentially expressed genes associated with cytokines, growth factors, and differentiation and migration of antigen-presenting cells. Additionally, the authors conducted a lipidomic analysis of treated cells using liquid chromatography–mass spectrometry (LC-MS). Lipid metabolites (40 upregulated and 56 downregulated) were identified in PFOS-exposed lymphocytes relative to control lymphocytes. Clusters of lipids associated with immune function were dysregulated, including lipids involved in glycerophospholipid metabolism, sphingolipid metabolism, glycerolipid metabolism, adipocytokine signaling, regulation of autophagy, and arachidonic acid metabolism. Taken together with the transcriptomic and functional analyses reported by Lv et al. (2015, 3981558) and Pennings et al. (2016, 3352001), these findings suggest that PFOS exposure may disrupt adaptive immunity through dysregulation of genes and lipids involved in lymphocyte survival, proliferation, and anergy.

The potential for PFOS to suppress immune responses to vaccines and infection are also informed by studies investigating PFOS-mediated effects on TH1/TH2-type cytokines in mice {Zhong, 2016, 3748828}, glycosylation of immunoglobulins in humans {Liu, 2020, 6833599}, and lymphocyte toxicity *in vitro* {Zarei, 2018, 5079848}. Zhong et al. (2016, 3748828) exposed pregnant female C57BL/6 mice to PFOS (0.1, 1.0, or 5.0 mg/kg/day) from GD 1–17 and cultured splenocytes of male pups at 4 and 8 weeks of age. Spontaneous IL-4 formation was increased and spontaneous production of TH1 cytokines (i.e., IL-2) was decreased in the 5 mg/kg/day group at 8 weeks. Functionally, lymphocyte proliferation was significantly decreased in splenocytes from both males and females exposed to the highest dose at 4 weeks, and natural killer (NK) cell activity exhibited a decreasing trend with dose (males only at 4 weeks, males and females at 8 weeks). Given the reductions in serum testosterone at 4 and 8 weeks of age, and increased estradiol levels in male pups at 4 weeks of age (discussed in Section 3.4.2.2), these findings suggest that in utero exposure may elicit sex-specific alterations in TH1 and TH2 cytokine profiles in immune cells as well as diminished lymphocyte and NK functions.

A recent study suggests that PFOS may also alter antibody glycosylation patterns {Liu, 2020, 6833599}. Altered IgG glycosylation patterns are associated with disease states and immune functions including cancer immunosurveillance and anti-inflammatory reactions {Cobb, 2020, 10284268}. The N-glycome profiles of immunoglobulins from serum samples of adults and children were analyzed by subjecting the IgG fraction to glycan release, derivatization, and matrix-assisted laser desorption/ionization-MS (MALDI-MS) analysis. Specifically, increasing PFOS exposure was associated with decreased galactosylation, increased fucosylation and sialylation in adults, and increased agalactosylation, bisecting GlcNAcylation, sialylation and decreased galactosylation in children. The authors suggested several mechanisms by which altered IgG glycosylation impacts immunity including antibody-dependent cellular cytotoxicity (ADCC). While no functional studies were conducted, these preliminary findings provide a potential mechanism for altered antibody-dependent immune responses in PFOS-exposed persons.

Zarei et al. (2018, 5079848) isolated lymphocytes from the blood of healthy humans and analyzed cytotoxicity *in vitro* in response to exposure to 100-500 µM PFOS for 12 hours. The IC50 for cytotoxicity was calculated to be 163.5 µM. Exposure to 75, 150, and 300 µM PFOS for 2, 4, 6, 8, 10, or 12 hours was associated with increased reactive oxygen species (ROS)

formation, lipid peroxidation, and glutathione depletion. PFOS also damaged mitochondrial and lysosomal membranes and was associated with significantly increased levels of cellular proteolysis and caspase 3 activity. These findings suggest that PFOS could mediate immunosuppressive effects through direct cytotoxicity of lymphocytes.

3.4.2.3.2.2 Mechanistic data informing autoimmune diseases

As described in Section 3.4.2.1, two studies reported that PFOS levels in healthy controls were either higher than in ulcerative colitis (UC) cases {Steenland, 2018, 5079806} or lower than in multiple sclerosis (MS) cases {Ammitzbøll, 2019, 5080379}. While no mechanistic studies directly investigated the mechanism by which PFOS could promote the development of autoimmunity, one study evaluated PFOS effects on TH17 cells, implicated in the pathophysiology of both MS and UC {Chen, 2020, 10284264; Fu, 2020, 10284269}. Suo et al. (2017, 3981310) examined the effects of 2 mg/kg PFOS in a mouse model of *Citrobacter rodentium* infection. PFOS was administered for 7 days by oral gavage before mice were infected with C. rodentium and throughout the early and late phases of infection. Large intestinal lamina proprial lymphocytes were isolated 5 days after infection and analyzed by flow cytometry after treatment with immune stimulators. Levels of IL-17 and IL-22 produced by Th17 cells were significantly elevated in PFOS-treated mice compared to the control group. These findings support that PFOS-mediated effects on pathogenic TH17 cells may impact development of autoimmune diseases as well as bacterial infections of the gut.

3.4.2.3.2.3 Mechanistic data informing allergic responses

Several studies were identified that evaluated associations between PFOS exposure and immune hypersensitivity, including asthma, allergy, and eczema as described in Section 3.4.2.1.2. Five new mechanistic studies informed allergy and asthma. Oulhote et al. (2017, 3748921) observed a significant association between PFAS exposures and increased basophil counts between birth and age 5 in human children. Although PFAS exposure was analyzed collectively (included PFOA, PFOS, PFHxS, PFNA, and perfluorodecanoic acid [PFDA]), PFOS showed the highest serum concentrations at all ages. The authors suggested that enhanced basophil levels could be associated with dysregulated allergic and asthma-related responses, possibly by promoting TH2-type responses.

Zhu et al. (2016, 3360105) evaluated 231 asthmatic children and 225 non-asthmatic control children from Northern Taiwan. A significant positive association was identified for PFOS blood levels and TH2 cytokines while a non-significant inverse association was found for TH1 cytokines among asthmatic children. Male asthmatics exhibited elevated IgE levels with increasing PFOS levels. Also, in males only, significant positive associations between PFOS levels in blood and TH2:TH1 cytokine ratios were observed for both the IL-4/IFN-γ ratio and IL-5/IFN-γ ratio. This finding suggests that PFOS may exacerbate asthma by altering availability of key TH1 and TH2 cytokines. However, the effects of PFOS on TH1- and TH2-type cytokine profiles may be dependent on disease context or the cell types under study. For example, in earlier studies of human peripheral blood leukocytes (PBLs) treated with phytohemagglutinin (PHA), PFOS exposure led to diminished IL-4, IL10, and IFN-γ {NTP, 2016, 5080063; Corsini, 2011, 1937246; Corsini, 2012, 1937239}.

Lee et al. (2018, 5085013) used an albumin-induced active systemic anaphylaxis model to evaluate type I hypersensitivity in mice. After sensitization with ovalbumin (OVA), PFOS (50-

150 mg/kg) was orally administered on days 9, 11, and 13. On day 14, OVA was administered by intraperitoneal (IP) injection, and mice were evaluated for signs of allergy. PFOS significantly aggravated allergic symptoms such as hypothermia and significantly increased serum histamine, TNF-α, IgE, and IgG1 relative to controls. Further findings suggest the mechanism of aggravated allergic responses mediated by PFOS is through release of histamine and β hexosaminidase associated with up-regulation of intracellular calcium in IgE-stimulated mast cells. Elevated levels of inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) were also observed in PFOS-exposed non-sensitized rat basophilic leukemia cells, which were linked to NF-kB activation. Together, these findings provide a plausible pathway for PFOS-mediated exacerbation of allergic responses.

3.4.2.3.2.4 Mechanistic Evidence for PFOS-mediated Effects on Innate Immune Responses

As described in Sections 3.4.2.2.3 and 3.4.2.2.4, several studies in animals suggest PFOS may negatively impact NK cells and macrophage function, indicating innate immune effector cells are susceptible to perturbations by PFOS. Very few studies were identified that evaluated the mechanisms by which PFOS may alter innate immunity and no studies evaluated the mechanisms by which PFOS alters NK cell activity. Among the studies reporting NK activity in Table 3-7 in section 3.4.2.2.4, most studies observed decreased NK activity, though at least one study observed enhanced NK responses at low doses of exposure {Dong, 2009, 1424951}. In all of these studies, NK cells were obtained from animals exposed *in vivo* and analyzed *in vitro* using target cells that were not exposed to PFOS, suggesting PFOS directly alters NK maturation or activity. Whether PFOS alters the spectrum of activating and inhibiting receptors on NK cells or some other aspect of NK activity is not known. At least one study treated NK and target YAC-1 cells *in vitro*, though neither NK receptor nor ligand expression were evaluated {Wirth, 2014, 1937219}. Thus, an important outstanding mechanistic question that may directly impact observations of dose- and sex-dependent effects is whether PFOS alters expression of NK cell receptors or target cell ligands for NK receptors.

Two studies were identified that evaluated mechanisms of PFOS activity on innate immune responses mediated by macrophages, and one evaluated PFOS effects on gut immunity and innate lymphoid cells (ILC3). Rainieri et al. (2017, 3860104) measured PFOS effects in TREM-like transcript (TLT) cells, a human macrophage-derived cell line. Treatment of cells with 15.6-500 mg/L PFOS for 24 hours increased cell viability relative to controls, which was associated with a significant decrease in the number of apoptotic cells. Using non-confluent cell cultures, 500 mg/L PFOS treatment significantly decreased the number of cells in the G2/M phase. PFOS treatment significantly increased ROS production. However, Berntsen et al. (2018, 4167035) found no PFOS-specific effects on macrophage phagocytosis in primary cells including peritoneal macrophages (PCM) from adult Wistar rats and C57Bl/6 mice, non-obese diabetic (NOD) mice, IL-1 knockout (KO) mice, and newly born rats. In addition, PFOS did not alter phagocytosis in human or rat monocyte-derived macrophages (MDM). Taken together, these limited findings suggest that while PFOS does not alter macrophage function, it may affect viability and induce ROS and lipid peroxidation in macrophage cell lines.

Suo et al. (2017, 3981310) examined effects of PFOS in a mouse model of *C. rodentium* infection. PFOS at 2 mg/kg or vehicle control was administered for 7 days before infecting mice with *C. rodentium* and throughout the observation period of infection. Part of this study

evaluated effects on ILC3s, which have been suggested to be important in controlling *C. rodentium* at the early phase of infection prior to induction of adaptive immune responses. ILC3s secrete IL-17 and IL-22 that act to stimulate epithelial cells to secrete anti-microbial peptides or through recruitment of neutrophils {Takatori, 2009, 9811595; Zheng, 2008, 10284265; Ishigame, 2009, 10284267}. PFOS inhibited the expansion of *C. rodentium* by promoting IL-22 production in ILC3 cells in an aryl hydrocarbon receptor (AhR)-dependent manner. However, PFOS also led to decreased mucin production from goblet cells, which may contribute to the observation that PFOS altered the gut microbiome. Specifically, PFOS-exposed mice at late stages of infection exhibited decreased levels of *Lactobacillus casei* and *Lactobacillus johnsonii*, and increased levels of *E. coli*. The authors crossed Ahrf/f mice (in which the Ahr gene is flanked by loxP sites) to mice in which the cre recombinase gene is driven by the RAR-related orphan receptor gamma promoter (RORc-cre) to delete Ahr in ILC3 and T cells (Ahrf/f RORc-cre). Cells isolated from either Ahrf/f RORc-cre or Ahrf/f mice were exposed to PFOS, and cytokines were analyzed using flow cytometry. PFOS-exposed mice exhibited increased IFN-γ production from CD3− non-T cells compared to control mice, indicating a pro-inflammatory role of PFOS. Taken together, PFOS-associated dysbiosis and persistent inflammation in the intestine ultimately led to a failure to clear *C. rodentium* at the late phase of infection. These findings suggest PFOS may impact gastrointestinal health in animals (See PFOS Appendix) and raises the possibility that immune mechanisms associated with AhR activation are disrupted by PFOS.

3.4.2.3.2.5 Mechanistic Evidence for PFOS-mediated Effects on Intrinsic Cellular Defense Pathways

There is limited evidence of PFOS exposure related to the disruption of intrinsic cellular defense pathways. Sørli et al. (2020, 5918817) used HBEC3-KT human bronchial epithelial cells to study inflammatory changes in response to PFOS, including modulation of the inflammatory response induced by polyinosinic:polycytidylic acid (Poly I:C), a toll-like receptor 3 (TLR3) ligand. In cells exposed to 30 or 60 μM PFOS for 48 hours, IL-1α/β release was elevated, indicative of a pro-inflammatory response. In cells treated with 5 μg/mL poly I:C for 3 hours followed by exposure to 10 μM PFOS for 48 hours, release of the chemokines CXCL8 and CXCL10 was suppressed, but IL-1 α/β release was enhanced. The authors hypothesized that IL- β release may be related to the fact that it requires only proteolytic cleavage of preformed IL-1 in the cytosol, and thus may not be dependent on TLR3-dependent gene expression. The authors also hypothesized that PFOS may inhibit NF-κB activation in a cell type-dependent manner in the lung. TLR3 stability and/or function, other double-stranded RNA sensors in these cells, or associated signal transduction pathways were not evaluated. These results indicate that PFOS can exert divergent effects on chemokine and cytokine release in a dose-dependent manner in human bronchial epithelial cells and modulates the activity of intrinsic cellular defense responses mediated by toll receptors and/or other double-stranded RNA sensors.

3.4.2.3.2.6 Mechanistic Evidence for PFOS-mediated Effects on Inflammation

PFOS-mediated effects on inflammation may impact a wide range of diseases given that chronic inflammation can be a key driver of many diseases such as cancer, cardiovascular, metabolic, and neurological diseases {Hunter, 2012, 10284266}. Earlier studies suggest that PFOS differentially impacts pro-inflammatory cytokine release in a cell type and tissue-specific manner. For example, as described in 2016 PFOS HESD {U.S. EPA, 2016, 3603365}, cells isolated from the peritoneal cavity and bone marrow, but not spleen, of mice exposed to high

levels of PFOS had enhanced levels of the pro-inflammatory cytokines, TNF-α and IL-6, in response to stimulation by lipopolysaccharide (LPS). The levels of these cytokines in the serum were not elevated {Qazi, 2009, 1937259}. Since the 2016 document, 9 additional mechanistic studies reported correlations between PFOS exposure and modulation of pro-inflammatory cytokines or serum markers of inflammation. Consequences of PFOS exposure are not consistent across species and are summarized in Table 3-9. Pro-inflammatory cytokines were elevated in PFOS-exposed rodents and in human and animal cells in culture. In both studies evaluating human subjects {Bassler, 2019, 5080624; Mitro, 2020, 6833625}, either no significant changes were observed in serum cytokine or marker levels (IL-6, IFN-γ, C-reactive protein [CRP], or C3a) or levels were reduced (TNF-α, IL-8) relative to subjects with lower PFOS exposures.

Table 3-9. Effects of PFOS Exposure on Pro-Inflammatory Cytokines and Markers of Inflammation

Study	Species or Cell Type	Cytokine or Inflammatory Marker	Matrix and Measurement	Direction of Change Following PFOS Exposure
Mitro et al. (2020, 6833625)	Human females 3 years postpartum, Project Viva	IL-6	blood protein (ELISA)	None
		CRP	blood protein (immunoturbidimetric high-sensitivity assay)	None
Bassler et al. (2019, 5080624)	Human males and females, C8 Health Project	IL-6	serum protein (Multispot Immunoassay)	None
		TNF-α	serum protein (Multispot Immunoassay)	↓
		IL-8	serum protein (Multispot Immunoassay)	↓
		IFN-γ	serum protein (Multispot Immunoassay)	None
		C3a	serum protein (ELISA)	↓
Li et al. (2020, 6833655)	Human lymphocytes	IL-1	culture supernatant protein (ELISA)	↑
		IL-6	culture supernatant protein (ELISA)	↑
Sørli et al. (2020, 5918817)	Human bronchial epithelial cell line	IL-1α	culture supernatant protein (ELISA)	↑
		IL-1β	culture supernatant protein (ELISA)	↑
Liao et al. (2013, 1937227)	Human umbilical vein endothelial cells (HUVECs)	IL-6	cellular mRNA (qRT-PCR)	↑
		IL-1β	cellular mRNA (qRT-PCR)	↑
Han et al. (2018, 4355066)	Sprague-Dawley male rats	IL-6	serum protein (ELISA)	↑
		TNF-α	serum protein (ELISA)	↑
Su et al. (2019, 5080481)	ICR male mice	IL-6	serum protein (ELISA)	↑
		TNF-α	serum protein (ELISA)	↑

Study	Species or Cell Type	Cytokine or Inflammatory Marker	Matrix and Measurement	Direction of Change Following PFOS Exposure
Han et al. (2018, 4355066)	Primary rat hepatocytes and Kupffer cells	IL-6	cellular mRNA (PCR) and culture supernatant protein (ELISA)	↑
		TNF-α	cellular mRNA (PCR) and culture supernatant protein (ELISA)	↑
Zhu et al. (2015, 2850996)	Murine microglial cell line	IL-6	cellular mRNA (PCR) and culture supernatant protein (ELISA)	↑
		TNF-α	cellular mRNA (PCR) and culture supernatant protein (ELISA)	↑

Notes: C3a = cohort 3a; CRP = C-reactive protein; ELISA = enzyme-linked immunosorbent assay; IL-1α = interleukin 1 alpha; IL-1β = interleukin 1 beta; IL-6 = interleukin 6; IL-8 = interleukin 8; PCR = polymerase chain reaction; TNF-α = tumor necrosis factor alpha; qRT-PCR = quantitative reverse transcription polymerase chain reaction.

3.4.2.3.2.6.1 Animal Toxicological Studies

Han et al. (2018, 4355066) investigated PFOS-effects on hepatic inflammation in male Sprague-Dawley (SD) rats exposed to 1 or 10 mg/kg body weight PFOS by gavage and in isolated primary rat Kupffer cells cultured *in vitro*. *In vivo*, PFOS induced Kupffer cell activation and elevated serum TNF-α and IL-6 and stimulated release of these cytokines from cultured primary Kupffer cells *in vitro*. Studies with a Kupffer cell-blocking and depleting agent, gandolinium chloride (GdCL3), demonstrated that PFOS exposure stimulated Kupffer cell release of TNF-α and IL-6 *in vivo* (measured by ELISA) and *in vitro* (increased mRNA expression measured by PCR and protein expression measured by ELISA). Furthermore, Kupffer cell activation was mitigated by treatment with anti-TNF-α or anti-IL-6 antibodies. *In vivo*, PFOS exposure upregulated the protein expression of proliferating cell nuclear antigen (PCNA), c-Jun, c-MYC, and Cyclin D1 (CyD1) in liver, a finding mirrored in Kupffer cells cultured *in vitro*. Treatment with a drug inhibitor of NF-κB (pyrrolidine dithiocarbamate [PDTC]) and a c-Jun N-terminal kinase (JNK) inhibitor (SP600125) significantly inhibited production of PFOS-induced TNF-α and IL-6. Together, these findings suggest that PFOS induces Kupffer cell activation, leading to NF-κB/TNF- α/IL-6-dependent hepatocyte proliferation.

Su et al. (2019, 5080481) also examined liver-specific immunotoxicity. Male ICR mice were dosed with 10 mg/kg/day for 21 days. TNF-α and IL-6 were significantly elevated, whereas fibroblast growth factor 21 (FGF21) was significantly reduced in sera from these mice. Co-treatment with 200 mg/kg per day of vitamin C led to a significant reversal in PFOS-induced changes in serum TNF-α, IL-6, and FGF21, consistent with results of immunostaining for TNF-α and FGF21 in liver cells. The mechanism by which vitamin C exerts protection from inflammatory responses in this model was not elucidated.

3.4.2.3.2.6.2 *In Vitro* Studies

Four studies demonstrated increased inflammatory cytokine expression in human cells cultured *in vitro*. PFOS exposure at concentrations of ≥30 μM led to increased IL-1α/β release in HBEC3-KT human bronchial epithelial cells {Sørli, 2020, 5918817}. Li et al. (2020, 6833655) demonstrated induction of IL-1 and IL-6 in human lymphocytes that were isolated from human

donors and exposed in culture to 50 mM PFOS for 72 hours. Giménez-Bastida and Surma (2015, 3981569) investigated inflammatory cytokine responses in human CCD-18 Co myofibroblasts as a model of colonic subepithelial myofibroblasts in the intestinal lamina propria. Cells were exposed to PFOS at concentrations ranging from 0.6 to 100 μM in combination with IL-1β (1 ng/mL). Exposure to PFOS reduced IL-1β-induced IL-6 production at all doses except 100 μM, but this reduction only reached significance at 6 μM. Liao et al. (2013, 1937227) pretreated human umbilical cord endothelial cells (HUVECs) with 100 mg/L PFOS for 5 hours and then co-treated with polyphenols (Flos Lonicerae extract and chlorogenic acid) for 24 or 48 hours. PFOS exposure resulted in increased levels of mRNA transcripts for inflammatory cytokines (IL-1β, IL-6) as well as COX-2 (cyclooxygenase 2) and NOS3 (nitric oxide synthase 3), the protein products of which function in cellular defense and prostaglandin synthesis. PFOS exposure also led to upregulation of transcripts for adhesion molecules P-Selectin (SELP) and ICAM1 (intercellular adhesion molecule 1). Functionally, PFOS treatment for 48 h increased adhesion of THP-1 monocytes to HUVECs. These PFOS-mediated changes in HUVECs were mitigated by co-treatment of cells with polyphenols.

In immortalized murine BV2 microglial cells, which are brain resident macrophage-like cells that are considered central to inflammatory responses in the brain, PFOS exposure increased inflammatory cytokine expression {Zhu, 2015, 2850996} via similar pathways observed in primary rat hepatocytes and Kupffer cells exposed to 100 μM PFOS {Han, 2018, 4355066}. Zhu et al. (2015, 2850996) reported that treatment with 10 μM PFOS for 6 hours resulted in increased levels of Tnfα and Il6 gene expression. Time course studies were performed using 1 μM PFOS and indicated that elevated Tnf-α and IL-6 mRNA expression occurs within 1 hour, peaks at 3 hours, and begins to diminish by 6 hours of PFOS exposure. Protein levels of these cytokines in culture supernatant continually increased with 6, 12, and 24 hours of 1 μM PFOS treatment. Transcriptional activation of TNF-α and IL-6 correlated with activation of NF-κB (measured by immunoblot of the phosphorylated form) and was mitigated by targeting JNK and the extracellular regulate kinase (ERK1/2) with a drug inhibitor (SP600125) or blocker (PD98059). Together, the data support a role for MAPK signaling pathways and NF-κB activation in PFOS-mediated inflammatory gene expression in cultured microglial cells and primary Kupffer cells.

In addition to activation of MAPK signal transduction pathways, epigenetic mechanisms may impact inflammatory gene expression mediated by PFOS. Park et al. (2019, 5412425) found increased gene expression of sirtuin (SIRT) genes in RAW 264.7 macrophage cells (cell line derived from BALB/c mice). The SIRT family of proteins act to deacetylate the lysine residues of histone proteins, but they also can deacetylate nonhistone substrates, such as inflammation-related transcription factors including NF-κB (Frescas, 2005, 10284417; Yeung, 2004, 10284418}. PFOS exposure increased expression of Sirt2, Sirt3, Sirt5, and Sirt6. The authors did not investigate the effect of increased expression of Sirt genes observed after PFOS on the acetylation status or expression of inflammatory proteins.

3.4.2.3.2.6.3 Human Studies

Bassler et al. (2019, 5080624) examined 200 adult participants of the C8 Health Project to test the hypothesis that environmental perfluoroalkyl acids (PFAAs) are associated with increased hepatocyte apoptosis and decreased pro-inflammatory cytokines in serum. In support of this hypothesis, PFOS levels were associated with significantly reduced serum TNF-α and IL-8 serum levels. However, there was no correlation between PFOS serum levels and other cytokines

(IL-6, IFN-γ), inflammatory markers (cleaved complement C3a) or markers of hepatocyte cell death (caspase 3 cleaved cytokeratin 18). The authors hypothesized that under certain circumstances such as with nonalcoholic fatty liver disease (NAFLD), PFAAs are associated with immunotoxic suppressive effects on innate immunity and inflammation.

Mitro et al. (2020, 6833625) set out to evaluate PFAS exposures and cardiometabolic health in pregnant women and in the years postpartum as part of Project Viva. The study obtained 3-year postpartum anthropometry measurements and blood biomarker measurements of inflammation including IL-6 and CRP. While exposure to some PFAS was associated with elevated IL-6 levels 3 years postpartum, no significant associations were observed for PFOS. None of the PFAS chemicals examined other than 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (MeFOSAA) showed a strong association with CRP levels in this study.

3.4.2.3.2.7 Summary

Since publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}, new mechanistic information has emerged informing immune system physiology, innate and adaptive immune functions, intrinsic cellular defense, and inflammation. Earlier studies summarized in the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} linked PFOS-mediated PPARγ activation to decreased spleen and thymus weight and reduced spleen and thymus cellularity {Yang, 2002, 1332453; NTP, 2016, 4613766}. Recent studies such as Zhong et al. (2016, 3748828) suggest a role for PFOS in disrupting spleen and thymic weights and cellularity through sex hormones, activation of MAPK signaling pathway and/or oxidative stress genes associated with apoptosis in lymphocytes {Lv, 2015, 3981558}, and reduced numbers of myeloid, pro/pre-B, immature B, and early mature B cells in bone marrow {Qazi, 2012, 1937236}.

New mechanistic insights into PFOS-mediated suppression of adaptive immune responses include PFOS-mediated effects on TH1/TH2-type cytokines and IgE titers in response to allergens in mice and humans {Zhong, 2016, 3748828; Zhu, 2016, 3360105}, glycosylation of immunoglobulins in humans {Liu, 2020, 6833599}, and lymphocyte toxicity *in vitro* {Zarei, 2018, 5079848}. Effects of PFOS exposure on allergy {Lee, 2018, 5085013} included release of histamine and β hexosaminidase associated with up-regulation of intracellular calcium in IgE-stimulated mast cells and release of inflammatory cytokines linked to NF-kB activation. PFOS was also found to stimulate release of IL-17 and IL-22 from TH17 cells in an animal model of intestinal infection {Suo, 2017, 3981310}. Additional insights were provided by transcriptomic and lipidomic studies {Lv, 2015, 3981558; Li, 2020, 6833655; Pennings, 2016, 3352001}. Transcriptomic studies identified candidate genes that may mediate immunotoxicity in children exposed in utero to PFOS including SHC4, PPARD, CYTL1, IL-27, and ADORA2A {Pennings, 2016, 3352001}. In mice, PFOS exposure upregulated THEMIS and CD3G and altered calcium homeostasis, cell cycle genes that may impact T cell immunophenotypes observed in spleen, and T cell function through inhibition of T cell proliferation and induction of T cell anergy {Lv, 2015, 3981558}.

With respect to innate immune responses, PFOS is associated with a depression of NK cell activity. An important outstanding mechanistic question that may directly impact observations of dose- and sex-dependent effects is whether PFOS alters NK cells directly or influences NK cell receptor ligand expression on potential target cells. Two new studies evaluated mechanisms of PFOS activity on innate immune responses mediated by macrophages and ILC3 {Rainieri, 2017,

3860104; Berntsen, 2018, 4167035}. Together, these findings suggest that while PFOS does not alter macrophage function, it may induce ROS and lipid peroxidation in macrophage cell lines. Also, Suo et al. (2017, 3981310) examined effects of PFOS in a mouse model of *C. rodentium* infection. PFOS inhibited the expansion of *C. rodentium* by promoting IL-22 production in ILC3 cells in an AhR-dependent manner and increased IFN-γ production from CD3− non-T cells compared to control mice.

Very little information is available regarding whether PFOS impacts intrinsic cellular defenses. One recent study, Sørli et al. (2020, 5918817), demonstrated that PFOS exerts divergent effects on chemokine and cytokine release in a dose-dependent manner in human bronchial epithelial cells. This study also proposed that PFOS can modulate the activity of intrinsic cellular defense responses mediated by toll receptors and/or other double-stranded RNA sensors.

Nine recent studies reported correlations between PFOS exposure and modulation of pro-inflammatory cytokines or serum markers of inflammation; however, the inflammatory responses to PFOS exposure are not consistent across species. Pro-inflammatory cytokines were elevated in PFOS-exposed rodents and in human and animal cells in culture through activation of MAPK signaling pathways and activation of NF-κB {Han, 2018, 4355066; Zhu, 2015, 2850996}. In contrast, the available studies evaluating human subjects observed either no changes in serum cytokine or marker levels (IL-6, IFN-γ, or CRP) or reduced levels (TNF-α, IL-8, or C3a) relative to subjects with lower PFOS exposures.

Despite recent research informing a range of immunotoxicity endpoints, a comprehensive understanding of the mechanisms by which PFOS alters immune system development, physiology, and function is lacking. Data from transcriptomic studies have advanced the understanding regarding the potential of PFOS to disrupt lymphocyte signaling and function. A particularly promising area of research relates to the observation that PFOS exposure in human lymphocytes is associated with dysregulated lipid profiles that encompass glycerophospholipid metabolism, sphingolipid metabolism, glycerolipid metabolism, adipocytokine signaling, regulation of autophagy, and arachidonic acid metabolism {Li, 2020, 6833655}. However, further studies are needed to determine if these gene expression changes result in altered protein accumulation and if gene expression and lipid profile changes mediate functional changes in immunity.

3.4.2.4 Evidence Integration

There is *moderate* evidence for an association between PFOS exposure and immunosuppressive effects in human studies based on largely consistent decrease in antibody response following vaccinations (against three different infectious agents) in multiple *medium* confidence studies in children. Reduced antibody response is an indication of immunosuppression and may result in increased susceptibility to infectious disease. Changes in antibody levels of 10–20% per doubling of PFOS exposure were observed in the Faroe Islands cohorts. The variability in the results, including null and positive associations, could be related to differences in sample sizes, individual variation, vaccine type, and differences in timing of the boosters, as well as differences in timing of antibody measurements in relation to the last booster. However, these factors cannot be explored further with currently available data. Overall, the evidence indicates an association between increased serum PFOS levels and decreased antibody production

following routine vaccinations in children. Evidence in adults does not indicate an association with immunosuppression, but *high* confidence studies are not available.

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

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

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

The animal evidence for an association between PFOS exposure and immunosuppressive responses is *moderate* based on decreased PFC responses and NK cell activities observed in 12 *high* or *medium* confidence rodent studies. Additionally, fluctuations in splenic and thymic cell populations and increased bone marrow hypocellularity in conjunction with extramedullary hematopoiesis were observed. Extramedullary hematopoiesis, blood cell production outside of the bone marrow, occurs when normal cell production is impaired. Bone marrow hypocellularity in parallel with extramedullary hematopoiesis suggest that PFOS impedes hematopoiesis in the bone marrow. As such, EPA concluded that elevated extramedullary hematopoiesis and bone marrow hypocellularity, as well as reduced ability to generate an immune response to a bacteria-like challenge and reduced PFC response indicate toxicity of relevance to humans exposed to PFOS.

It is clear that PFOS can alter immune cells and signaling in experimental systems. However, the connection between various alterations to immune and inflammation signaling and immunologic effects reported in humans is not clear. Transcriptomics data represent some of the most informative findings in regard to potential underlying mechanisms of immunotoxicity of PFOS. Together, the findings from transcriptomic and functional analyses reported in human lymphocytes exposed to PFOS, in human cord blood samples from gestational exposure to PFOS, and in mice treated with PFOS suggest that PFOS exposure may disrupt adaptive immunity through the dysregulation of genes and lipids involved in lymphocyte survival, proliferation, and inactivation. PFOS effects on gene expression paralleled a dose-dependent increase in intracellular free calcium (which plays an important role in immune cell proliferation in response to foreign antigens) concentration in splenocytes of mice treated with PFOS, suggesting that activation of MAPK signaling pathway and/or oxidative stress genes in response

to PFOS may alter splenic architecture via induction of apoptosis in lymphocytes. Relatedly, additional *in vitro* transcriptomic data collected from mouse microglial cells and rat hepatocytes and Kuppfer cells demonstrate activation of TNF-α and IL-6, correlated with activation of NF-κB. These data support a role for MAPK signaling pathways and NF-κB activation in PFOS-mediated inflammatory gene expression *in vitro*. TNF-α, IL-6, and NF-κB are all related to inflammation, allergy, and other immune responses.

Despite recent research informing a range of immunotoxicity endpoints, a comprehensive understanding of the mechanisms by which PFOS alters immune system development, physiology, and function is lacking. A particularly promising area of research relates to the observation that PFOS exposure in human lymphocytes is associated with dysregulated lipid profiles that encompass glycerophospholipid metabolism, sphingolipid metabolism, glycerolipid metabolism, adipocytokine signaling, regulation of autophagy, and arachidonic acid metabolism. Additional research is needed to determine if these gene expression changes result in altered protein accumulation and if gene expression and lipid profile changes mediate functional changes in immunity; specifically, alterations to antibody response and susceptibility to infection, as reported in humans.

3.4.2.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the ***evidence indicates*** that PFOS exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans under relevant exposure circumstances (Table 3-10). The hazard judgment is driven primarily by consistent evidence of reduced antibody response from epidemiological studies at levels of 0.8 ng/mL PFOS (median exposure in studies observing an adverse effect). The evidence in animals showed coherent immunomodulatory responses at doses as low as 0.0017 mg/kg/day that are consistent with potential immunosuppression and supportive of the human studies, although issues with overt organ/systemic toxicity raise concerns about the biological significance of some of these effects. While there is some evidence that PFOS exposure might also have the potential to affect sensitization and allergic responses in humans given relevant exposure circumstances, the human evidence underlying this possibility is uncertain and with limited support from animal or mechanistic studies. Based on the antibody response data in humans, children and young individuals exposed during critical developmental windows may represent a potential susceptible population for the immunosuppressive effects of PFOS. The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental life stages represents a major source of uncertainty in the Immunotoxicity database of PFOS.

Table 3-10. Evidence Profile Table for PFOS Immune Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.2.1)					⊕⊕⊙ ***Evidence Indicates (likely)***
Immunosuppression 1 *High* confidence study 16 *Medium* confidence studies 6 *Low* confidence studies 2 *Mixed*[a] confidence studies	Studies conducted in the Faroe Islands examined antibody levels among children at various timepoints compared to exposure measured prenatally and throughout childhood. Lower antibody levels against tetanus and diphtheria were observed in children at birth, 18 months, age 5 years (pre-and post-booster), and at age 7 years, with some being statistically significant. Findings in the three studies examining adults and adolescents were less consistent than children. Infectious disease was examined in 11 studies of children. Studies examining infections of the respiratory system observed some positive associations (5/11), although many findings from other studies were not precise. Findings for infectious disease in adults were mixed, with two studies reporting	• *High* and *medium* confidence studies • *Consistent direction* of effect • *Coherence* of findings between antibody response and increased infectious disease	• *Low* confidence studies • *Imprecision* of findings	⊕⊕⊙ *Moderate* Evidence for immune effects is based on decreases in childhood antibody responses to pathogens such as diphtheria and tetanus. Reductions in antibody response were observed at multiple timepoints in childhood, using both prenatal and childhood exposure levels. An increased risk of upper and lower respiratory tract infections was observed among children, coherent with findings of reduced antibody response. There was also supporting evidence of increased risk of asthma, eczema, and autoimmune disease, however, the number of studies examining the same type of autoimmune disease was limited.	*Primary basis and cross-stream coherence*: Human data indicated consistent evidence of reduced antibody response. Evidence in animals showed coherent immunomodulatory responses that are consistent with potential immunosuppression and supportive of the human studies, although issues with overt organ/systemic toxicity raise concerns about the biological significance of some of these effects. While there is some evidence that PFOS exposure might also have the potential to affect sensitization and allergic responses in humans given relevant exposure circumstances, the human evidence underlying this possibility is uncertain and with limited support from animal or mechanistic studies.

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	inconsistent results for COVID-19 infections.				*Human relevance and other inferences:* Based on the antibody response data in humans, children and young individuals exposed during critical developmental windows may represent a potential susceptible population for the immunosuppressive effects of PFOS. The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental life stages represents a major source of uncertainty in the immunotoxicity database of PFOS.
Immune hypersensitivity 1 *High* confidence study 17 *Medium* confidence studies 3 *Low* confidence studies 2 *Mixed*[a] confidence studies	Examination of immune hypersensitivity includes outcomes such as asthma, allergies, and eczema. Increased odds of asthma were reported in most *medium* confidence studies (6/9), although associations were often inconsistent by subgroups. *Low* confidence studies supported the findings of increased odds of asthma or higher exposure levels among asthmatics, although results were not always consistent or precise. Seven studies examined allergies, rhinitis, or rhinoconjunctivitis. Some positive associations (3/7) were observed, although this varied by outcome timing and were at times inconsistent. Significantly increased odds of eczema were observed in three (3/7) studies for those in the highest exposure group, however, inverse associations were also observed (3/7).	• *High* and *medium* confidence studies • *Consistent direction* of effect for asthma across *medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effect between subpopulations		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Autoimmune disease 1 *Medium* confidence study 3 *Low* confidence studies	Lower exposure levels were observed in healthy controls compared to multiple sclerosis cases in one study of adults. An increased risk of celiac disease was also observed in a study of children and young adults. Another study observed lower exposure levels among ulcerative colitis cases compared to health controls. There was no significant difference in exposure levels based on type 1 diabetes status.	• *Medium* confidence study	• *Low* confidence studies • *Limited number* of studies examining outcome		
Evidence from *In Vivo* Animal Toxicological Studies (Section 3.4.2.2)					
Immune response 4 *Medium* confidence studies	In response to a SRBC challenge, decreased IgM response in the PFC assay was reported (2/2) in a subchronic and developmental study in mice, and was dose-dependent in males. In the developmental study, NK cell activity was reduced up to 8 weeks after a gestational exposure (1/1). One short-term study in rats examined the effect of PFOS on a delayed-type hypersensitivity response to a KLH challenge (1/1)	• *Medium* confidence studies • *Dose-response* relationship seen within multiple studies	• *Limited number* of studies examining specific outcomes	⊕⊕⊙ *Moderate* Evidence is based on decreased immune responses and NK cell activities observed in several *high* or *medium* confidence rodent studies. Additionally, fluctuations in splenic and thymic cell populations and increased bone marrow hypocellularity in conjunction with extramedullary	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	and observed no changes in IgG levels (1/1) or footpad swelling (1/1). Another short-term study observed no changes in circulating white blood cells but an increase in IgE after an OVA challenge (1/1).			hematopoiesis were observed. Extramedullary hematopoiesis, blood cell production outside of the bone marrow, occurs when normal cell production is impaired. Bone marrow hypocellularity in parallel with extramedullary hematopoiesis suggest that PFOS impedes hematopoiesis in the bone marrow.	
Immune cellularity 2 *High* confidence studies 6 *Medium* confidence studies	Of the studies that measured circulating WBCs and differentials (5/8), one short-term rat study found decreases in WBCs and segmented neutrophils in males only, while a chronic rat study found increases in segmented neutrophils in males only. In another short-term study in rats, a negative trend for subsets of T-cells and a positive trend for B-cells were observed in males. In females a positive trend was observed for WBCs, lymphocytes, and subsets of T-cells; a negative trend was observed for B-cells. No effects on WBCs or differentials were seen in a short-term study of male mice and in a chronic study in monkeys.	• *High* and *medium* confidence studies • *Coherence* of findings across circulating immune cells, splenic cellularity, and thymic cellularity and with histopathological changes	• *Inconsistent direction* of effects across studies and sex		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	Decreases in total spleen cellularity and/or subsets of splenic cells were observed in 2 short-term studies in male and female rats and mice. Similar decreases were seen in the thymus in these studies; however, no changes were observed in females.				
Histopathology 1 *High* confidence studies 5 *Medium* confidence studies	In 1 *high* confidence short-term study, a dose-dependent increase in both extramedullary hematopoiesis in the spleen and hypocellularity in the bone marrow was observed in male and female rats. No changes were observed in the thymus or lymph nodes. None of the *medium* confidence studies (5) reported histopathologic changes in the spleen (4), thymus (2), or lymph nodes (2).	• *High* and *medium* confidence studies • *Dose-response* relationship observed • *Coherent* changes with those observed in circulating immune cells, splenic cellularity, and thymic cellularity	• *Inconsistent direction* of effects across studies and sex		
Organ weights 2 *High* confidence studies 5 *Medium* confidence studies	Mixed results were reported for absolute and relative spleen (7) and thymus (5) weights. Both studies in male and female rats reported decreases in absolute spleen (2/2) (males only) and thymus	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects across species and sex • *Confounding* variables such as decreases in body weights		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	weights (2/2) (males and females), which generally coincided with decreases in body weights. Relative spleen weights were unchanged (2/2) or increased (1/2) in rats, while relative thymus weights were unchanged (1/2) or decreased (1/2). In mouse studies, absolute spleen and thymus weights were not reported. Decreased relative spleen weights were observed in mice (4/5); however, this result was not always consistent between sex and timepoint. Relative thymus weights were decreased in male mice (2/2) and unchanged in female mice (1/1).		• Lack of *dose-response* relationship		
Globulins and immunoglobulins 1 *High* confidence studies 4 *Medium* confidence studies	Two short-term studies found decreased globulin levels (2/3) in male rats and no changes in female rats. One short-term study found increases in subsets of immunoglobulins (1/1) in both male and female rats, and one short-term study found no changes in IgE (1/1) in male mice.	• *High* and *medium* confidence studies	• *Limited number* of studies examining specific outcomes • *Inconsistent direction* of effects across sex		
Mechanistic Evidence and Supplemental Information (Section 3.4.2.3)					

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Biological events or pathways	**Summary of Key Findings, Interpretation, and Limitations**			**Evidence Stream Judgement**	
Immune system development and physiology	**Key findings and interpretation:** • Changes in WBC and alterations in expression of immune and inflammation-related genes in human cord blood have been reported. • Reduction in immune organ weight, cellularity, and morphology (spleen and thymus) in mice and rats. • Disrupted splenic architecture and reduction in T helper and cytotoxic T cells in the spleen in mice. **Limitations:** • No direct effects related to immune system development or physiology in humans to anchor mechanistic findings.			PFOS can alter immune cells and signaling in experimental systems. However, the connection between various alterations to immune and inflammation signaling and immunologic effects reported in humans is not clear.	
Effects on adaptive immune responses	**Key findings and interpretation:** • Inverse association between PFOS exposure and vaccine-induced antibody levels in human studies (*in utero* exposure to PFOS). • Dysregulation of genes and lipids involved in lymphocyte survival, proliferation, and anergy *in vitro* in human lymphocytes. • Alterations to the expression of genes involved in adaptive immune responses (i.e., immunological and/or hematopoietic functions) in cord blood of samples from cases of maternal exposure to PFOS, as well as in spleens of PFOS-exposed mice, and in human lymphocytes exposed to PFOS *in vitro*. **Limitations:** • Association between gene expression changes and apical endpoints need further confirmation.				
Autoimmune diseases	**Key findings and interpretation:** • PFOS-mediated effects on pro-inflammatory T helper cells, specifically increased IL-17 and IL-22 production, in mice. **Limitations:** • Only a single study; no studies directly evaluated the mechanism by which PFOS could promote autoimmunity.				

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Allergic responses	**Key findings and interpretation:** • Serum levels PFAS, including PFOS, was positively associated with basophil counts in children between birth and age 5. • PFOS blood levels were associated with alterations in cytokines in asthmatic and non-asthmatic children, with some effects being specific to asthmatic children. **Limitations:** • Human data include exposure to other PFAS in addition to PFOS.				
Innate Immunity	**Key findings and interpretation:** • Conflicting results for NK cell activity across studies of cells from animals exposed to PFOS *in vivo*. • Alterations to apoptosis and cell cycle stage in a human macrophage-derived cell line. **Limitations:** • Limited database, no human studies of innate immunity endpoints.				
Effects on Intrinsic Cellular Defense Pathways	**Key findings and interpretation:** • PFOS-induced elevation of IL-1α/β release, indicative of a pro-inflammatory response, in human bronchial epithelial cells exposed *in vitro*. **Limitations:** • Only a single study.				
Effects on Inflammation	**Key findings and interpretation:** • Altered levels of pro-inflammatory cytokines or serum markers of inflammation have been reported in humans, mice, and rats both *in vivo* as well as *in vitro*. • No association between PFOS exposure and increased acute or chronic inflammatory responses in humans *in vivo*. **Limitations:** • Limited database.				

Notes: COVID-19 = coronavirus disease 2019; SRBC = sheep red blood cells; IgM = immunoglobulin M; PFC = plaque forming cell; NK = natural killer; KLH = keyhole limpet hemocyanin; IgG = immunoglobulin G; IgE = immunoglobulin E; OVA = ovalbumin; WBC = white blood cells; IL-17 = interleukin 17; IL-22 = interleukin 22; IL-1α/β = interleukin 1 alpha/beta.

[a]Studies may be of mixed confidence due to differences in how individual outcomes within the same study were assessed (e.g., clinical test vs self-reported data).

3.4.3 Cardiovascular

EPA identified 106 epidemiological and 12 animal toxicological studies that investigated the association between PFOS and cardiovascular effects. Of the 46 epidemiological studies addressing cardiovascular endpoints, 4 were classified as *high* confidence, 24 as *medium* confidence, 11 as *low* confidence, 3 as *mixed* (1 *high/medium* and 2 *medium/low*) confidence, and 4 were considered *uninformative* (Section 3.4.3.1). Of the 80 epidemiological studies addressing serum lipid endpoints, 2 were classified as *high* confidence, 29 as *medium* confidence, 26 as *low* confidence, 16 as *mixed* (1 *high/medium* and 15 *medium/low*) confidence, and 7 were considered *uninformative* (Section 3.4.3.1). Of the animal toxicological studies, 2 were classified as *high* confidence, 7 as *medium* confidence, 2 as *low* confidence, 1 and was considered *mixed* (*medium/low*) (Section 3.4.3.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.3.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.3.1.1 Cardiovascular Endpoints

3.4.3.1.1.1 Introduction

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

The 2016 Health Advisory {U.S. EPA, 2016, 3982043} and HESD {U.S. EPA, 2016, 3603365} assessments did not assess evidence for associations between CVD diseases and PFOS, besides the review of its effects on serum lipids which are further described in subsequent sections. There are 2 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and cardiovascular effects. Study quality evaluations for these 2 studies are shown in Figure 3-30.

The developmental section in the 2016 Health Advisory describes results from Geiger et al. (2014, 2851286) which reported no association with hypertension in 1,655 children aged 12–18 years from the NHANES (1999–2000 and 2003–2008 cycles). An occupational study {Alexander, 2003, 1291101} reported an inverse association for mortality from heart disease among all cohort members. The decreased SMR was consistent in sensitivity analyses of cohort members ever employed in a high exposure job and those only working in non-exposed jobs. The study was considered *low* confidence due to concerns about healthy work effect and potential residual confounding by smoking status and race/ethnicity.

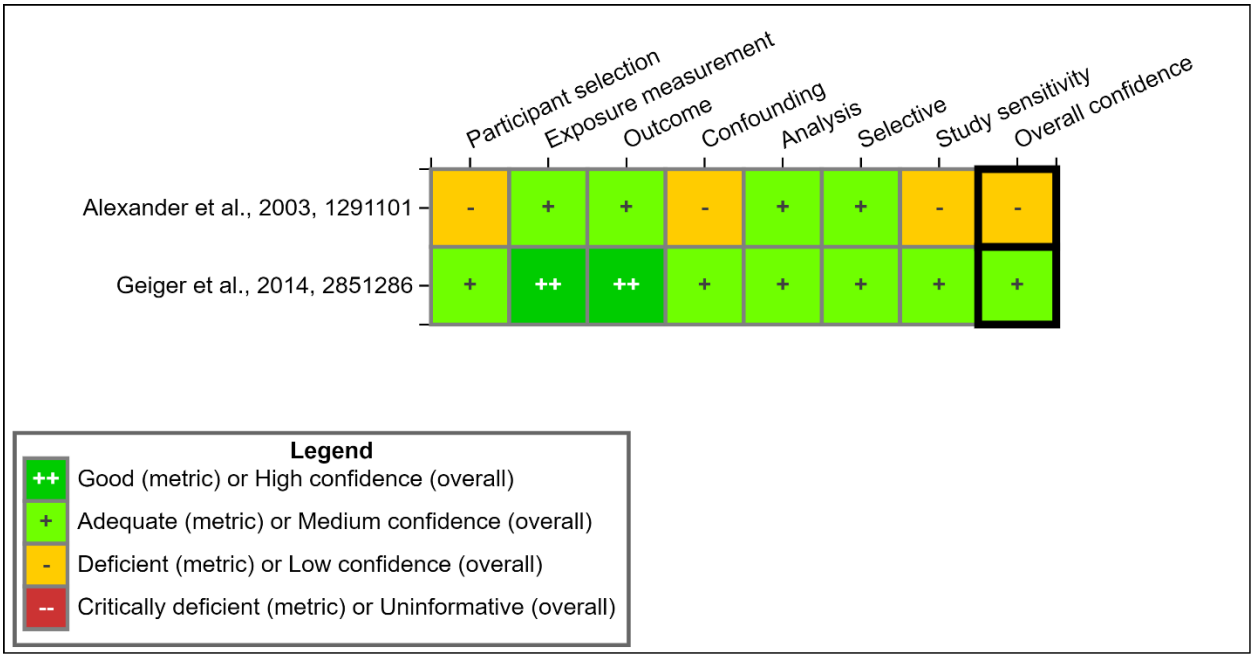


Figure 3-30. Summary of Study Evaluation for Pre-2016 Epidemiology Studies of PFOS and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

Since publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}, 44 new epidemiological studies report on the association between PFOS and CVD, including outcomes such as hypertension, CAD, congestive heart failure (CHF), microvascular diseases, and mortality. Of these, 19 examined blood pressure or hypertension in adults. Pregnancy-related hypertension is discussed in Section 3.4.3.1.1. All studies were conducted on the general population with six {Honda-Kohmo, 2019, 5080551; Hutcheson, 2020, 6320195; Bao, 2017, 3860099; Mi, 2020, 6833736; Yu, 2021, 8453076; Ye, 2021, 6988486} conducted in a high-exposure community in China (i.e., C8 Health Project and "Isomers of C8 Health Project" populations), and three studies {Canova, 2021, 10176518; Pitter, 2020, 6988479;Zare Jeddi, 2021, 7404065} were conducted in a high-exposure community in Italy (i.e., Vento Region). Different study designs were also used including three controlled trial studies {Cardenas, 2019, 5381549; Liu, 2018, 4238396; Osorio-Yáñez, 2021, 7542684}, 11 cohort studies {Fry, 2017, 4181820; Donat-Vargas, 2019, 5080588; Lin, 2020, 6311641; Manzano-Salgado, 2017, 4238509; Matilla-Santander, 2017, 4238432; Mitro, 2020, 6833625; Warembourg, 2019, 5881345; Li, 2021, 7404102; Papadopoulou, 2021, 9960593}, one case-control study {Mattsson, 2015, 3859607}, and 33 cross-sectional studies {Bao, 2017, 3860099; Chen, 2019, 5387400; Christensen, 2016, 3858533; Christensen, 2019, 5080398; Graber, 2019, 5080653; Honda-Kohmo, 2019, 5080551; Huang, 2018, 5024212; Hutcheson, 2020, 6320195; Jain, 2020, 6311650; Jain, 2020, 6833623; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Liao, 2020, 6356903; Lin, 2013, 2850967; Lin, 2016, 3981457; Lind, 2017, 3858504; Liu, 2018, 4238514; Ma, 2019, 5413104; Mi, 2020, 6833736; Mobacke, 2018, 4354163; Yang, 2018, 4238462; Averina, 2021, 7410155; Canova, 2021, 10176518; Jain, 2020, 6988488; Zare Jeddi, 2021, 7404065; Khalil, 2020, 7021479; Koskela, 2022, 10176386; Leary, 2020, 7240043; Lin, 2020,

6988476; Pitter, 2020, 6988479; Yu, 2021, 8453076; Ye, 2021, 6988486}. The two controlled trial studies {Cardenas, 2019, 5381549; Liu, 2018, 4238396} were not controlled trials of PFAS exposures, but rather health interventions: prevention of type 2 diabetes in Diabetes Prevention Program and Outcomes Study (DPPOS) {Cardenas, 2019, 5381549; Osorio-Yáñez, 2021, 7542684} and weight loss in the Prevention of Obesity Using Novel Dietary Strategies Lost (POUNDS-Lost) Study {Liu, 2018, 4238396}. Thus, these studies could be interpreted as cohort studies for evaluating cardiovascular risk purposes.

The studies were conducted in different study populations with the majority of studies conducted in the United States {Cardenas, 2019, 5381549; Christensen, 2016, 3858533; Christensen, 2019, 5080398; Fry, 2017, 4181820; Graber, 2019, 5080653; Honda-Kohmo, 2019, 5080551; Huang, 2018, 5024212; Hutcheson, 2020, 6320195; Jain, 2020, 6311650; Jain, 2020, 6833623; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Liao, 2020, 6356903; Lin, 2020, 6311641; Liu, 2018, 4238396; Liu, 2018, 4238514; Ma, 2019, 5413104; Mi, 2020, 6833736; Mitro, 2020, 6833625; Jain, 2020, 6988488; Khalil, 2020, 7021479; Koskela, 2022, 10176386; Leary, 2020, 7240043; Li, 2021, 7404102; Jain, 2020, 6988488; Osorio-Yáñez, 2021, 7542684}. The remaining studies were conducted in China {Bao, 2017, 3860099; Yang, 2018, 4238462; Yu, 2021, 8453076; Ye, 2021, 6988486}, Taiwan {Lin, 2013, 2850967; Lin, 2016, 3981457}, Spain {Manzano-Salgado, 2017, 4238509; Matilla-Santander, 2017, 4238432}, Croatia {Chen, 2019, 5387400}, Sweden {Donat-Vargas, 2019, 5080588; Lind, 2017, 3858504; Mattsson, 2015, 3859607; Mobacke, 2018, 4354163}, Denmark {Jensen, 2020, 6833719}, Italy {Canova, 2021, 10176518; Ye, 2021, 6988486; Zare Jeddi, 2021, 7404065; Pitter, 2020, 6988479}, Norway {Averina, 2021, 7410155}, and two studies conducted in several European countries {Papadopoulou, 2021, 9960593; Warembourg, 2019, 5881345}. All the studies measured PFOS in blood components (i.e., serum or plasma) with three studies measuring levels in maternal serum {Papadopoulou, 2021, 9960593; Li, 2021, 7404102;Warembourg, 2019, 5881345}, and four studies measuring levels in maternal plasma {Papadopoulou, 2021, 9960593; Warembourg, 2019, 5881345; Manzano-Salgado, 2017, 4238509; Mitro, 2020, 6833625}.

3.4.3.1.1.2 Study Quality

There are 45 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and cardiovascular effects. Study quality evaluations for these 45 studies are shown in Figure 3-31 and Figure 3-32.

Of the 45 studies identified since the 2016 assessment, 4 studies were *high* confidence, 23 were *medium* confidence, 10 were *low* confidence, 4 studies were *mixed* (1 *high/medium* due to difference exposure estimates and 3 *medium/low* for different cardiovascular endpoints) confidence, and 4 studies included an outcome considered *uninformative* {Jain, 2020, 6833623; Jain, 2020, 6311650; Seo, 2018, 4238334; Leary, 2020, 7240043}. The main concerns with the *low* confidence studies included the possibility of outcome misclassification (e.g., reliance on self-reporting) in addition to the potential for residual confounding or selection bias (e.g., unequal recruitment and participation among subjects with outcome of interest, lack of consideration and potential exclusion due to medication usage). Residual confounding was possible due to socioeconomic status (SES), which can be associated with both exposure and the cardiovascular outcome. Although PFOS has a long half-life in the blood, concurrent measurements may not be appropriate for cardiovascular effects with long latencies. Further,

temporality of PFOS exposure could not be established for several *low* confidence studies due to their cross-sectional design. Several of the *low* confidence studies also had sensitivity issues due to limited sample sizes {Christensen, 2016, 3858533; Girardi, 2019, 6315730; Graber, 2019, 5080653;Khalil, 2018, 4238547}. Two studies were rated *adequate* for all domains, indicating lower risk-of-bias; however, both studies treated PFOS as the dependent variable, resulting in both studies being considered *uninformative* {Jain, 2020, 6833623; Jain, 2020, 6311650}. Analyses treating PFOS as the dependent variable support inferences for characteristics (e.g., kidney function, disease status, race/ethnicity, etc.) that affect PFOS levels in the body, but it does not inform the association between exposure to PFOS and incidence of cardiovascular disease. Small sample size (n = 45) and missing details on exposure measurements were the primary concerns of the remaining *uninformative* study {Leary, 2020, 7240043}. Studies considered *uninformative* were not considered further.

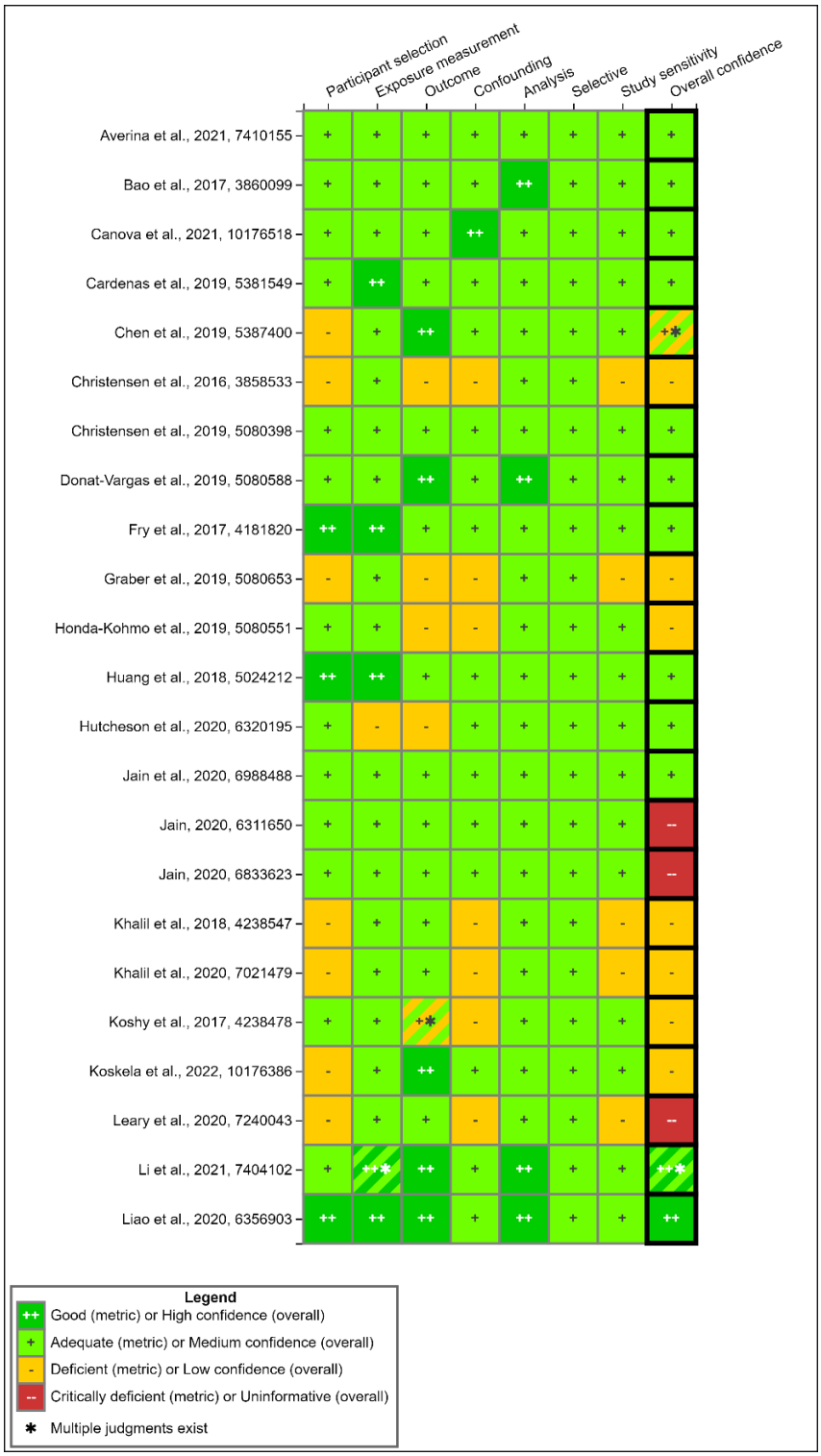


Figure 3-31. Summary of Study Evaluation for Epidemiology Studies of PFOS and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

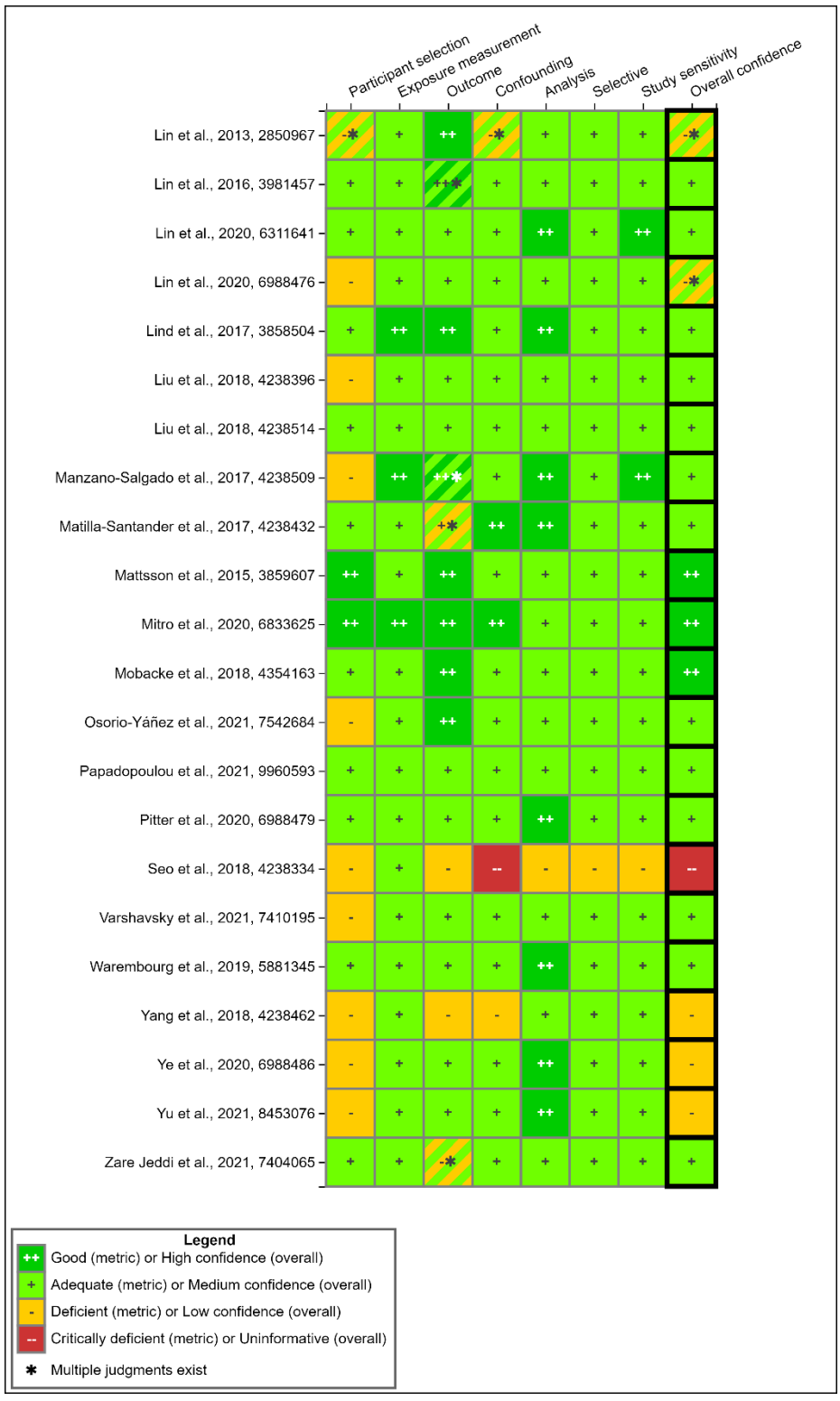


Figure 3-32. Summary of Study Evaluation for Epidemiology Studies of PFOS and Cardiovascular Effects (Continued)

Interactive figure and additional study details available on HAWC.

3.4.3.1.1.3 Findings from Children

The single *high* confidence study examined the association between PFOS at several ages (prenatal, cord blood, 3 years, 8 years, and 12 years) and blood pressure at age 12 and all observed associations were essentially null. Of the six *medium* confidence studies that examined blood pressure in children and adolescents, one reported positive association with diastolic blood pressure (DBP) only {Ma, 2019, 5413104}, one reported an inverse association with systolic blood pressure (SBP) and DBP in adolescents, and one reported an increased risk of hypertension among first-level high school students {Averina, 2021, 7410155}. Results from the remaining *medium* confidence studies were essentially null} (see PFOS Appendix). Among 2,251 NHANES (2003–2012) adolescents (mean age 15.5 years) Ma et al. (2019, 5413104) observed a positive association with DBP, but significant only in boys (0.025; 95% CI: 0.001, 0.049). The study also reported that male adolescents with PFOS levels in the highest quintile (> 18 ng/mL) had mean DBP values that were 2.70% greater (95% CI: 0.32%, 5.02%) than the lowest quartile (< 6.2 ng/mL). Blood pressure also was examined in children (n = 2,693) and adolescents (n = 6,669) participating in a health surveillance program in a high-exposure community (Italy, Veneto Region). Inverse associations were observed for both SBP and DBP in adolescents which were significant for DBP in continuous analyses. Inverse associations for DBP were observed in quartile analyses of children, but none reached significance. No association was observed for SBP in children. In contrast, an increased risk of hypertension was observed among first-level high school students (n = 940) participating in the Fit Futures Study {Averina, 2021, 7410155}. In quartile analyses, the association was positive for the second to fourth quartiles compared to the first but was only significant for the fourth quartile comparison. No association was observed for DBP among female adolescents, or for SBP among all adolescents. Manzano-Salgado et al. (2017, 4238509) reported that maternal PFOS was not associated with blood pressure in combined or in gender-stratified analyses at age 4 and 7 years. In a cohort of 1,277 children (age 6–11 years), Warembourg et al. (2019, 5881345) observed that PFOS measured in maternal blood during the pre-natal period, and in plasma during the post-natal period were not associated with blood pressure in single-pollutant models. Results from an overlapping study {Papadopoulou, 2021, 9960593} on the same cohort were consistent with Warembourg et al. (2019, 5881345)

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

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

Overall, the limited evidence available among children and adolescents was inconsistent and indicates PFOS is not associated with blood pressure in these age groups. The evidence for an

association between PFOS and other CVD-related endpoints assessed in this study population was limited and inconsistent.

3.4.3.1.1.4 Findings from the General Adult Population

Most of the studies identified since the last assessment were conducted among general population adults (see PFOS Appendix). A total of 16 studies examined PFOS in association with SBP, DBP, hypertension, and elevated blood pressure {Bao, 2017, 3860099; Chen, 2019, 5387400; Christensen, 2016, 3858533; Christensen, 2019, 5080398; Donat-Vargas, 2019, 5080588; Mitro, 2020, 6833625; Liao, 2020, 6356903; Lin, 2020, 6311641; Liu, 2018, 4238514; Liu, 2018, 4238396; Mi, 2020, 6833736; Yang, 2018, 4238462; Pitter, 2020, 6988479; Zare Jeddi, 2021, 7404065; Ye, 2021, 6988486; Yu, 2021, 8453076}.

Of the eight studies that examined blood pressure as a continuous measure, five observed statistically significant positive associations {Liao, 2020, 6356903; Mitro, 2020, 6833625; Bao, 2017, 3860099; Mi, 2020, 6833736; Liu, 2018, 4238396}. However, the results were not always consistent between SBP and DBP. A *high* confidence study in 6,967 participants 20 years and older in NHANES (2003–2012) reported a statistically significant positive association with SBP (per 10-fold change in PFOS: 1.35; 95% CI: 0.18, 2.53) {Liao, 2020, 6356903}. Using a generalized additive model and restricted cubic splines, a non-linear (J-shaped) relationship between PFOS and DBP was observed, with the inflection point of PFOS at 8.20 ng/mL. Each 10-fold increase in PFOS was inversely associated with DBP (OR: −2.62; 95% CI: −4.73, −0.51) on the left side of the inflection point and positively associated on the right side of the inflection point (OR: 1.23; 95% CI: −0.42, 2.88). A *high* confidence study {Mitro, 2020, 6833625} conducted in 761 women that examined associations between PFOS concentrations measured during pregnancy and blood pressure assessed at 3 years post-partum reported significantly higher SBP levels among all women (beta per doubling of PFOS: 1.2; 95% CI: 0.3, 2.2) and among women 35 years or older (percent difference per doubling of PFOS: 2.3; 95% CI: 0.9, 3.6). No association was observed with DBP.

Two *medium* confidence cross-sectional studies with overlapping data from the "Isomers of C8 Health Project", a high-exposed population of Shenyang, China {Mi, 2020, 6833736; Bao, 2017, 3860099} also reported positive associations for blood pressure. In adults with very high PFOS levels (median 24.22 ng/mL), Bao et al. (2017, 3860099) observed statistically significant increases in DBP (2.70; 95% CI: 1.98, 3.42) and SBP (4.84; 95% CI: 3.55, 6.12). A positive trend for the association between PFOS, linear (n-PFOS), and branched isomers, and blood pressure was highly significant ($p < 0.001$). In adults with high PFOS levels (median 10.33 ng/mL) Mi et al. (2020, 6833736) reported statistically significant increases in SBP (2.23; 95% CI: 0.58, 3.89). After stratification by sex, significant positive associations were observed in women only for SBP, the estimate was 3.08 (95% CI: 1.53, 4.62; p-value for interaction by sex = 0.03). For DBP, the associations were positive but non-significant overall or among women. Another high-exposure community study {Pitter, 2020, 6988479} examined risk of hypertension in a large population (n = 15,786) of young adults (20–39 years old) living in a PFAS-contaminated region of Italy (Veneto Region) and observed an increased risk of hypertension. The risk of hypertension was significantly increased in continuous analyses (OR per ln-ng/mL PFOS: 1.12; 95% CI: 1.02, 1.22), but quartile analyses indicated the association may have been driven by males in the highest two quartiles of exposure. An overlapping study {Zare Jeddi, 2021, 7404065} on the same population examined blood pressure as a criterion for

metabolic syndrome and results were consistent with an increased risk of hypertension among the whole population.

Lin et al. (2020, 6311641) using data from the Diabetes Prevention Program, a randomized controlled health intervention trial, reported that higher baseline PFOS concentrations were significantly associated with a decrease in SBP over time (year 2: −2.13 mmHg; 95% CI: −3.54, −0.71) among participants assigned to the lifestyle intervention arm, but no association was observed in participants in the placebo-medication arm. However, the study authors attribute the negative findings for BP trajectories (decreases over time) in the lifestyle group to regression towards the mean, a statistical phenomenon in which a more extreme value from the population mean can experience a greater change toward the mean; however, it is unclear why this phenomenon would apply only to the lifestyle arm.

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

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

Of the eight studies that examined risk of elevated blood pressure (hypertension), two reported statistically significant associations {Bao, 2017, 3860099; Mi, 2020, 6833736}. Hypertension was defined as average SBP > 140 mmHg and average DBP > 90 mmHg, or self-reported use of prescribed anti-hypertensive medication. Mi et al. (2020, 6833736) and Bao et al. (2017, 3860099), which had overlapping data on high exposed Isomers of C8 Health Project participants, reported significant associations. Bao et al. (2017, 3860099) reported significantly higher odds of hypertension (OR: 1.24; 95% CI: 1.08, 1.44) for PFOS, and for several PFOS isomers. The associations remained significant in women for PFOS (OR: 1.63; 95% CI: 1.24, 2.13; p-value for interaction by sex = 0.016), and some isomers. These results suggest branched PFOS isomers have a stronger association with increased risk of hypertension compared to linear isomers (n-PFOS). Mi et al. (2020, 6833736) reported a significant positive association for hypertension (OR: 2.52; 95% CI: 1.91, 3.33) overall, and in women (OR 2.32; 95% CI: 1.38, 3.91; p-value for interaction by sex < 0.01).

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

Increased risk of elevated blood pressure was also observed in both *low* confidence studies {Ye, 2021, 6988486; Yu, 2021, 8453076}, both of which examined participants of the Isomers of C8 Health Project (overlapping with Mi et al. (2020, 6833736) and Bao et al. (2017, 3860099)). Yu

et al. (2021, 8453076) examined components of metabolic syndrome and reported significantly increased risk of elevated blood pressure. The association was significant in continuous analyses and the trend was significant in quartile analyses. When stratified by sex, the association was more pronounced in women and was not significant in men. Ye, 2020, 6988486 reported a non-significant increased risk in elevated blood pressure. The magnitude of association for total PFOS was similar to individual PFOS isomers.

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

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

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

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

Cardenas, 2019, 5381549 reported significant increases in risk of any microvascular disease, that were significant only in the lifestyle arm of a health interventions-controlled trial (OR: 1.37; 95% CI: 1.04, 1.84). No associations were observed for nephropathy, retinopathy, or neuropathy.

Two studies assessed potential PFOS-associated changes in heart structure {Mobacke, 2018, 4354163} and carotid atherosclerosis {Lind, 2017, 3858504} in participants 70 years and older, with mixed results. Mobacke, 2018, 4354163 evaluated alterations of left ventricular geometry, a risk factor for CVD and reported that serum PFOS (linear isomer) was significantly associated with higher left ventricular end-diastolic diameter (0.47; 95% CI: 0.08, 0.87; p = 0.02) and lower relative wall thickness (−0.01; 95% CI: −0.01, −0.001; p = 0.03). PFOS was not significantly associated with left ventricular mass. Lind et al. (2017, 3858504) reported that plasma PFOS was

not associated with markers of carotid artery atherosclerosis, including atherosclerotic plaque, the intima-media complex, and the CIMT, a measure used to diagnose the extent of carotid atherosclerotic vascular disease. Aortic and coronary artery calcification was examined in a *medium* confidence study {Osorio-Yáñez, 2021, 7542684} on prediabetic participants from the DPPOS. A significantly increased risk of ascending aortic calcification was reported along with increased risk of coronary artery calcification. Coronary artery calcification was represented as a score of severity (Agatston score) indicating mild, moderate, or severe calcification. The odds of a moderate score (11–400) compared to a mild score (< 11) was increased with respect to PFOS exposure, and the odds of a severe score (> 400) compared to a mild score were significantly increased. Koskela, 2022, 10176376, a *low* confidence study, examined abdominal aortic calcification among participants aged 40 years and older in NHANES (2013–2014) did not observe an association.

No association between PFOS and C-reactive protein levels, a risk factor for CVD, was observed in two studies of pregnant and post-partum women {Mitro, 2020, 6833625; Matilla-Santander, 2017, 4238432}.

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

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

3.4.3.1.2 Serum Lipids

3.4.3.1.2.1 Introduction

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

There are 14 studies (15 publications)[13] from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and serum lipid effects. Study quality evaluations for these 15 studies are shown in Figure 3-33.

In the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}, the epidemiologic evidence overall supported an association between PFOS and increased TC. An association between PFOS and small increases in TC in the general population was observed in several studies {Steenland,

[13] Olsen (2003, 1290020) is the peer-review paper of Olsen (2001, 10228462).

2009, 1291109; Geiger, 2014, 2850925; Eriksen, 2013, 2919150; Frisbee, 2010, 1430763; Nelson, 2010, 1291110}. Steenland {2009, 1291109} examined serum PFOS levels among over 46,000 C8 Health Project participants and reported significant positive associations for all serum lipids except HDL. A cross-sectional study {Frisbee, 2010, 1430763} of children enrolled in the C8 Health Project also reported significantly increased TC and LDL, with increasing serum PFOS. Positive associations were seen in another general population study {Eriksen, 2013, 2919150} conducted among Danish adults (50–65 years old). A positive association between PFOS and hypercholesterolemia also was observed in two cohorts {Steenland, 2009, 1291109, C8 Health Project; Fisher, 2013, 2919156, Canadian Health Measures Survey}. Cross-sectional occupational studies {Olsen, 2001, 10228462; Olsen, 2003, 1290020} reported positive associations between PFOS and increased TC and triglycerides (TG), however, the association was not observed in longitudinal analyses. Evidence for associations between other serum lipids and PFOS was mixed including HDL, LDL, VLDL, non-HDL cholesterol, and triglycerides.

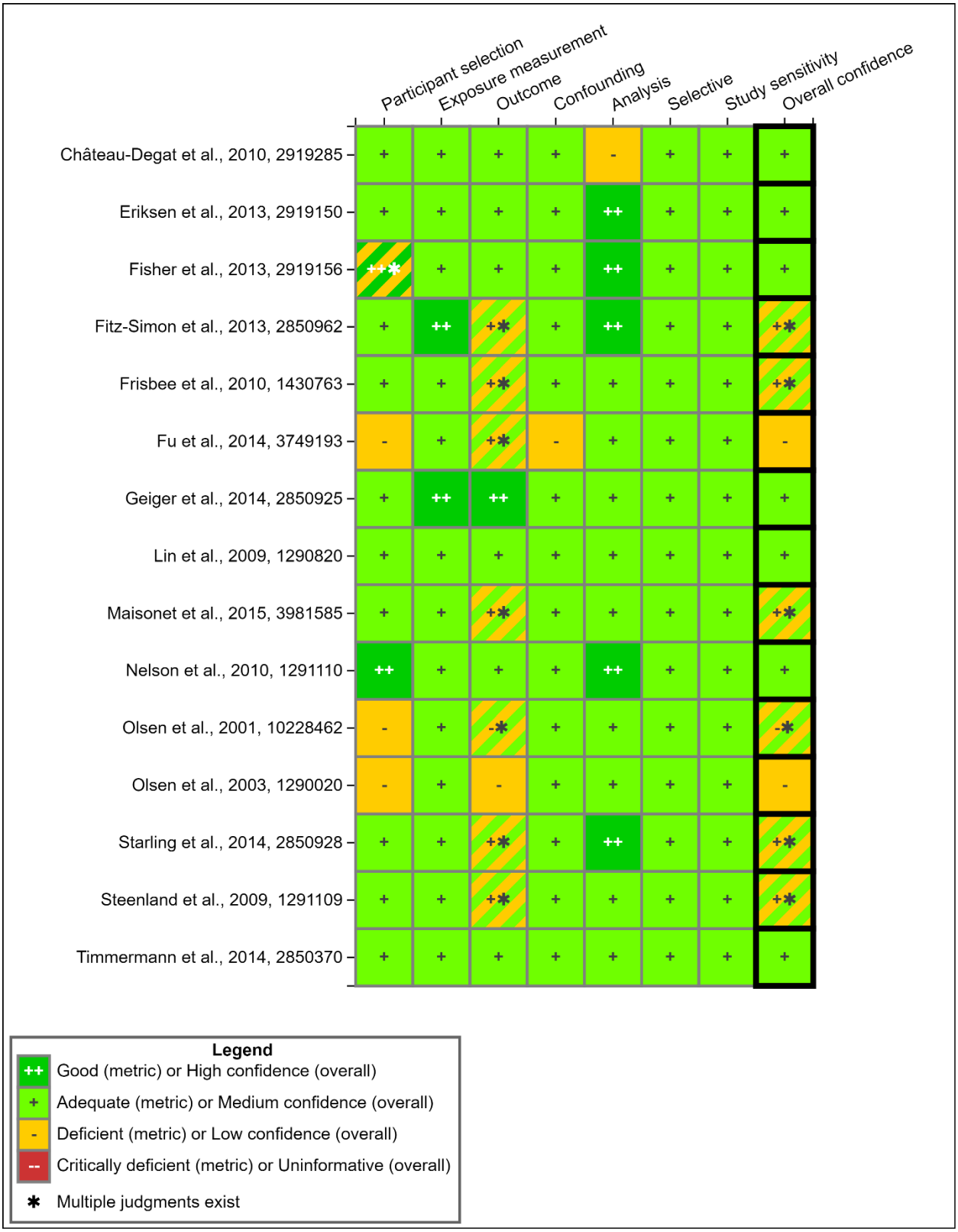


Figure 3-33. Summary of Study Evaluation for Pre-2016 Epidemiology Studies of PFOS and Serum Lipids

Interactive figure and additional study details available on HAWC.

Since publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}, 66 new epidemiologic studies (65 publications)[14] were identified. These studies examined the associations between PFOS and serum lipids in children (n = 24), in pregnant women (n = 7), in the general adult population (n = 32), and in workers (n = 3). Except for ten studies {Olsen, 2012, 2919185; Domazet, 2016, 3981435; Lin, 2019, 5187597; Liu, 2020, 6318644; Donat-Vargas, 2019, 5080588; Liu, 2018, 4238396; Blomberg, 2021, 8442228; Sinisalu, 2020, 7211554; Li, 2021, 7404102; Tian, 2020, 7026251}, all studies were cross-sectional. Some cohort studies provided additional cross-sectional analyses {Blomberg, 2021, 8442228; Sinisalu, 2020, 7211554; Li, 2021, 7404102}. Most studies assessed exposure to PFOS using biomarkers in blood, and measured serum lipids with standard clinical biochemistry methods. Serum lipids were frequently analyzed as continuous outcomes, but some studies examined the prevalence or incidence of hypercholesterolemia, hypertriglyceridemia, and low HDL based on the clinical cut-points, medication use, doctor's diagnosis, or criteria for metabolic syndrome.

3.4.3.1.2.2 Study Quality

All studies were evaluated for risk of bias, selective reporting, and sensitivity following the EPA IRIS protocol. Three considerations were specific to evaluating the quality of studies on serum lipids. First, because lipid-lowering medications strongly affect serum lipid levels, unless the prevalence of medication use is expected to be low in the study population (e.g., children), studies that did not account for the use of lipid-lowering medications by restriction, stratification, or adjustment were rated as *deficient* in the *participant selection* domain. Second, because triglycerides levels are sensitive to recent food intake {Mora, 2016, 9564968}, outcome measurement error is likely substantial when TG is measured without fasting. Thus, studies that did not measure triglycerides in fasting blood samples were rated *deficient* in the *outcome measures* domain for triglycerides. The *outcome measures* domain for LDL was also rated *deficient* if LDL was calculated based on triglycerides. Fasting status did not affect the *outcome measures* rating for TC, directly measured LDL, and HDL because the serum levels of these lipids change minimally after a meal {Mora, 2016, 9564968}. Third, measuring PFOS and serum lipids concurrently was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOS (median half-life = 3.5 years) {Li, 2018, 4238434}, current blood concentrations are expected to correlate well with past exposures. Furthermore, although reverse causation due to hypothyroidism {Dzierlenga, 2020, 6833691} or enterohepatic cycling of bile acids {Fragki, 2021, 8442211} has been suggested, there is yet clear evidence to support these reverse causal pathways.

There are 65 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and serum lipid effects. Study quality evaluations for these 65 studies are shown in Figure 3-34, Figure 3-35, and Figure 3-36.

Based on the considerations mentioned, 2 studies were considered *high* confidence, 1 study was rated *high* for one exposure measurement and *medium* for the other, 22 studies were rated *medium* confidence for all lipid outcomes, 9 studies were rated *medium* confidence for TC or HDL, but *low* confidence for triglycerides or LDL, 24 studies were rated *low* confidence for all

[14] Dong 2019, 5080195 counted as two studies, one in adolescents and one in adults.

lipid outcomes, and 7 studies were rated *uninformative* for all lipid outcomes {Seo, 2018, 4238334; Abraham, 2020, 6506041; Predieri, 2015, 3889874; Huang, 2018, 5024212; Leary, 2020, 7240043; Sinisalu, 2021, 9959547}. Notably, nine studies {Zeng, 2015, 2851005; Manzano-Salgado, 2017, 4238509; Canova, 2020, 7021512; Matilla-Santander, 2017, 4238432; Blomberg, 2021, 8442228; Canova, 2021, 10176518; Dalla Zuanna, 2021, 7277682; Tian, 2020, 7026251; Yang, 2020, 7021246} 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;van den Dungen, 2017, 5080340; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Christensen, 2016, 3858533; Graber, 2019, 5080653; Sun, 2018, 4241053; Rotander, 2015, 3859842; Liu, 2018, 4238396; Cong, 2021, 8442223; Khalil, 2020, 7021479; Kobayashi, 2021, 8442188; Liu, 2021, 10176563; Ye, 2021, 6988486; Yu, 2021, 8453076}, outcome measures {Koshy, 2017, 4238478; Yang, 2018, 4238462; Christensen, 2016, 3858533; Kishi, 2015, 2850268; Graber, 2019, 5080653; Rotander, 2015, 3859842; Kobayashi, 2021, 8442188}, confounding {Wang, 2012, 2919184; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Olsen, 2012, 2919185; Lin, 2013, 2850967; Lin, 2020, 6315756; van den Dungen, 2017, 5080340; Li, 2020, 6315681; Yang, 2018, 4238462; Christensen, 2016, 3858533; Graber, 2019, 5080653; Khalil, 2020, 7021479; Liu, 2021, 10176563; Sinisalu, 2020, 7211554}, analysis {He, 2018, 4238388; Sun, 2018, 4241053; Liu, 2018, 4238396}, sensitivity {Wang, 2012, 2919184; Khalil, 2018, 4238547; Olsen, 2012, 2919185; Christensen, 2016, 3858533; Graber, 2019, 5080653; Rotander, 2015, 3859842; van den Dungen, 2017, 5080340; Khalil, 2020, 7021479; Sinisalu, 2020, 7211554}, or selective reporting {Dong, 2019, 5080195} (adolescent portion).

The most common reason for a *low* confidence rating was concerns for participant selection. These concerns include a lack of exclusion based on use of lipid-lowering medications {Wang, 2012, 2919184; Lin, 2020, 6315756; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Sun, 2018, 4241053; van den Dungen, 2017, 5080340; Liu, 2018, 4238396; Cong, 2021, 8442223; Liu, 2021, 10176563; Ye, 2021, 6988486; Yu, 2021, 8453076}, potential for self-selection {Li, 2020, 6315681; Christensen, 2016, 3858533; Graber, 2019, 5080653; Rotander, 2015, 3859842; van den Dungen, 2017, 5080340}, highly unequal recruitment efforts in sampling frames with potentially different joint distributions of PFOS and lipids {Lin, 2013, 2850967}, and missing key information on the recruitment process {Khalil, 2018, 4238547; Yang, 2018, 4238462; Khalil, 2020, 7021479}. Another common reason for *low* confidence was a serious risk for residual confounding by SES {Wang, 2012, 2919184; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Olsen, 2012, 2919185; Lin, 2013, 2850967; Lin, 2020, 6315756; van den Dungen, 2017, 5080340; Li, 2020, 6315681; Yang, 2018, 4238462; Christensen, 2016, 3858533; Graber, 2019, 5080653; Sinisalu, 2020, 7211554}. Frequently, deficiencies in multiple domains contributed to an overall *low* confidence rating. The *uninformative* studies had *critical deficiencies* in at least one domain or were *deficient* in several domains. These *critical deficiencies* include a lack of control for confounding {Seo, 2018, 4238334; Huang, 2018, 5024212; Abraham, 2020, 6506041}, convenience sampling {Sinisalu, 2021, 9959547}, and treating PFOS as an outcome of all lipids instead of an exposure, which limits the ability to make causal inference for the purpose of hazard determination {Predieri, 2015, 3889874}. Small sample size (n = 45) and missing details on exposure measurements were the primary concerns of the remaining *uninformative* study {Leary, 2020, 7240043}. Studies considered *uninformative* were not considered further. 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.

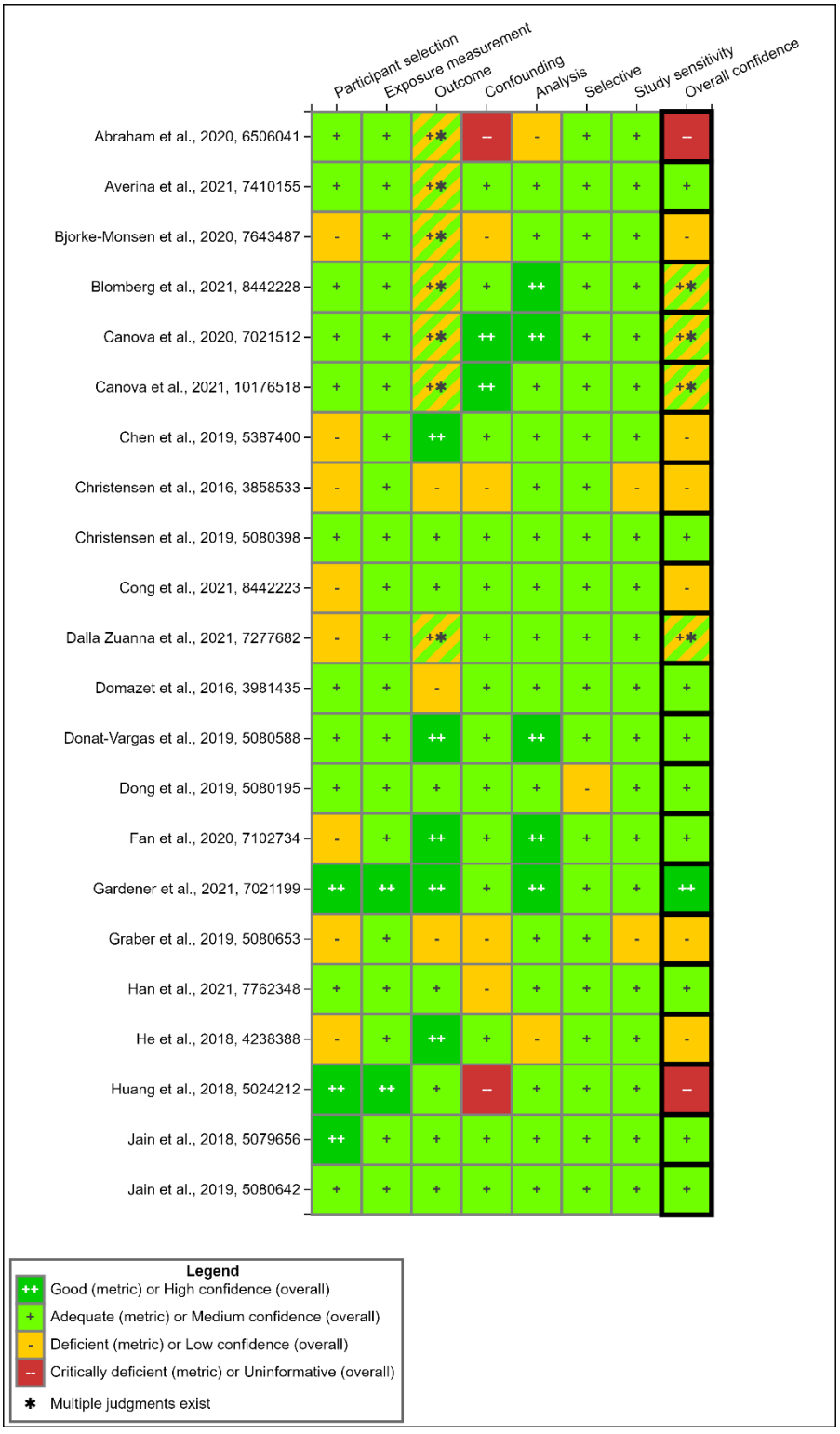


Figure 3-34. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids

Interactive figure and additional study details available on HAWC.

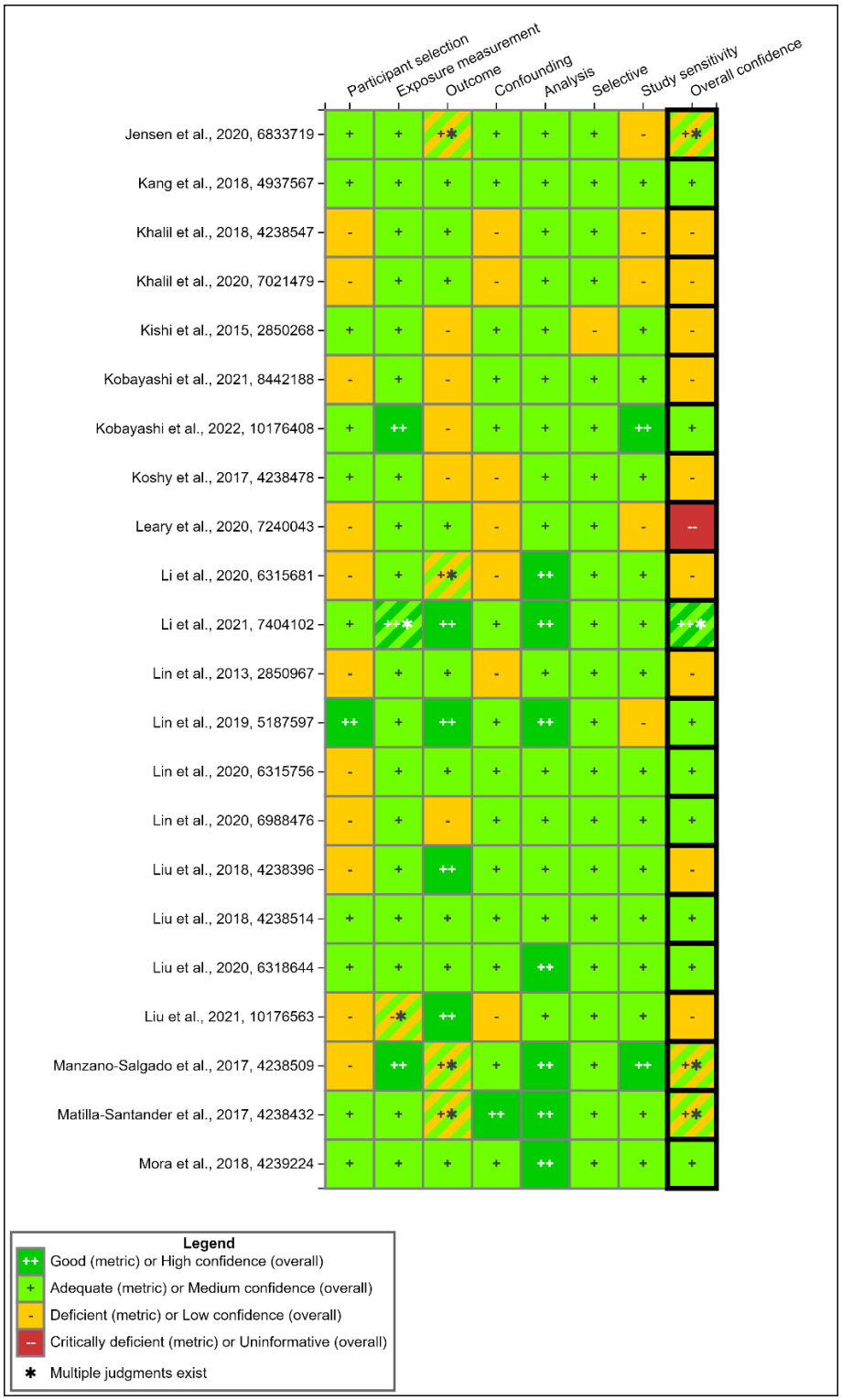


Figure 3-35. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

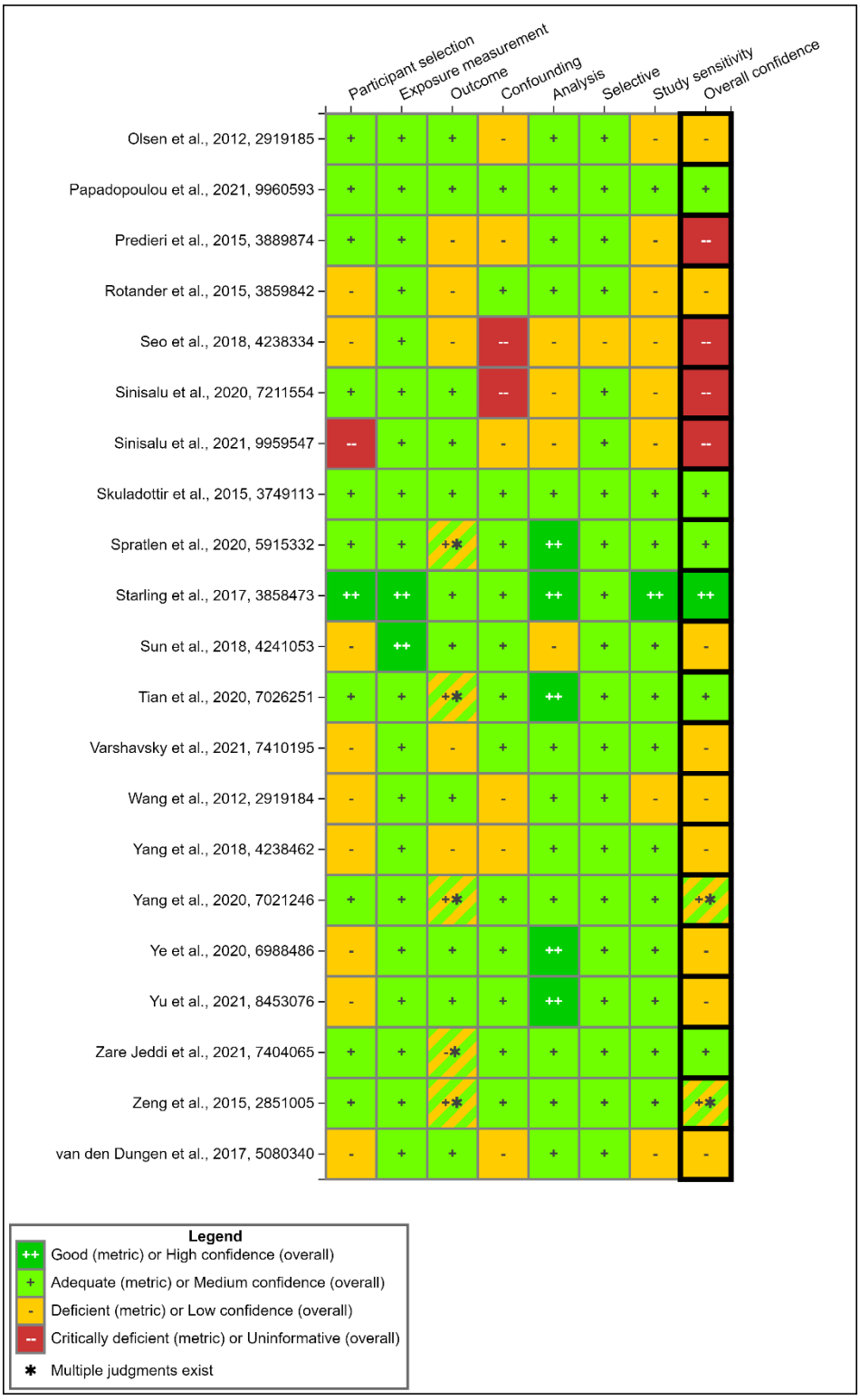


Figure 3-36. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

3.4.3.1.2.3 Findings from Children

Results for the studies that examined TC in children are presented in the Appendix (see PFOS Appendix). Eleven *medium* confidence and three *low* confidence studies examined the association between PFOS and TC in children. Of these, four studies examined the association between prenatal PFOS exposure and TC in childhood {Spratlen, 2020, 5915332; Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2018, 4239224}, one examined exposure and TC at multiple timepoints throughout childhood {Blomberg, 2021, 8442228}, and ten examined the association between childhood PFOS exposure and concurrent TC {Mora, 2018, 4239224; Jain, 2018, 5079656; Zeng, 2015, 2851005; Kang, 2018, 4937567; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Averina, 2021, 7410155; Canova, 2021, 10176518; Tian, 2020, 7026251; Dong, 2019, 5080195} (adolescent portion). Higher PFOS was significantly associated with higher TC in all children in five *medium* confidence studies {Jain, 2018, 5079656; Zeng, 2015, 2851005; Canova, 2021, 10176518; Averina, 2021, 7410155; Blomberg, 2021, 8442228}. Notably, significant positive associations were observed among children {n = 2,693} and adolescents (n = 6,669) of a high-exposure community in Italy (Veneto Region). The associations were significant in continuous and all quartile analyses and were more prominent in children compared to adolescents. Significant positive associations were observed in nine-year old cross-sectional analyses and one prospective comparison (PFOS measured at 5 years, TC measured at 9 years of age) of children belonging to a Faroese cohort {Blomberg, 2021, 8442228}. Comparisons of PFOS and TC measured at other timepoints were less consistent. Positive associations were also found in four other *medium* confidence studies {Spratlen, 2020, 5915332; Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2018, 4239224}, but the associations were small and statistically not significant except for girls in mid-childhood {Mora, 2018, 4239224}. In contrast, one *medium* confidence study {Tian, 2020, 7026251} reported inverse associations, however, this analysis was only conducted concurrently in cord blood. In two out of three *low* confidence studies, positive associations were reported, including a statistically significant finding in Koshy 2017, 4238478 {Khalil, 2018, 4238547; Koshy, 2017, 4238478}. However, residual confounding by SES may have positively biased the results of both studies. Taken together, these studies support a positive association between PFOS and TC in children, particularly for childhood exposure.

Five *medium* confidence and seven *low* confidence studies examined the association between PFOS and LDL in children. Of these, three examined prenatal exposure {Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2018, 4239224}, one examined prenatal and childhood exposure {Papadopoulou, 2021, 9960593} and nine examined childhood exposure {Mora, 2018, 4239224; Zeng, 2015, 2851005; Kang, 2018, 4937567; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Averina, 2021, 7410155; Canova, 2021, 10176518; Tian, 2020, 7026251; Dong, 2019, 5080195} (adolescent portion). The *medium* studies generally found small, positive associations between PFOS and LDL, but only one study in first-level high school students reported a significant association {Averina, 2021, 7410155}. None of the associations were statistically significant in the remaining *medium* confidence studies (see PFOS Appendix) {Jensen, 2020, 6833719; Mora, 2018, 4239224; Kang, 2018, 4937567}. Most *low* confidence studies found a positive association between PFOS and LDL {Khalil, 2018, 4238547; Koshy, 2017, 4238478; Manzano-Salgado, 2017, 4238509; Zeng, 2015, 2851005; Canova, 2021, 10176518}, including statistically significant findings in three studies {Khalil, 2018, 4238547; Koshy, 2017, 4238478; Canova, 2021, 10176518}. However, residual confounding by SES {Khalil, 2018, 4238547; Koshy, 2017, 4238478} and the use of non-fasting samples {Canova, 2021, 10176518; Zeng,

2015, 2851005; Manzano-Salgado, 2017, 4238509} were concerns in these studies. Overall, increases in LDL with increasing PFOS were observed in children, but the magnitudes were small.

One *high* confidence, eleven *medium* confidence, and three *low* confidence studies examined the association between PFOS and HDL in children. Of these, three examined prenatal exposure {Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2018, 4239224}, one examined prenatal and postnatal exposure {Papadopoulou, 2021, 9960593}, two examined exposure and HDL at multiple timepoints throughout childhood {Blomberg, 2021, 8442228;Li, 2021, 7404102}, and six examined childhood exposure {Mora, 2018, 4239224; Jain, 2018, 5079656; Zeng, 2015, 2851005; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Dong, 2019, 5080195; Averina, 2021, 7410155; Canova, 2021, 10176518; Tian, 2020, 7026251} (adolescent portion). The only *high* confidence study {Li, 2021, 7404102} reported significant positive associations for HDL at 12 years of age among child participants of the HOME study. PFOS measured at 8 years of age and concurrently at 12 years of age was significantly associated with increased HDL. The associations for PFOS measured prenatally, at birth, and at 3 years of age were all non-significantly positive. Higher PFOS was significantly associated with higher HDL in children in mid-childhood in two *medium* confidence studies {Mora, 2018, 4239224; Canova, 2021, 10176518}. The positive association observed in Canova, 2021, 10176518 was consistent when examining adolescent participants. In Faroese children {Blomberg, 2021, 8442228}, higher PFOS was significantly associated with higher HDL when measured concurrently at 9 years of age. Comparisons of other timepoints (18-month concurrent measurements, 18-month PFOS and 9-year HDL, and 5-year PFOS and 9-year HDL) were all positively associated with HDL with increasing PFOS concentrations. Other *medium* confidence studies found positive {Jain 2018, 5079656}, inverse (HDL at 18 months in Jensen et al. (2020, 6833719); Papadopoulou et al. (2021, 9960593), prenatal PFOS; Manzano-Salgado et al. (2017, 4238509); Zeng et al. (2015, 2851005); Tian et al. (2020, 7026251)), or close to zero (HDL at 3 months in Jensen et al. (2020, 6833719); Papadopoulou et al. (2021, 9960593), postnatal PFOS) associations; none of these associations were statistically significant. Two of the three *low* confidence studies found positive associations between PFOS and HDL {Khalil, 2018, 4238547; Koshy, 2017, 4238478}. In summary, mixed associations were found between PFOS and HDL in children.

Five *medium* confidence studies and four *low* confidence studies examined the association between PFOS and triglycerides in children. Of these, four examined prenatal exposure {Spratlen, 2020, 5915332; Jensen, 2020, 6833719; Manzano-Salgado, 2017, 4238509; Mora, 2018, 4239224} and six examined childhood exposure {Domazet, 2016, 3981435;Mora, 2018, 4239224; Zeng, 2015, 2851005; Kang, 2018, 4937567; Khalil, 2018, 4238547; Koshy, 2017, 4238478}. Higher mid-childhood PFOS exposure was significantly associated with lower triglycerides in one *medium* confidence study {Mora, 2018, 4239224}. The other *medium* confidence studies reported positive {Spratlen, 2020, 5915332; Kang, 2018, 4937567}, inverse (triglycerides at 3 months in Jensen et al. (2020, 6833719); PFOS exposure at age 9 years in Domazet et al. (2016, 3981435)), or close to zero associations (triglycerides at 18 months in Jensen et al. (2020, 6833719); PFOS exposure at age 15 years in Domazet et al. (2016, 3981435)); none of these associations were statistically significant. Of note, in Jensen et al. (2020, 6833719) and Domazet et al. (2016, 3981435), the direction of association changed depending on the timing of outcome or exposure assessment. One *medium* confidence study

{Kobayashi, 2022, 10176408} and one *low* confidence study {Kobayashi, 2021, 8442188} conducted on mother-child pairs from the Hokkaido Study on Environment and Children's Health examined the association between prenatal PFOS exposure, maternal polymorphisms of nuclear receptor genes, and triglyceride levels in infants. Inverse associations for PFOS and TG were observed, but both studies reported no significant interaction between maternal nuclear gene polymorphisms and PFOS exposure on triglyceride levels. All other *low* confidence studies reported positive associations between PFOS and triglycerides, but all associations were small and not statistically significant {Manzano-Salgado, 2017, 4238509; Zeng, 2015, 2851005; Khalil, 2018, 4238547; Koshy, 2017, 4238478; Sinisalu, 2020, 7211554}. The use of non-fasting samples and residual confounding by SES may have biased these results upwards. Overall, mixed associations were found between PFOS and triglycerides in children.

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

3.4.3.1.2.4 Findings from Pregnant Women

Four *medium* confidence studies examined the association between PFOS and TC in pregnant women and three reported positive associations between PFOS and TC (see PFOS Appendix) {Matilla-Santander, 2017, 4238432; Skuladottir, 2015, 3749113; Dalla Zuanna, 2021, 7277682}. Skuladottir 2015, 3749113, reported a statistically significant linear trend of increasing TC with increasing PFOS. Positive associations also were observed in an Italian high-exposure community study {Dalla Zuanna, 2021, 7277682} on pregnant women. The association from continuous analyses indicated non-significantly increased TC, which was supported by positive associations when analyzing the second and fourth quartile of exposure but not the second. No association between PFOS and TC was observed in a Chinese study of pregnant women {Yang, 2020, 7021246}. No association was found in the single *low* confidence study {Varshavsky, 2021, 7410195} on total serum lipids after adjustment for race/ethnicity, insurance type, and parity. These findings suggest a consistently positive association between PFOS and TC in pregnant women.

Two studies {Dalla Zuanna, 2021, 7277682; Yang, 2020, 7021246} considered *low* confidence for LDL due to lack of fasting did not observe an association between PFOS exposure and LDL in pregnant women. Three *medium* confidence studies examined the association between PFOS and HDL, and two reported positive associations. In a high-exposure community study {Dalla Zuanna, 2021, 7277682}, serum HDL was significantly increased among pregnant Italian women (beta per ln-ng/mL PFOS: 4.84; 95% CI: 2.15, 7.54), and the association was consistent in quartile analyses. A study on pregnant women in the Healthy Start Study reported a positive, though statistically non-significant, association between PFOS and HDL (see PFOS Appendix) {Starling, 2017, 3858473}. No association between PFOS and HDL was observed in a Chinese study of pregnant women {Yang, 2020, 7021246}.

One *medium* confidence and three *low* confidence studies examined the association between PFOS and triglycerides in pregnant women. The *medium* confidence study reported no association between PFOS and triglycerides (see PFOS Appendix) {Starling, 2017, 3858473}. Two *low* confidence studies reported statistically significant, inverse associations between PFOS and triglycerides {Matilla-Santander, 2017, 4238432; Kishi, 2015, 2850268} while the remaining study {Yang, 2020, 7021246} reported a non-significant inverse association. All *low*

confidence studies were limited by their use of non-fasting blood samples. Given that recent food intake is associated with increased triglycerides and may be a source of PFOS, using non-fasting blood samples is expected to positively bias the PFOS- triglycerides association. That inverse associations were still observed in the *low* confidence studies provides support for an inverse association between PFOS and triglycerides. This inverse association is inconsistent with the finding in the only *medium* confidence study. In sum, the available evidence suggests an inverse association between PFOS and triglycerides in pregnant women. However, high-quality evidence is lacking to confirm this association.

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

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

3.4.3.1.2.5 Findings from the General Adult Population

Ten *medium* confidence and twelve *low* confidence studies examined PFOS and TC or hypercholesterolemia in adults. All studies examined the cross-sectional association {Dong, 2019, 5080195, adult portion; Jain, 2019, 5080642; Liu, 2018, 4238514; Liu, 2020, 6318644; Lin, 2019, 5187597; Donat-Vargas, 2019, 5080588; Wang, 2012, 2919184; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Christensen, 2016, 3858533; Graber, 2019, 5080653; Sun, 2018, 4241053; Liu, 2018, 4238396; Canova, 2020, 7021512; Fan, 2020, 7102734; Lin, 2020, 6988476; Han, 2021, 7762348; Bjorke-Monsen, 2020, 7643487; Cong, 2021, 8442223; Liu, 2021, 10176563; Khalil, 2020, 7021479}; two studies additionally examined the association between baseline PFOS and changes in TC or incident hypercholesterolemia {Liu, 2020, 6318644; Lin, 2019, 5187597}.

Of the ten *medium* confidence studies, nine reported positive associations. In a population of young adults aged 20 to 39 years in Veneto region, Italy, an area with water contamination by PFAS, Canova 2020, 7021512 reported statistically positive associations with TC. Canova 2020, 7021512 also reported a concentration-response curve when PFOS was categorized in quartiles or deciles, with a higher slope at higher PFOS concentrations. Another high-exposure community study {Lin, 2020, 6988476} conducted in Taiwan provided a sensitivity analysis of older adults (age 55–75 years), restricting to those participants not taking lipid lowering or anti-hypertensive medications. In quartile analyses of TC, the association was significantly positive for the second (beta for Q2 vs. Q1: 15.06; 95% CI: 4.66, 25.46) and third quartile (beta for Q3 vs. Q1: 11.47; 95% CI: 1.03, 21.91) of exposure. The magnitude of association was similar for the fourth quartile of exposure but did not reach significance.

Four *medium* studies using overlapping data from NHANES 2003–2014 reported positive associations between PFOS and TC in adults {Dong, 2019, 5080195, adult portion; Jain, 2019, 5080642; Liu, 2018, 4238514; Fan, 2020, 7102734} (see PFOS Appendix). The association was statistically significant when data from all cycles were pooled in analyses {Dong, 2019,

5080195}. A cross-sectional analysis {Han, 2021, 7762348} of type 2 diabetes cases and healthy controls in China reported a positive association for TC, but it did not reach significance. PFOS also was associated with slightly higher TC at baseline in the POUNDS-Lost cohort {Liu, 2020, 6318644} and the DPPOS {Lin, 2019, 5187597}, but neither association was statistically significant. The DPPOS also reported that PFOS was associated with a slightly higher prevalence of hypercholesterolemia at baseline (OR = 1.02, 95% CI: 0.85, 1.21)) and a slightly higher incidence of hypercholesterolemia prospectively (HR = 1.01, 95% CI: 0.91, 1.12). In contrast to these findings, Donat-Vargas 2019, 5080588 reported inverse associations between PFOS and concurrently measured TC. Further, it reported positive associations between PFOS averaged between baseline and follow-up and TC at follow-up {Donat-Vargas, 2019, 5080588}. All associations in Donat-Vargas 2019, 5080588 were small and few were statistically significant. It is noteworthy that all participants in Lin {2019, 5187597} were prediabetic, approximately half of all participants in Han {2021, 7762348} were diabetic, all participants in Liu {2020, 6318644} were obese and enrolled in a weight loss trial, and all participants in Donat-Vargas {2019, 5080588} were free of diabetes for at least 10 years of follow-up. It is unclear if differences in participants' health status explained the studies' conflicting findings.

In *low* confidence studies, positive associations between PFOS and TC or hypercholesterolemia were reported in ten of twelve studies {Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Christensen, 2016, 3858533; Graber, 2019, 5080653; Sun, 2018, 4241053; Liu, 2018, 4238396; Bjorke-Monsen, 2020, 7643487; Cong, 2021, 8442223; Liu, 2021, 10176563}. However, oversampling of persons with potentially high PFOS exposure and health problems was a concern in three of these studies {Li, 2020, 6315681; Christensen, 2016, 3858533; Graber, 2019, 5080653}. Medication status and potential residual confounding by SES was a concern in three studies {Bjorke-Monsen, 2020, 7643487; Cong, 2021, 8442223; Liu, 2021, 10176563}. Further, He 2018, 4238388 used similar data as the four *medium* NHANES studies and thus added little information. Considering *medium* and *low* confidence studies together, small increases in TC with increased PFOS were observed, though less consistently.

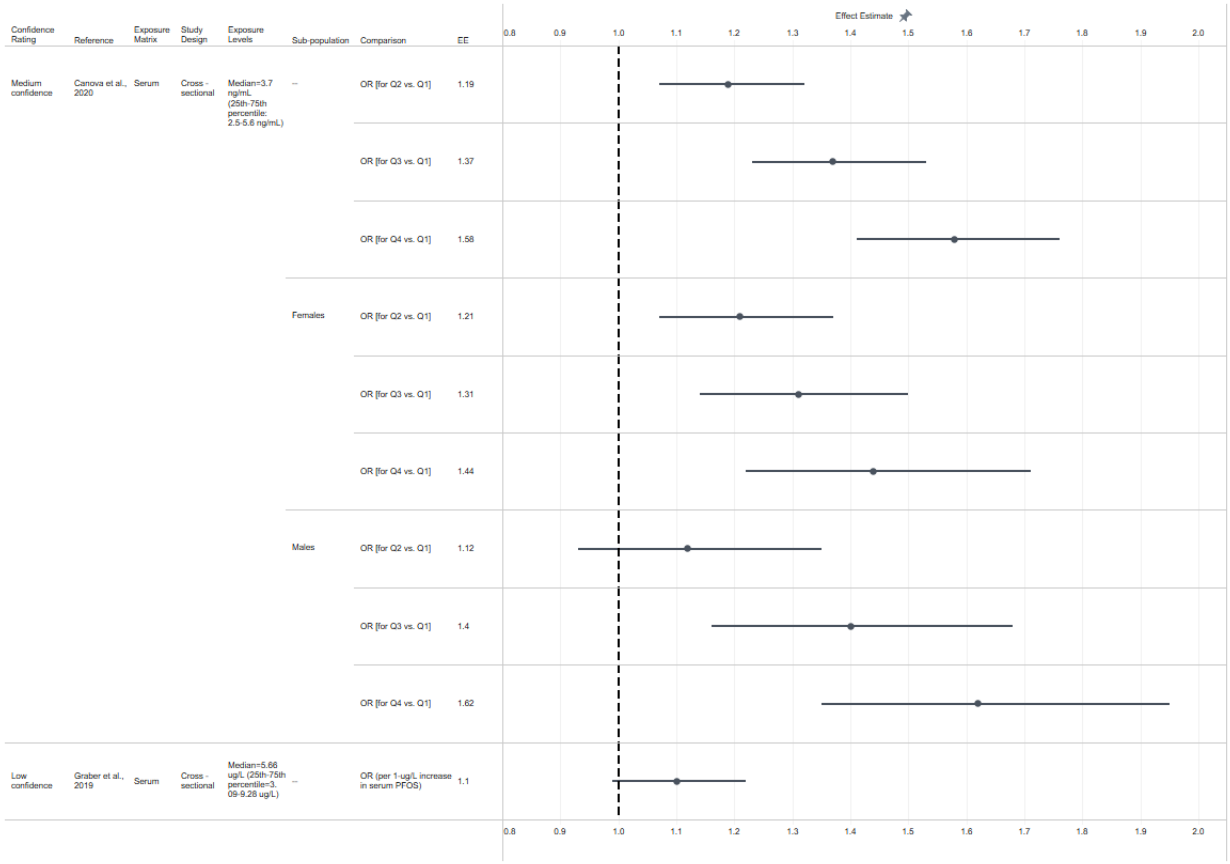


Figure 3-37. Odds of High Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

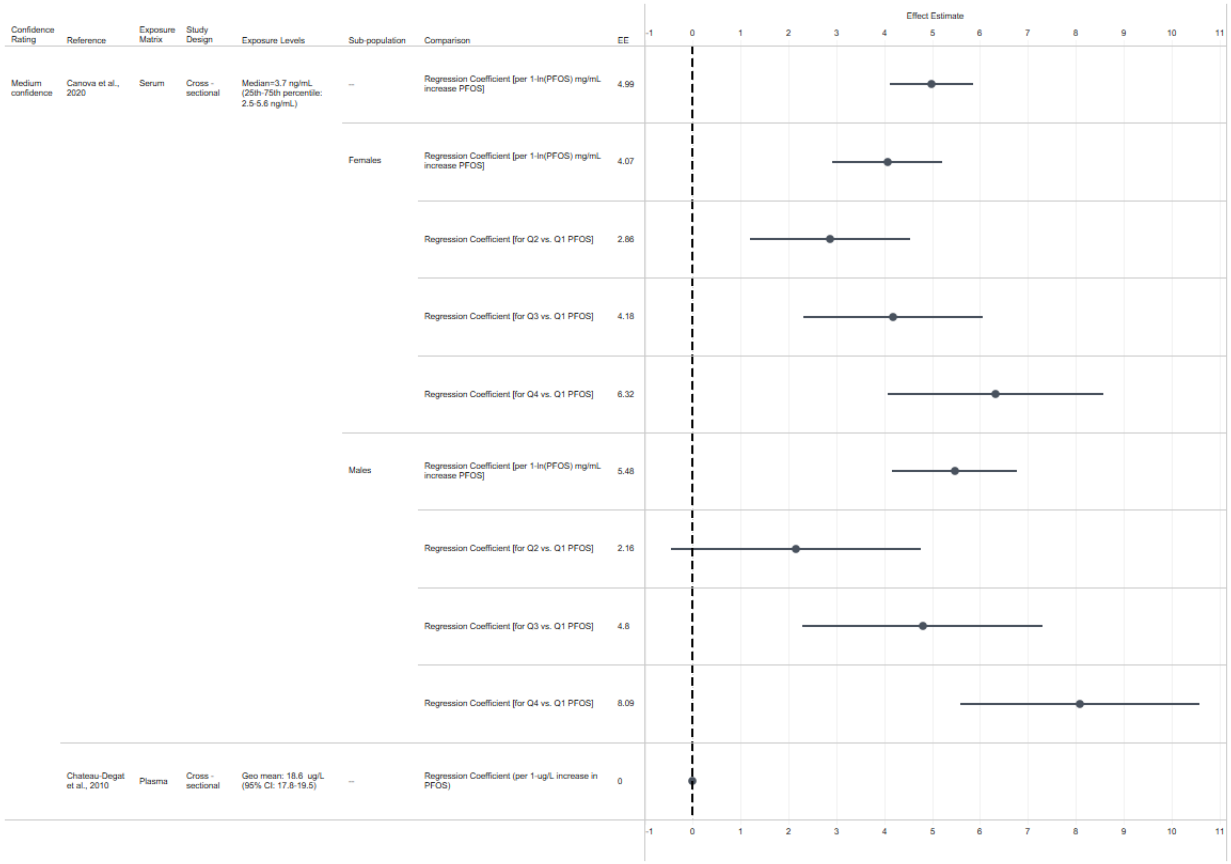


Figure 3-38. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

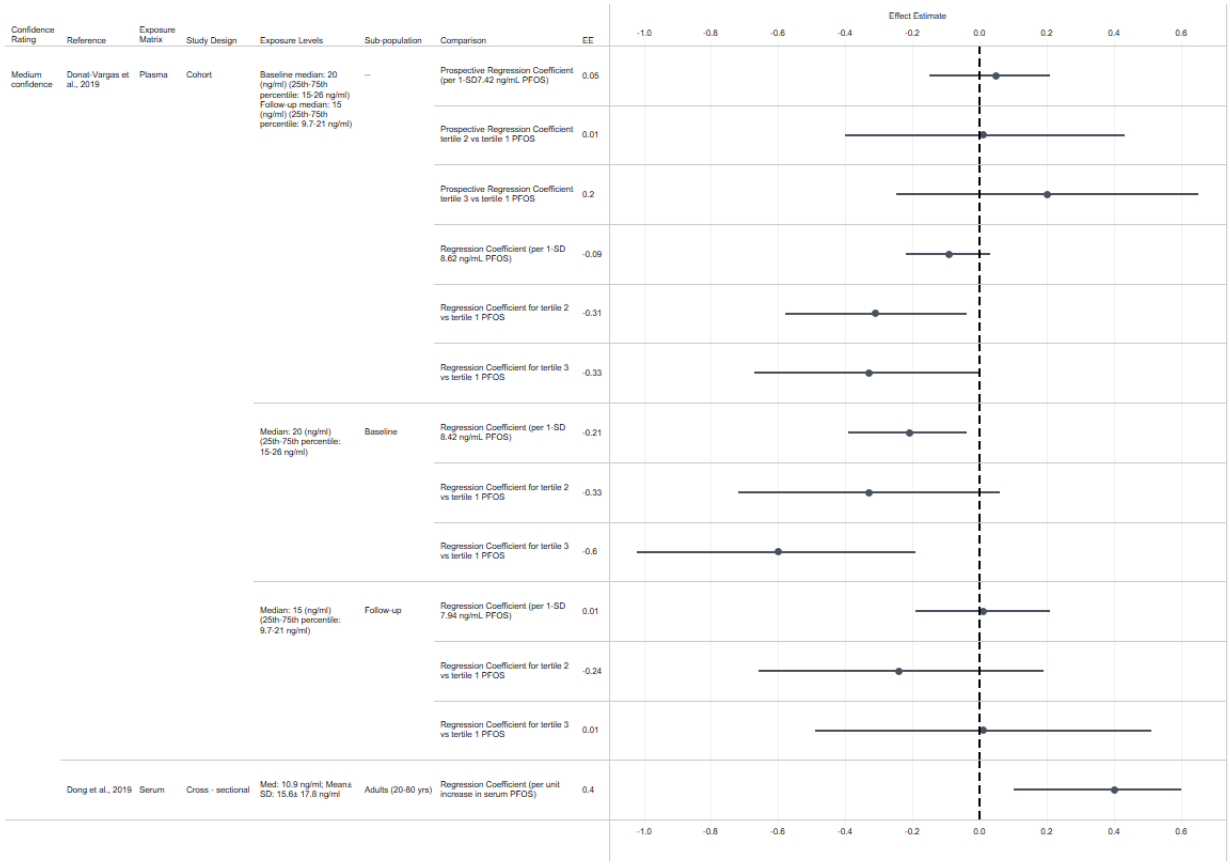


Figure 3-39. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on Tableau.

Figure 3-40. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on Tableau.

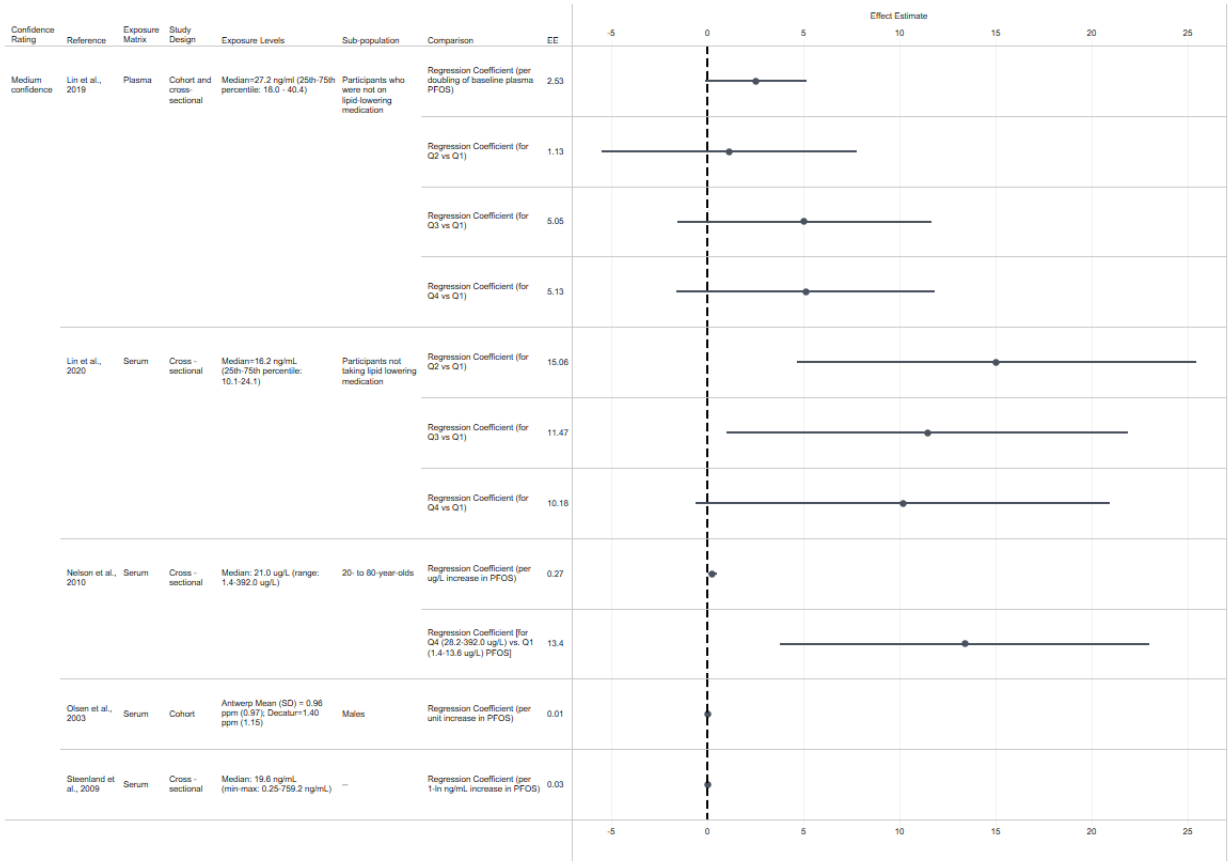


Figure 3-41. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on Tableau.

Six *medium* confidence studies examined PFOS and LDL in adults and all reported positive associations. The four studies using overlapping data from NHANES 2003–2014 reported positive associations between PFOS and LDL {Dong, 2019, 5080195, adult portion; Jain, 2019, 5080642; Liu, 2018, 4238514}, but the association was statistically significant in obese women only {Jain, 2019, 5080642} (see PFOS Appendix). The association was inverse, but not statistically significant, in non-obese persons {Jain, 2019, 5080642}. A cross-sectional analysis {Han, 2021, 7762348} of a case-control study conducted in China reported a significant positive association among 55–75-year-olds. This analysis combined cases of type 2 diabetes and healthy controls, and it is unclear if the health status of cases explained some of the association. Positive association between PFOS and LDL also was reported at baseline in the DPPOS, but this association was not statistically significant {Lin, 2019, 5187597}. This study additionally reported that PFOS was significantly associated with higher VLDL and non-HDL {Lin, 2019, 5187597}, which are cholesterol species related to LDL and known to increase cardiovascular risks. Liu 2020, 6318644 reported that PFOS was associated with slightly higher cholesterol in combined fractions of intermediate-density (IDL) and LDL that contained apolipoprotein C-III (ApoC-III), but this association was not statistically significant. ApoC-III-containing IDL and LDL are strongly associated with increased cardiovascular risks. Thus, the positive associations with cholesterol in ApoC-III-containing fractions of IDL and LDL were coherent with the

positive associations found for LDL in the other *medium* confidence studies. APOB was also examined in a single *medium* confidence NHANES study {Jain, 2020, 6988488} that reported a significantly positive association among non-diabetic, non-lipid-lowering medication users. Consistent with these findings, nine of the ten *low* confidence studies reported positive associations between PFOS and LDL {Lin, 2020, 6315756; Lin, 2013, 2850967; Li, 2020, 6315681; He, 2018, 4238388; Canova, 2020, 7021512; Liu, 2018, 4238396; Bjorke-Monsen, 2020, 7643487; Cong, 2021, 8442223; Khalil, 2020, 7021479}. However, residual confounding by SES {Lin, 2020, 6315756; Lin, 2013, 2850967; Bjorke-Monsen, 2020, 7643487; Cong, 2021, 8442223} and oversampling of persons with potentially high PFOS and health problems {Li, 2020, 6315681} were major concerns in these studies. In addition, He 2018, 4238388 provided little new information because it used similar data as the four *medium* confidence NHANES studies. Altogether, the available evidence supports a positive association between PFOS and LDL. Few available findings were statistically significant, however, suggesting that the association between PFOS and LDL may be relatively small.

Eleven *medium* confidence and thirteen *low* confidence studies examined PFOS and HDL or clinically defined low HDL in adults. All studies examined the cross-sectional association {Dong, 2019, 5080195, adult portion; Jain, 2019, 5080642; Christensen, 2019, 5080398; Liu, 2018, 4238514; Liu, 2020, 6318644; Lin, 2019, 5187597; Wang, 2012, 2919184; van den Dungen, 2017, 5080340; Lin, 2020, 6315756; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Fan, 2020, 7102734; Canova, 2020, 7021512; Liu, 2018, 4238396; Bjorke-Monsen, 2020, 7643487; Cong, 2021, 8442223; Khalil, 2020, 7021479; Lin, 2020, 6988476; Han, 2021, 7762348; Zare Jeddi, 2021, 7404065; Ye, 2021, 6988486}. Two studies additionally examined the association between baseline PFOS and changes in HDL {Liu, 2020, 6318644; Liu 2018, 4238396}. In a population of young adults aged 20 to 39 years in Veneto region, Italy, an area with water contamination by PFAS, Canova et al. (2020, 7021512) reported statistically positive associations with HDL. Canova et al. (2020, 7021512) also reported a concentration-response curve when PFOS was categorized in deciles. An overlapping study {Zare Jeddi, 2021, 7404065} in the same community was consistent with Canova et al. (2020, 7021512), reporting significantly decreased odds of reduced HDL (< 40 mg/L, male; < 50 mg/L, female) in young adults (aged 20 to 39 years). PFOS was associated with lower HDL at baseline in the DPPOS, but this association was not statistically significant {Lin, 2019, 5187597} (see PFOS Appendix). The POUNDS-Lost study {Liu, 2020, 6318644}, most cycles of NHANES 2003–2014 {Dong, 2019, 5080195}, a study conducted in a Taiwanese high-exposure community {Lin, 2020, 6988476}, and a cross-sectional analysis {Han, 2021, 7762348} of type 2 diabetes cases and healthy controls reported no association between PFOS and HDL. In *low* confidence studies, PFOS was positively associated with HDL in five of thirteen studies {Lin, 2020, 6315756; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Liu, 2018, 4238396} (association with concurrent HDL). Of note, in Lin 2020 6315756, the positive association was limited to linear PFOS only; the association between branched PFOS and HDL was inverse and statistically significant {Lin, 2020, 6315756}. The *low* confidence studies had limitations in participant selection, residual confounding by SES, and analysis. It is unclear to what extent these limitations explained the inconsistent findings between *medium* and *low* confidence studies. Overall, the available evidence does not support a consistently inverse association between PFOS and HDL in adults.

Nine *medium* confidence and thirteen *low* confidence studies examined the association between PFOS and TG or hypertriglyceridemia. All studies examined the cross-sectional association {Jain, 2019, 5080642; Christensen, 2019, 5080398; Liu, 2018, 4238514; Liu, 2020, 6318644; Lin, 2019, 5187597; Donat-Vargas, 2019, 5080588; Wang, 2012, 2919184; Lin, 2013, 2850967; Lin, 2020, 6315756; Chen, 2019, 5387400; Li, 2020, 6315681; He, 2018, 4238388; Yang, 2018, 4238462; Sun, 2018, 4241053; Canova, 2020, 7021512; Fan, 2020, 7102734; Liu, 2018, 4238396; Cong, 2021, 8442223; Khalil, 2020, 7021479; Ye, 2021, 6988486; Han, 2021, 7762348; Zare Jeddi, 2021, 7404065}; three studies additionally examined the association between baseline PFOS and changes in TG or incident hypertriglyceridemia {Liu, 2020, 6318644; Lin, 2019, 5187597; Liu, 2018, 4238396}. Higher PFOS was significantly associated with higher levels of TG in the DPPOS {Lin, 2019, 5187597} (see PFOS Appendix). This study also reported that PFOS was associated with higher odds of hypertriglyceridemia at baseline and higher incidence of hypertriglyceridemia prospectively; the prospective association was particularly strong in participants enrolled in the placebo arm of the DPPOS {Lin, 2019, 5187597}. In contrast, PFOS was not associated with triglycerides or changes in triglycerides in the POUNDS-Lost study {Liu, 2020, 6318644}, a cross-sectional analysis {Han, 2021, 7762348} of type 2 diabetes cases and healthy controls, and a high-exposure community study in Italian young adults (aged 20–39 years) {Zare Jeddi, 2021, 7404065}. Furthermore, PFOS was inversely associated with TG in the three studies using overlapping NHANES data {Jain, 2019, 5080642; Christensen, 2019, 5080398; Liu, 2018, 4238514} and in Donat-Vargas 2019, 5080588. In this latter study, there was a statistically significant, linear trend of lower TG with increasing PFOS, regardless of whether PFOS was measured concurrently with TG or averaged between baseline and follow-up {Donat-Vargas, 2019, 5080588}. In *low* confidence studies, five reported inverse associations {Lin, 2013, 2850967; Lin, 2020, 6315756; Li, 2020, 6315681; He, 2018, 4238388; Liu, 2018, 4238396}, six reported essentially null associations {Chen, 2019, 5387400; Sun, 2018, 4241053; Canova, 2020, 7021512; Cong, 2021, 8442223; Khalil, 2020, 7021479; Ye, 2021, 6988486}, one reported a positive association {Yang, 2018, 4238462}, and one stated the association was not statistically significant {Wang, 2012, 2919184}. Altogether, the association between PFOS and TG was inconsistent.

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

3.4.3.1.2.6 Findings from Occupational Studies

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

Three *low* confidence studies examined the association between PFOS and TC in workers. Of these, two examined the cross-sectional association between PFOS and TC in fluorochemical plant workers or firefighters exposed to AFFF {Wang, 2012, 2919184; Rotander, 2015, 3859842}; one investigated the association between baseline PFOS and changes in TC over the

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

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

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

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

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

3.4.3.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 4 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and 8 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and cardiovascular effects. Study quality evaluations for these 12 studies are shown in Figure 3-42.

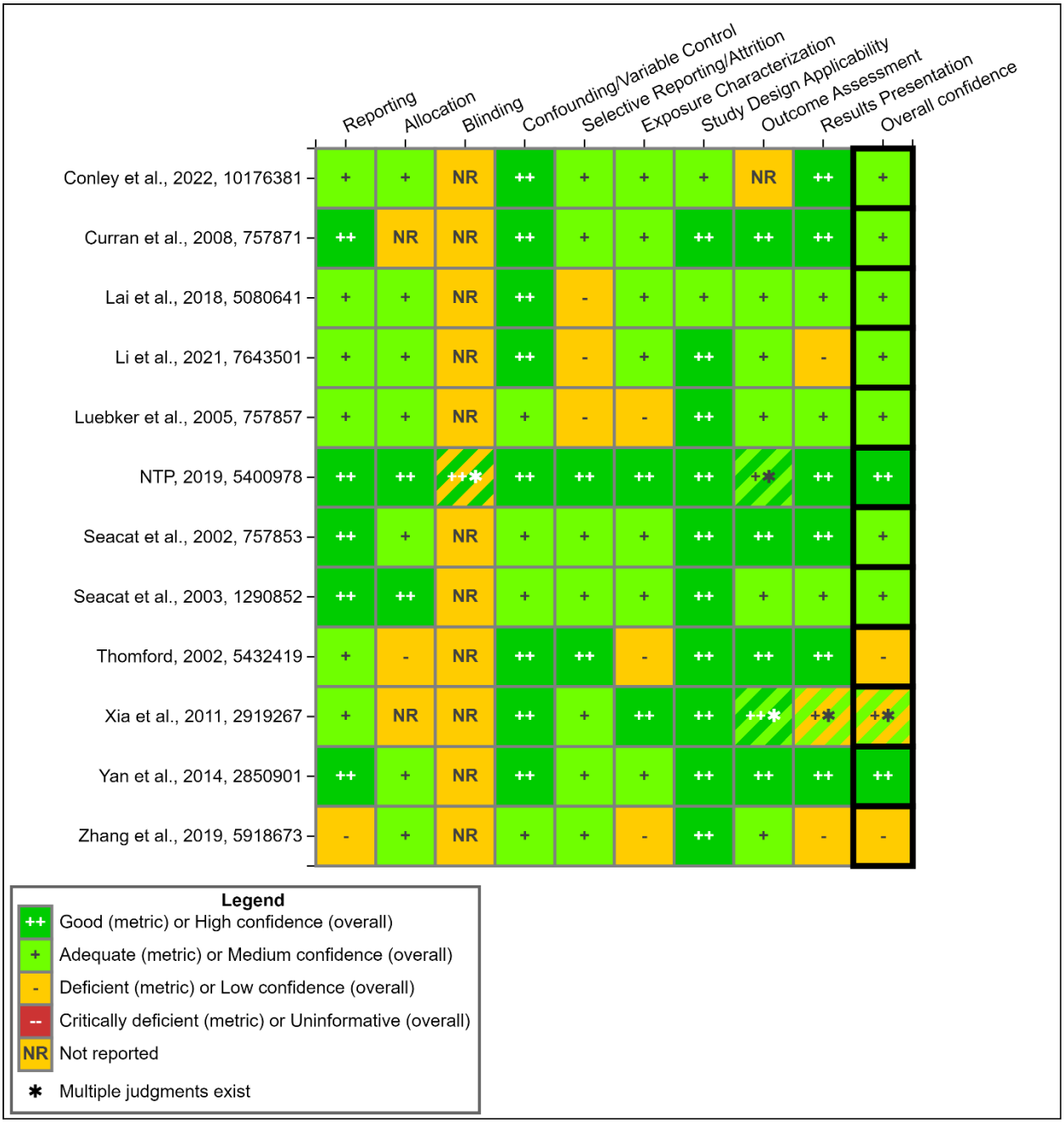


Figure 3-42. Summary of Study Evaluation for Toxicology Studies of PFOS and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

Cardiovascular effects, including blood pressure, heart weight, heart histopathology, and/or serum lipid levels, following exposure to PFOS were minimal {Curran, 2008, 757871; NTP, 2019, 5400978; Rogers, 2014, 2149155; Li, 2021, 7643501; Xia et al., 2011, 2919267}. In male and female mice (sexes combined), relative heart weight was increased at PND 21 after gestational exposure (GD 2–GD 21) to 2 mg/kg/day PFOS; however, this was confounded by decreased body weights; absolute heart weights were unchanged {Xia et al., 2011, 2919267}. In

10–11-week-old Sprague-Dawley rats exposed daily by gavage for 28 days, a decrease in absolute (14% relative to control animals) and relative (9% relative to control animals) heart weight were reported in females exposed to 5 mg/kg/day while a decrease in absolute (9% relative to control animals) heart weight was reported in male rats exposed to 5 mg/kg/day. The authors note that the biological significance of this is not clear. No alterations were observed in the heart following histopathological analysis in either sex {NTP, 2019, 5400978}. It should be noted that this study design (e.g., 28-day duration) is not sufficient to address whether PFOS exposure leads to injuries in the cardiovascular system like plaque formation in atherosclerosis as this often requires 10–12 weeks for development to accurately be evaluated in a rodent model {Daugherty, 2017, 5932343}. H&E staining of tissues extracted from PFOS-exposed female BALB/c mice revealed that exposure (0.1 or 1 mg/kg/day for 2 months) accumulated in the epicardial area of the heart that correlated regionally with inflammatory cell infiltration (results reported qualitatively) {Li, 2021, 7643501}.

Curran et al. (2008, 757871) measured blood pressure in 35–37-day old Sprague-Dawley rats exposed to PFOS in the diet (doses up to approximately 6.34 mg/kg/day for males and 7.58 mg/kg/day for females) for 28 days; no significant change in blood pressure measurements were observed across the groups, though results were not quantitatively reported. Adult Sprague-Dawley offspring of dams treated with PFOS (18.75 mg/kg/day) via oral gavage from GD 2–GD 6 had increased blood pressure measurements {Rogers, 2014, 2149155}. Male offspring exhibited an 18% and 12% increase in systolic blood pressure at 7 and 52 weeks of age, respectively. Female offspring exhibited a 24% and 19% increase in systolic blood pressure at 37 and 65 weeks of age, respectively; no change in blood pressure was noted at the 7-week timepoint. In male offspring, increased systolic blood pressure was associated with a significantly decreased number of nephrons in the kidney (measurements were taken at PND 22; body weights and kidney weights were not significantly different compared to control animals). Rogers et al. (2014, 2149155) discussed that the association is a consequence of a higher load on the available nephrons. The higher load results in a cycle of sclerosis and pressure natriuresis that can increase blood pressure. However, the exact mechanisms have yet to be elucidated. In contrast to the results of Rogers et al. (2014, 2149155), no changes in blood pressure were observed at PND 21 in male and female mice gestationally exposed to 0.2–2 mg/kg/day PFOS {Xia et al., 2011, 2919267}. Heart rate was also unchanged in this study.

PFOS has been observed to cause perturbations in lipid homeostasis, which may have effects on the cardiovascular system. Alterations in serum lipid levels have been observed in non-human primates and rodent models in subchronic, chronic, and developmental studies of oral exposure to PFOS (Figure 3-43). Decreased serum TC, triglycerides, HDL, LDL, and/or VLDL levels occurred in rhesus monkeys {Goldenthal, 1979, 9573133}, cynomolgus monkeys {Seacat, 2002, 757853}, rats {Seacat, 2003, 1290852; Thibodeaux, 2003, 5082311; Luebker, 2005, 757857; Curran, 2008, 757871; NTP, 2019, 5400978; Conley, 2022, 10176381}, and mice {Bijland, 2011, 1578502; Wan, 2012, 1332470; Wang, 2014, 2851252; Yan, 2014, 2850901; Lai, 2018, 5080641} following PFOS exposure. In Sprague-Dawley rats exposed daily by gavage for 28 days, significant decreases in serum TC (males) and triglyceride (females) levels were reported following PFOS exposure as low as 0.312 and 2.5 mg/kg/day, respectively {NTP, 2019, 5400978}. Serum triglyceride levels were significantly decreased in female CD-1 mice exposed daily by gavage to 3 mg/kg/day PFOS for 7 weeks {Lai, 2018, 5080641}. One study reported decreased serum HDL levels but an approximate 2-fold increase in serum LDL levels in male

BALB/c mice following exposure to 5 mg/kg/day PFOS by gavage for 28 days {Yan, 2014, 2850901}.

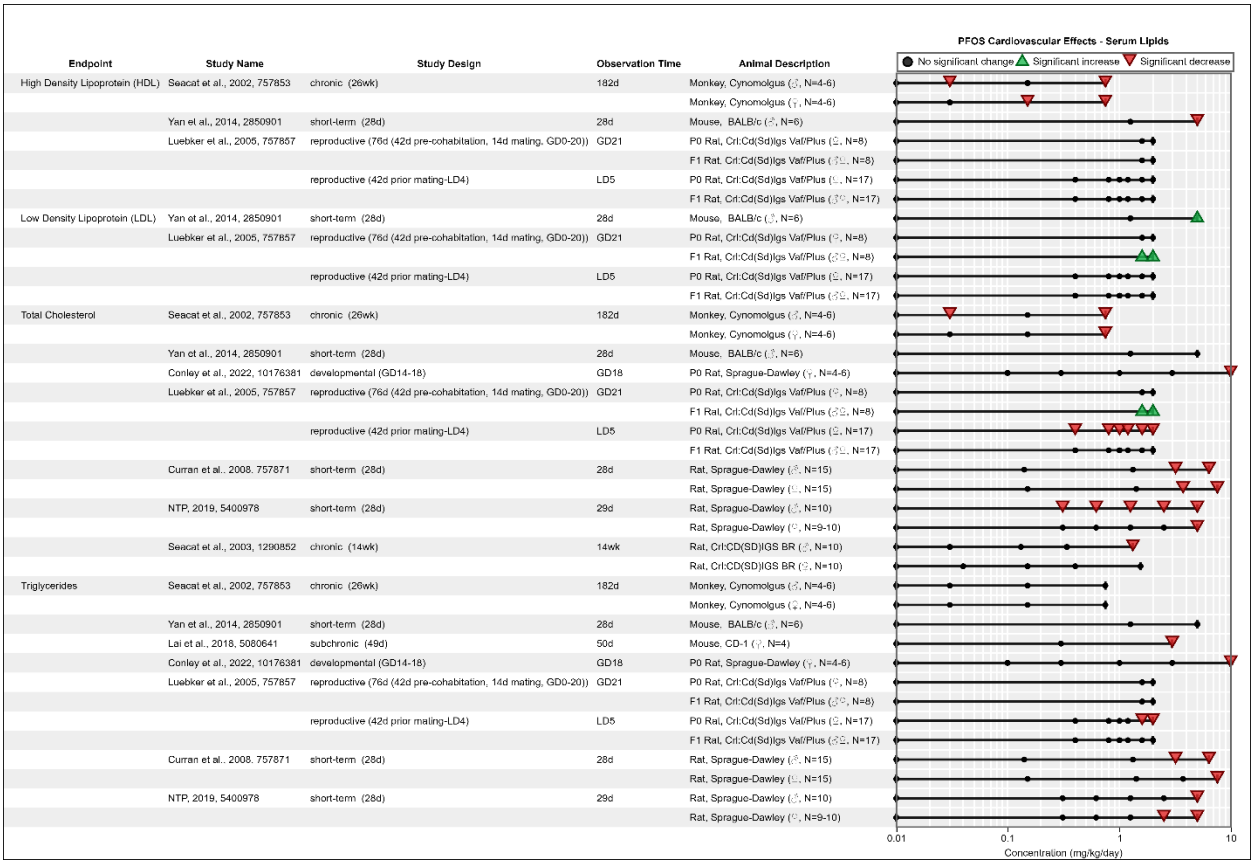


Figure 3-43. Serum Lipid Levels in Animal Models Following Exposure to PFOS

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.
GD = gestation day; P_0 = parental generation; PND = postnatal day; PNW = postnatal week; F_1 = first generation.

Conclusions from these studies are limited by differences in serum lipid composition between humans and commonly used rodent models, which may impact the relevance of the results to human exposures {Getz, 2012, 1065480; Oppi, 2019, 5926372}. Some rodent studies {Yan, 2014, 2850901} exhibit a biphasic dose response where low exposure concentrations lead to increased serum lipid levels while high exposure concentrations lead to decreased serum lipid levels. This has called in the validity of using rodent models to predict human lipid outcomes. Additionally, food consumption and food type may confound these results {Cope, 2021, 10176465; Schlezinger, 2020, 6833593; Fragki, 2021, 8442211}, as diet is a major source of lipids, yet studies do not consistently report a fasting period before serum collection and laboratory diets contain a lower fat content compared to typical Westernized human diets. More research is needed to understand the influence of diet on the response of serum cholesterol levels in rodents treated with PFOS.

3.4.3.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse cardiovascular outcomes is discussed in Section 3.2.6 of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 9 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to cardiovascular effects. A summary of these studies is shown in Figure 3-44.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	0	1	1	2
Atherogenesis And Clot Formation	1	1	2	4
Cell Growth, Differentiation, Proliferation, Or Viability	0	1	1	2
Cell Signaling Or Signal Transduction	0	0	2	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	1	2
Inflammation And Immune Response	0	0	2	2
Oxidative Stress	0	2	2	4
Grand Total	2	3	4	9

Figure 3-44. Summary of Mechanistic Studies of PFOS and Cardiovascular Effects

Interactive figure and additional study details available on Tableau.

3.4.3.3.1 Fatty acid synthesis, metabolism, storage, transport, and binding

One study published in 2019 found that *in vivo* exposure to PFOS significantly upregulated the expression of genes associated with fatty acid metabolism in zebrafish heart tissue {Khazaee, 2019, 5918850}. Fatty acid binding proteins are highly expressed in tissues involved in active lipid metabolism, such as the heart and liver, and they act as intracellular lipid chaperones {Nguyen, 2020, 727986}. In this study, adult male and female zebrafish were exposed to 0.1 or 1 mg/L PFOS for 30 days, and the expression of genes that encode fatty acid binding proteins *fabp1a*, *fabp10a*, and *fabp2* was measured in several tissues (liver, heart, intestine, and ovary) at four timepoints. PFOS upregulated the expression of fatty acid binding proteins *fabp10a* and *fabp2* in the heart tissue of males and females at all timepoints, while *fabp1a* expression was not detected in heart tissue. The authors found that the heart had the most consistent results out of all tissues examined {Khazaee, 2019, 5918850}. For additional information on the disruption of fatty acid synthesis, metabolism, transport, and storage in the liver following PFOS exposure, please see Section 3.4.1.3.2.

3.4.3.3.2 Serum Lipid Homeostasis

Epidemiological studies (Section 3.4.3.1) provide consistent evidence that PFOS alters serum lipid levels, demonstrated by significant positive associations between PFOS and TC and LDL cholesterol. The mechanisms underlying these associations have not yet been determined. One

study summarized in EPA's 2016 Health Effects Support Document {U.S. EPA. 2016, 3603365} provides mechanistic evidence related to these outcomes {Fletcher, 2013, 2850968}. The authors of this study evaluated a subset of 290 adults in the C8 Health Project for evidence that PFOS can influence the expression of genes involved in cholesterol metabolism, mobilization, or transport measured in whole blood. When both sexes were analyzed together, a positive association was found between PFOS and a gene involved in cholesterol mobilization (Neutral Cholesterol Ester Hydrolase 1 (*NCEH1*)), and a negative relationship was found between PFOS and a transcript involved in cholesterol transport (Nuclear Receptor Subfamily 1, Group H, Member 3 (*NR1H3*)). When males and females were analyzed separately, serum PFOS was positively associated with expression of genes involved in cholesterol mobilization and transport in females (*NCEH1* and *PPARα*), but no associations were found in males. For additional information on the disruption of lipid metabolism, transport, and storage in the liver following PFOS exposure, please see Section 3.4.1.3.2.

3.4.3.3.3 Oxidative stress, apoptosis, inflammation, and vascular permeability leading to atherogenesis

Epidemiological studies (Section 3.4.3.1) provide consistent evidence for an association between PFOS and blood pressure in some human populations, and limited evidence for an association between PFOS and increased risk of hypertension. The biological mechanisms underlying the association between PFOS and elevated blood pressure are still largely unknown, but pathways that have been proposed include PFOS-induced oxidative stress leading to endothelial dysfunction and impaired vasodilation, intra-uterine exposure leading to reduced number of nephrons at birth, interference with signaling pathways of thyroid hormones that regulate blood pressure, and transcriptional induction of aldosterone {Pitter, 2020, 6988479}.

Oxidative damage, inflammation, and increased vascular permeability are all pathways associated with the early stages of atherosclerosis. Atherosclerosis is an inflammatory disease of vessel walls characterized by plaque build-up inside arteries caused by high blood lipid levels and endothelial dysfunction. Atherosclerosis is an established risk factor for cardiovascular diseases including myocardial infarction and stroke {Nguyen, 2020, 7279862}. One epidemiological study found no significant associations between PFOS and carotid artery atherosclerotic plaque or CIMT {Lind, 2017, 3858504}, but two other studies found significant associations between PFOS and CIMT {Lin, 2013, 2850967; Lin, 2016, 3981457}.

3.4.3.3.4 Endothelial disfunction

3.4.3.3.4.1 *In Vivo* Evidence

A cross-sectional study in adolescents and young adults in Taiwan (1992–2000) studied the associations between serum PFOS, CIMT, circulating endothelial and platelet microparticles, and urinary 8-hydroxydeoxyguanosine (8-OHdG) {Lin, 2016, 3981457}. CIMT is a measure used to diagnose the extent of carotid atherosclerotic vascular disease. Cluster of differentiation 31 (CD31), also known as platelet endothelial cell adhesion molecule (PECAM-1), is a protein involved in cell-to-cell adhesion. CD42 is a protein expressed on the surface of platelets that is involved in platelet adhesion and plug formation at sites of vascular injury. This study evaluated serum CD31+/CD42a- as a marker of endothelial apoptosis and serum CD31+/CD42a+ as a marker of platelet apoptosis. The results showed that both markers of apoptosis increased significantly across quartiles of PFOS exposure. No significant associations were found between

PFOS and CD62E, a marker of endothelial activation, or between PFOS and CD62P, a marker of platelet activation. In addition, no significant associations were found between serum PFOS and urinary 8-OhdG, a marker of DNA oxidative stress. The authors observed a positive association between PFOS and CIMT that was stronger when serum markers of endothelial and platelet apoptosis were higher. The adjusted odds ratio (OR) for CIMT with PFOS was 2.86 (95% CI: 1.69, 4.84), P < 0.001) when the levels of CD31+/CD42a- and CD31+/CD42a+ were both above 50%, compared to the OR of 1.72 (95% CI: 0.84, 3.53, P = 0.138) when both apoptosis markers were below 50%. The authors postulated that PFOS may play a role in atherosclerosis by inducing apoptosis of endothelial and platelet cells {Lin, 2016, 3981457}.

Another cross-sectional study in Taiwanese adults (2009–2011) evaluated the associations between serum PFOS and urinary 8-OhdG and 8-nitroguanine (8-NO2Gua) as biomarkers of DNA oxidative and nitrative stress {Lin, 2020, 6315756}; however, unlike Lin et al. (2016, 3981457), this study found significant associations between PFOS and biomarkers of oxidative DNA damage. Linear PFOS levels were positively associated with adjusted levels of 8-OhdG and 8-NO2Gua, while no association was found for branched PFOS levels. The authors also evaluated the associations between PFOS and serum lipid profiles (LDL, small dense LDL, HDL, triglycerides), and found that the adjusted OR for elevated LDL (> 75th percentile) with linear PFOS was higher when each DNA stress marker was above 50% compared to below 50% (OR 3.15, 95% CI: 1.45, 6.64, P = 0.003 for both stress markers above 50% vs. OR 1.33, 95% CI: 0.78, 2.27, P = 0.302 for both stress markers below 50%). Linear PFOS levels were also positively correlated with HDL, but the relationship with stress markers was not studied.

3.4.3.3.4.2 *In Vitro* Evidence

Liao et al. (2013, 1937227) found that expression of peroxisome proliferator-activated receptor-gamma (*PPARγ)* and estrogen receptor-alpha (*Erα*) were significantly upregulated in human umbilical vein endothelial cells (HUVECs) exposed to PFOS (100 mg/L) for 48 hours. PFOS exposure also significantly upregulated expression of six inflammatory response-related genes (interleukin-1-beta (*IL-1β*), interkeukin-6 (*IL-6*), prostaglandin-endoperoxide synthase 2 (*PTGS2*) also known as COX2, nitric oxide synthase 3 (*NOS3*), *P-Selectin*, and intracellular adhesion molecule 1 (*ICAM1*)) and increased the generation of intracellular reactive oxygen species (ROS) in HUVECs. In addition, adhesion of monocytes onto HUVECs was increased 2.1-fold over the control when the cells were treated with PFOS (100 mg/L) for 48 hours. The authors postulated that the PFOS-induced inflammatory response in this *in vitro* system was mediated by *PPARγ*, *Erα*, and ROS, and that PFOS upregulation of *ICAM1* and *P-Selectin* may play an important role in adhesion of monocytes to vascular epithelium leading to vascular inflammation.

Similarly, Qian et al. (2010, 2919301) found that PFOS induced ROS production in human microvascular endothelial cells (HMVECs) even at low concentrations (2–5 μM) within one hour. These authors also studied permeability changes in HMVEC monolayers following PFOS exposure by measuring transendothelial electrical resistance. The results showed that PFOS induced endothelial permeability in a concentration-dependent manner. Confocal microscopy imaging analysis revealed many gaps in the PFOS-treated HMVEC monolayers that increased in a concentration-dependent manner. PFOS also induced actin filament remodeling. Pretreating HMVEC monolayers with catalase, a ROS scavenger, prior to PFOS exposure substantially blocked the PFOS-induced gap formation and actin filament remodeling.

Two studies evaluated the potential for PFOS and other PFAS to activate the plasma kallikrein-kinin system (KKS) using *in vitro* and *ex vivo* activation assays and *in silico* molecular docking analysis {Liu, 2017, 4238579; Liu, 2018, 4238499}. The plasma KKS plays important roles in regulating inflammation, blood pressure, coagulation, and vascular permeability. Activation of the plasma KKS can release the inflammatory peptide, bradykinin (BK), which can lead to dysfunction of vascular permeability {Liu, 2018, 4238499}. The cascade activation of KKS involves autoactivation of Hageman factor XII (FXII), cleavage of plasma prekallikrein (PPK), and activation of high-molecular-weight kininogen (HK) {Liu, 2018, 4238499}. These studies examined the potential for PFOS and other PFAS chemicals to act as FXII activators due to their structural similarities to natural long-chain fatty acids {Liu, 2017, 4238579}. The addition of PFOS (1–5 mM) to mouse plasma *ex vivo* resulted in dose-dependent PPK activation measured by analysis of PPK and plasma kallikrein expression levels after 2 hours of incubation, and the approximate lowest-observed-effect concentration (LOEC) for PFOS was 3 mM {Liu, 2017, 4238579}. This demonstrated the potential for PFOS to activate the plasma KKS, but at a relatively high concentration compared to typical human exposure levels in the general population. PFAS with longer carbon chain lengths activated the KKS at a much lower concentration compared to PFOS (e.g., PFHxDA activated the KKS at 30 µM). Time course experiments showed that PPK activation occurred within 5 min after addition of PFOS or other PFAS to mouse plasma {Liu, 2017, 4238579}.

The potential effects of PFOS on KKS activation in mouse plasma *ex vivo* were also evaluated using protease activity assays. Plasma samples were incubated with PFOS (100–5,000 µM) for 15 minutes and then analyzed for FXIIa activity and kallikrein-like activity. PFOS significantly increased FXIIa activity only at the highest concentration tested (5 mM) Liu et al. (2018, 4238499), and kallikrein-like activity was significantly increased only at 3 and 5 mM PFOS {Liu, 2017, 4238579; Liu, 2018, 4238499}. Western blot analyses demonstrated that 5 mM PFOS could induce the KKS waterfall cascade activation both *in vitro*, utilizing human plasma zymogens FXII, PPK, and HK, and *ex vivo* utilizing plasma from human volunteers {Liu, 2017, 4238579}.

Binding of PFOS with purified human FXII was further evaluated by Liu et al. (2017, 4238579) using native PAGE separation and FXII Western blot assay. Two hours of incubation of FXII with PFOS (1 or 3 mM) reduced the amount of free FXII in a concentration-related manner. The results from *ex vivo, in vitro*, and *in silico* experiments were compared for different PFAS, and the authors concluded that the degree of KKS activation was related to structural properties such as carbon chain length, terminal groups, and fluorine atom substitution. For example, PFAS terminated with sulfonic acid, including PFOS, demonstrated a stronger binding affinity for FXII and higher capability of inducing KKS activation than PFAS terminated with carboxylic acid or other terminal groups. {Liu, 2017, 4238579}.

3.4.3.3.5 Coagulation and fibrinolysis

The coagulation and fibrinolytic pathways can contribute to the progression of atherosclerosis. Two studies from the literature published after the 2016 HESD evaluated the potential of PFOS to affect these pathways. Bassler et al. (2019, 5080624) evaluated a subset of 200 individuals from the C8 Health Project for a variety of disease biomarkers including plasminogen activator inhibitor (PAI-1), a glycoprotein that inhibits the formation of plasmin from plasminogen and thus prevents clot lysis in vessel walls. Elevated PAI-1 levels are associated with thrombotic risk,

but this study found no significant association between PFOS and PAI-1 levels. Likewise, Chang et al. (2017, 3981378) saw no significant changes in coagulation parameters measured in male and female cynomolgus monkeys following acute oral exposure to PFOS with serum concentrations up to 165 µg/mL, including measures of prothrombin time, activated partial thromboplastin time, and fibrinogen.

3.4.3.4 Evidence Integration

There is *moderate* evidence for an association between PFOS exposure and cardiovascular effects in humans based on consistent positive associations with serum lipid levels, specifically LDL and TC. Additional evidence of positive associations with blood pressure and hypertension in adults supported this classification. The available data for CVD and atherosclerotic changes was limited and addressed a wider range of outcomes, resulting in some residual uncertainty for the association between PFOS exposure and these outcomes.

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

Based on this systematic review of 44 epidemiologic studies, the available evidence supports a positive association between PFOS and TC in the general population, including children and pregnant women. The available evidence also generally supports a positive association between PFOS and LDL in children and adults in the general population. Although PFOS appeared not associated with elevated TC and LDL in workers, this conclusion is uncertain as the occupational studies included in this review are limited in both quantity and quality. Finally, for all populations, the association between PFOS and HDL and TG were mixed, suggesting no consistent associations between PFOS and reduced HDL and elevated TG. Overall, these findings are largely consistent with the 2016 Health Assessment. The positive associations with TC are also supported by the recent meta-analysis restricted to general population studies in adults {EPA, 2022, 10369698}. Similarly, a recent meta-analysis including data from 11 studies reported consistent associations between serum PFOS or a combination of several PFCs including PFOA and PFOS, and increased serum TC, LDL, triglyceride levels in children and adults {Abdullah Soheimi, 2021, 9959584}.

The animal evidence for an association between PFOS exposure and cardiovascular toxicity is *moderate* based on serum lipids effects observed in eight *high* or *medium* confidence studies.

The most consistent results are for total cholesterol and triglycerides, although direction of effect can vary by dose. In animal toxicological studies, no effects or minimal alterations were noted for blood pressure, heart weight, and histopathology in the heart. However, many of the studies identified may not be adequate in exposure duration to assess potential toxicity to the cardiovascular system. The biological significance of the decrease in various serum lipid levels observed in these animal models regardless of species, sex, or exposure paradigm is unclear; however, these effects do indicate a disruption in lipid metabolism.

The mechanisms underlying the positive associations between PFOS and serum TC, LDL, and blood pressure in humans have yet to be determined. Data from the C8 Health Project demonstrated that serum PFOS was positively associated with expression of genes involved in cholesterol mobilization and transport in samples from women (NCEH1 and PPARα), while there were no associations in men. The results for PFOS-induced changes to serum lipid levels are in contrast in rodents (generally decreased) compared to humans (generally increased). PFOS exposure led to up-regulation of genes that encode fatty acid binding proteins in zebrafish, which play a role in lipid binding, particularly in the heart. Evidence is ultimately limited in regard to clear demonstration of mechanisms of alterations to serum lipid homeostasis caused by PFOS exposure.

Regarding the potential for PFOS to lead to atherosclerosis as evaluated by related mechanisms or mechanistic indicators, one epidemiologic study found no association between PFOS and carotid artery atherosclerotic plaque or CIMT, while two other epidemiologic studies found significant associations between PFOS and CIMT. The two studies that reported PFOS-associated CIMT demonstrated endothelial dysfunction via increases in markers of endothelial and platelet apoptosis in the serum: increased serum CD31+/CD42a-, which is a marker of endothelial apoptosis, and increased serum CD31+/CD42a+, which is a marker of platelet apoptosis. Markers of serum and platelet activation were not changed, nor was there evidence of DNA oxidative damage (no change in urinary 8-OhdG). The authors of the study postulated that PFOS-induced apoptosis of endothelial and platelet cells may play a role in the development of atherosclerosis. In contrast, another human study reported increased urinary 8-OhdG and 8-nitroguanine (8-NO2Gua) resulting in limited and inconsistent results for oxidative damaging potential of PFOS. *In vitro,* PFOS was shown to induce oxidative stress and upregulate inflammatory response genes in human umbilical vein endothelial cells. The authors concluded that oxidative stress and changes in the expression of genes involved in adhesion of monocytes to vascular epithelium may lead to vascular inflammation. Binding of PFOS to human FXII was demonstrated, which is the initial zymogen of plasma kallikrein-kinin system (KKS) activation, an important regulator of inflammation, blood pressure, coagulation, and vascular permeability. The authors attributed the degree of KKS activation to structural properties of PFOS (among other PFAS). There was no association between PFOS and disease biomarkers related to clotting and coagulation in both human and non-human primate data. While there is mechanistic evidence that PFOS exposure can lead to molecular and cellular changes that are related to atherosclerosis, human studies identified herein reported a lack of an association between PFOS exposure and markers of atherosclerosis. Thus, the relevance of these mechanistic data is unclear.

3.4.3.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the ***evidence indicates*** that PFOS exposure is likely to cause adverse cardiovascular effects, specifically serum lipids effects, in humans under relevant exposure circumstances (Table 3-11). The hazard judgment is driven primarily by consistent evidence of serum lipids response from epidemiological studies at median PFOS levels between 3.7–36.1 ng/mL (range of median exposure in studies observing an adverse effect). The evidence in animals showed coherent results for perturbations in lipid homeostasis in non-human primates and rodent models in developmental, subchronic, and chronic studies following exposure to doses as low as 0.03 mg/kg/day PFOS. While there is some evidence that PFOS exposure might also have the potential to affect blood pressure and other cardiovascular responses in humans given relevant exposure circumstances, the human evidence underlying this possibility is uncertain and without support from animal or mechanistic studies.

Table 3-11. Evidence Profile Table for PFOS Cardiovascular Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.3.1)					⊕⊕⊙ ***Evidence Indicates (likely)***
Serum lipids 2 *High* confidence studies 20 *Medium* confidence studies 25 *Low* confidence studies 11 *Mixed*[a] studies	Among studies of children (20), several studies reported evidence of significant increases in TC (6/20) and LDL (5/20), though others observed no association. While some studies observed significantly increased HDL (6/20), others reported significant decreases or no associations. *Medium* and *mixed* confidence studies in adults (19) observed significant positive associations in HDL (7/19), LDL (7/19), and TC (8/19). Results for TG were mixed, with two studies reporting increased levels and three studies finding decreased levels, with one study in obese females. Non-significant inverse results were observed for HDL (4/19), LDL (3/19), TC (6/19), and TG (7/19). *Low* confidence studies followed the similar trend of mixed results. Studies examining pregnant	*High* and *medium* confidence studies C*onsistent findings* of positive associations for LDL and TC across study populations *Coherence* of observed associations in adults from the general population with previous evidence from serum lipid effects	*Low* confidence studies	⊕⊕⊙ *Moderate* Evidence for cardiovascular effects is based on numerous *medium* confidence studies reporting positive associations with serum lipids (HDL, LDL, TC, TG) in adults from the general population. Studies of children reported mixed findings in most serum lipids, but results were largely consistent for LDL and TC, with some reaching significance. *High* and *medium* confidence studies reported positive associations with blood pressure and increased risk of hypertension. *Low* confidence studies reported non-significant associations, while most *mixed* confidence studies reported significant associations. Observed effects were inconsistent for CVD and imprecise for	*Primary basis and cross-stream coherence*: Human evidence indicated consistent evidence of serum lipids response and animal evidence showed coherent results for perturbations in lipid homeostasis in non-human primates and rodent models in developmental, subchronic, and chronic studies following exposure to PFOS. While there is some evidence that PFOS exposure might also have the potential to affect blood pressure and other cardiovascular responses in humans given relevant exposure circumstances, the human evidence underlying this possibility is uncertain and without support from animal or mechanistic studies. *Human relevance and other inferences:* No specific factors are noted.

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	women were of *medium* and *mixed* confidence and reported mixed results (6). While three studies reported evidence of increased HDL and TC levels, the others failed to reach significance or reported inverse associations.			atherosclerotic changes across all study populations.	
Blood pressure and hypertension 2 *High* confidence studies 16 *Medium* confidence studies 7 *Low* confidence studies	Results from studies of varying confidence reported mixed results for changes in blood pressure, including DBP and SBP, and risk of hypertension for all study populations. Studies in children (10) reported mostly non-significant associations with blood pressure and/or hypertension, though two studies in adolescents reported significantly increased (1/10) and decreased (1/10) DBP in males. In adults (13), one study reported a significantly increased risk of hypertension (1/13), but associations from other studies did not reach significance (3/13). When stratified by sex, there were mixed results.	*High* and *medium* confidence studies	*Low* confidence studies *Inconsistent findings* of effects observed in children, likely due to variation in measured exposure windows		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	One study reported a higher risk of hypertension for males (1/13), while another reported higher risk for females (1/13). One study reported an inverse association for DBP (1/13), while others reported positive associations for DBP (6/13), but only three studies reached significance. SBP was significantly increased for all adults (4/13), in females only (2/13), and in males only (1/13). No studies examined blood pressure or hypertension in occupational populations.				
Cardiovascular disease 1 *High* confidence study 4 *Medium* confidence studies 2 *Low* confidence studies	In adults from the general population (6), significantly decreased odds of stroke (1/6) and significantly increased odds of MVD (1/6), heart attack and CVD in the third exposure group (1/6), CVD in males (1/6), and self-reported cardiovascular conditions (1/6) were observed. Other studies of stroke, CHD, and CVD reported	*High* and *medium* confidence studies	*Low* confidence studies *Inconsistent findings* for CVD-related outcomes *Imprecision* of findings, particularly for two studies with self-reported outcome measures		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	non-significant associations, including one *high* confidence study that reported no associations with CHD among Swedish men and a *medium* confidence study that reported no association with mortality from CVD or other heart diseases. One *low* confidence occupational study (1) examined male anglers over age 50 and did not observe an association with CHD or any cardiovascular conditions.				
Atherosclerotic changes 1 *High* confidence study 4 *Medium* confidence studies 1 *Low* confidence study	In studies of children (3), one study observed significant associations with CIMT across exposure groups, among females, and among those ages 12-19 (1/3). A cohort study of children and young adults reported significant increases in CIMT for all exposure groups and significant increases in some endothelial microparticle levels for atherosclerosis (1/3). Findings were mixed among adults older than 70 years of age. One	*High* and *medium* confidence studies	*Low* confidence study *Imprecision* of findings across children and adult study populations *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	study did not observe significant effects while a separate study reported a significant increase in left ventricular end-diastolic diameter and a significant decrease in relative wall thickness (1/3). One *medium* confidence study also reported significantly increased odds in Agatatson Scores of over 400, a measure of arterial calcification, in prediabetic adults aged over 25.				
Evidence from *In Vivo* Animal Toxicological Studies (Section 3.4.3.2)					
Serum lipids 2 *High* confidence studies 6 *Medium* confidence studies	Significant decreases in serum TG were observed in 5/7 studies that examined this endpoint, regardless of species, sex, or study design. No changes were observed in one monkey study and one short-term study in male mice. Similar decreases were observed in serum TC (6/7), with no changes being observed in one short-term study in male mice. In a developmental study, decreases were observed in dams, but no change	*High* and *medium* confidence studies *Consistency* of findings across species, sex, or study design *Dose-response* relationship observed within multiple studies	*Incoherence* of findings in other cardiovascular outcomes *Biological significance* of the magnitude of effect is unclear	⊕⊕⊙ *Moderate* Evidence based on eight *high* or *medium* confidence studies observed that PFOS affects serum lipids in animal models. The most consistent results are for total cholesterol and triglycerides, although direction of effect can vary by dose. The biological significance of the decrease in various serum lipid levels observed in	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	was observed in pups. Fewer studies examined HDL and LDL, with decreases in HDL (2/3) and increases in LDL (2/2) being observed.			these animal models regardless of species, sex, or exposure paradigm is unclear; however, these effects do indicate a disruption in lipid metabolism. No effects or minimal alterations were noted for blood pressure, heart weight, and histopathology in the heart. However, many of the studies identified may not be adequate in exposure duration to assess potential toxicity to the cardiovascular system.	
Histopathology 1 *High* confidence study 2 *Medium* confidence studies	No changes in heart histopathology were reported in 2 rat studies. One study in female mice qualitatively reported an increase in inflammatory cell infiltration.	*High* and *medium* confidence studies	*Limited number* of studies examining outcome		
Organ weight 1 *High* confidence study, 2 *Medium* confidence studies	Mixed results were reported for absolute and relative heart weight. Two short-term studies reported decreases in absolute heart weights in male and female rats, but mixed results (no change or decreases) were reported for relative heart weights. A developmental study reported no change in absolute heart weight and an increase in relative heart weight which was confounded by decreases in body weights.	*High* and *medium* confidence studies	*Limited number* of studies examining outcome *Confounding* variables such as decreases in body weights may limit ability to interpret these responses		
Blood pressure and heart rate 3 *Medium* confidence studies	A short-term and a developmental study found no effect on blood pressure in male and female rats. One developmental study	*Medium* confidence studies	*Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	found no effect on heart rate.				
Mechanistic Evidence and Supplemental Information (Section 3.4.3.3)					
Summary of Key Findings, Interpretation, and Limitations				**Evidence Stream Judgement**	
Key findings and interpretation: PFOS exposure was associated with changes in the expression of genes involved in cholesterol metabolism, mobilization, or transport in whole blood of adult humans. PFOS induced oxidative stress and upregulated inflammatory response genes in human umbilical vein endothelial cells exposed *in vitro*, which can lead to vascular inflammation. PFOS can bind to human FXII *in vitro*, which is the initial zymogen of plasma KKS activation, a regulator of inflammation, blood pressure, coagulation, and vascular permeability. **Limitations:** Small database; the only *in vivo* evidence is reported in two human studies with conflicting results for markers of platelet activation. Results regarding the association between PFOS exposure and carotid artery atherosclerotic plaques or CIMT, which are mechanisms of atherosclerosis, are inconsistent in human epidemiological studies.				Findings support the plausibility that PFOS exposure can lead to changes in the expression of genes involved in cholesterol regulation, as well as molecular and cellular changes that are related to atherosclerosis, although no association was observed between PFOS exposure and atherosclerosis in human epidemiological studies.	

Notes: CHD = coronary heart disease; CIMT = carotid intima-media thickness; CVD = cardiovascular disease; DBP = diastolic blood pressure; FXII = Factor XII; HDL = high density lipoprotein; KKS = kallikrein-kinin system; LDL = low density lipoprotein; density lipoprotein; SBP = systolic blood pressure; MVD = microvascular disease; TC = total cholesterol; TG = triglycerides.

[a]Mixed confidence studies had split confidence determinations for different serum lipid measures with some measures rated *medium* confidence and others rated *low* confidence.

3.4.4 Developmental

EPA identified 96 epidemiological and 19 animal toxicological studies that investigated the association between PFOS and developmental effects. Of the epidemiological studies, 28 were classified as *high* confidence, 37 as *medium* confidence, 20 as *low* confidence, 3 as *mixed* (2 *high/medium* and 1 *medium/low*) confidence, and 8 were considered *uninformative* (Section 3.4.4.1). Of the animal toxicological studies, 15 were classified as *medium* confidence, 3 as *low* confidence, and 1 was considered *mixed* (*medium/uninformative*) (Section 3.4.4.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.4.4.1 Human Evidence Study Quality Evaluation and Synthesis

3.4.4.1.1 Introduction

This section describes studies of PFOS exposure and potential *in utero* and perinatal effects or developmental delays, as well as effects attributable to developmental exposure. Developmental endpoints include gestational age, measures of fetal growth (e.g., birth weight), and miscarriage, as well as infant/child development.

The 2016 PFOS HESD {U.S. EPA, 2016, 3603365} summarized epidemiological studies of developmental effects in relation to PFOS exposure. There are 18 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and developmental effects. Study quality evaluations for these 18 studies are shown in Figure 3-45. Studies included ones conducted both in the general population as well as in communities known to have experienced *high* PFOS exposure (e.g., the C8 population in West Virginia and Ohio). Results of eleven *high* or *medium* confidence epidemiological studies (see Section 2.1.3 for information about study quality evaluations) discussed in the 2016 PFOS HESD are summarized below.

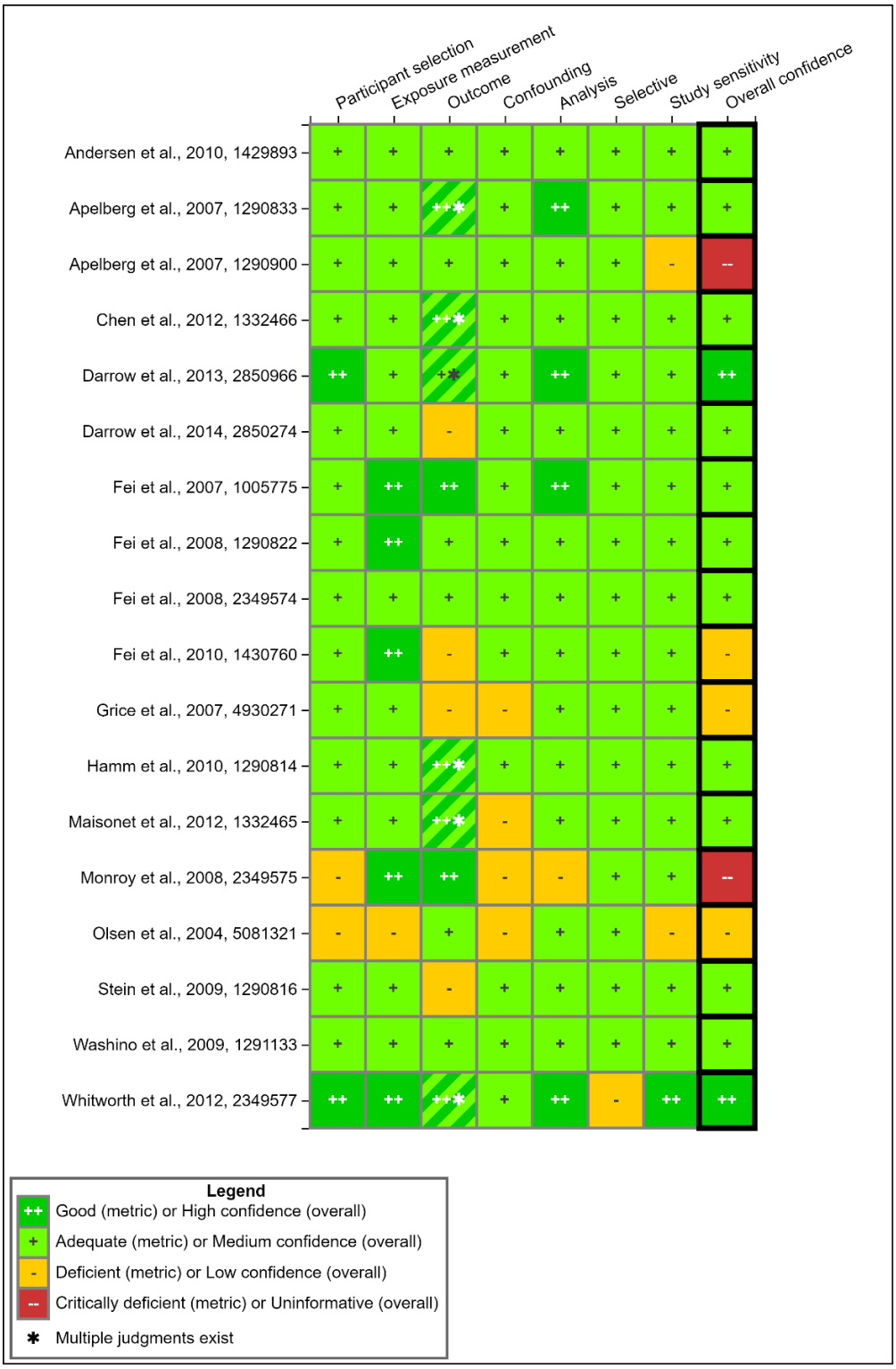


Figure 3-45. Summary of Study Evaluation for Pre-2016 Epidemiology Studies of PFOS and Developmental Effects

Interactive figure and additional study details available on HAWC.

As noted in the 2016 HESD, several available studies measured fetal growth outcomes. Apelberg et al. (2007, 1290833) found that birth weight, head circumference, and ponderal index were inversely associated with umbilical cord PFOS concentration in 293 infants born in Maryland in 2004–2005. In particular, large deficits in mean birth weight per one ln-unit increase in PFOS concentration were found (β = −69; 95% CI: −149, 10; PFOS was detected in > 99% of samples at a mean concentration of 0.005 µg/mL). Maisonet et al. (2012, 1332465) evaluated fetal growth outcomes in 395 singleton female births of participants in the Avon Longitudinal Study of Parents and Children (ALSPAC) and found that increased maternal PFOS concentration (median concentration of 0.0196 µg/mL) was associated with lower birth weights, but not with lower 20-month body weights. A study of 252 pregnant women in Alberta, Canada found no statistically significant association between birth weight or gestation length and PFOS concentration measured in maternal blood during the second trimester (mean concentration of 0.009 µg/mL) (Hamm, 2010, 1290814), although mean birth weight increased slightly by increasing PFOS tertiles (3,278 g for < 0.006 µg/mL; 3,380 g for 0.006–0.010 µg/mL; 3,387 g for > 0.010–0.035 µg/mL). In a prospective cohort study in Japan (2002–2005), Washino et al. (2009) found an inverse association between PFOS concentration in maternal blood during pregnancy (mean PFOS concentration of 0.006 µg/mL) and birth weight. As noted in the 2016 HESD, these researchers reported large reductions in mean birth weight (β = −149; 95% CI: −297.0, −0.5 g) for each log-10 change in maternal PFOS concentration, especially among female infants (β = −269.4; 95% CI: −465.7, −73.0 g). Chen et al. (2012, 1332466) examined 429 mother-infant pairs from the Taiwan Birth Panel Study and found that umbilical cord blood PFOS concentration (geometric mean of 5.94 ng/mL) was inversely associated with gestational age (β = −0.37, 95% CI −0.60, −0.13, weeks), birth weight (β = −110.2, 95% CI −176.0, −44.5, g), and head circumference (β = −0.25, 95% CI −0.46, −0.05, cm). Additionally, ORs for preterm birth, low birth weight, and small for gestational age increased with PFOS exposure (adjusted OR (95% CI) = 2.45 (1.47, 4.08), 2.61 (0.85, 8.03) and 2.27 (1.25, 4.15), respectively).

Some studies evaluated fetal growth parameters in the prospective Danish National Birth Cohort (DNBC; 1996–2002) {Andersen, 2010, 1429893; Fei et al., 2007, 1005775; Fei, 2008, 2349574}. Maternal blood samples were taken in the first and second trimester. The median maternal plasma PFOS concentration was 0.0334 µg/mL (range of 0.0064–0.1067 µg/mL). Fei et al. (2007, 1005775) found no associations between maternal PFOS concentration (blood samples taken in the first and second trimester) and birth weight. Also, as noted in the 2016 HESD, these researchers found that ORs for preterm birth (OR range: 1.43–2.94) were consistent in magnitude across the upper three PFOS quartiles, and that ORs for low birth weight (OR range: 3.39–6.00) were consistently elevated across the upper three quartiles. The HESD notes, however, that analyses in this study were limited by small cell sizes due to low incidence of these outcomes. Fei et al. (2008, 2349574) found an inverse association between maternal PFOS levels and birth length and ponderal index in the DNBC in a stratified analysis, but the associations were not statistically significant. Andersen et al. (2010, 1429893) examined the association between maternal PFOS concentrations and birth weight, birth length, and infant body mass index (BMI) and body weight at 5 and 12 months of age in DNBC participants. They found an inverse association between PFOS concentration and birth weight in girls (β = −3.2; 95% CI: −6.0, −0.3), 12-month body weight in boys (β = −9; 95% CI: −15.9, −2.2), and 12-month BMI in boys (β = −0.017; 95% CI: −0.028, −0.005).

Some studies described in the 2016 PFOS HESD evaluated developmental outcomes in the C8 Health Project study population, which comprises a community known to have been subjected to high PFAS exposure. The C8 Health Project included pregnancies within 5 years prior to exposure measurement, and many of the women may not have been pregnant at the time of exposure measurement. Stein et al. (2009, 1290816) found an association between maternal PFOS concentration and increased risk of low birth weight (adjusted OR = 1.5; 95% CI: 1.1,1.9; dose-related relationship for the 50th–75th, 75th–90th and > 90th percentile PFOS exposure concentrations), but not pre-term birth. Mean PFOS serum concentration was 0.014 μg/mL. Darrow et al. (2013, 2850966) evaluated birth outcomes in 1,630 singleton live births from 1,330 women in this study population and found an inverse association between maternal PFOS concentration and birth weight (−29 g per log unit increase; 95% CI: –66, –7); they found no association with preterm birth or low birth weight. Darrow et al. (2014, 2850274) and Stein et al. (2009, 1290816) found no association between maternal serum PFOS and increased risk for miscarriage in this population.

3.4.4.1.2 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., glomerular filtration rate and blood volume changes over the course of pregnancy), because these factors may be related to both PFOS levels and the developmental effects examined here. More confidence was placed in the epidemiologic studies that adjusted for glomerular filtration rate in their regression models or if they limited this potential source of confounding by sampling PFAS levels earlier in pregnancy. An additional source of uncertainty was the potential for confounding by other PFAS (and other co-occurring contaminants). Although scientific consensus on how best to address PFAS co-exposures remains elusive, this was considered in the study quality evaluations and as part of the overall weight of evidence determination.

For the Exposure domain, all the available studies analyzed PFAS in serum or plasma using standard methods. Given the estimated long half-life of PFOS in humans as described in Section 3.3, samples collected during all three trimesters, before birth or and shortly after birth) were considered adequately representative of the most critical *in utero* exposures for fetal growth and gestational duration measures. The postnatal anthropometric studies were evaluated with consideration of fetal programming mechanisms (i.e., Barker hypothesis) where *in utero* perturbations, such as poor nutrition, can lead to developmental effects such as fetal growth restriction and ultimately adult-onset metabolic-related disorders and related complications (see more on this topic in {De Boo, 2009, 6937194} and {Perng, 2016, 6814341}). There is some evidence that birth weight deficits can be followed by increased weight gain that may occur especially among those with rapid growth catch-up periods during childhood {Perng, 2016, 6814341}. Therefore, the primary critical exposure window for measures of postnatal (and early childhood) weight and height change is assumed to be *in utero* for study evaluation purposes, and studies of this outcome were downgraded in the exposure domain if exposure data were collected later during childhood or concurrently with outcome assessment (i.e., cross-sectional analyses).

Studies were also downgraded for study sensitivity, for example, if they had limited exposure contrasts and/or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present (e.g., for sex-stratified results). In the Outcome domain, specific considerations address validation and accuracy of specific endpoints and adequacy of case ascertainment for some dichotomous (i.e., binary) outcomes. For example, birthweight measures have been shown to be quite accurate and precise, while other fetal and early childhood anthropometric measures may result in more uncertainty. Mismeasurement and incomplete case ascertainment can affect the accuracy of effect estimates by impacting both precision and validity. For example, the spontaneous abortion studies were downgraded for incomplete case ascertainment in the outcome domain given that some pregnancy losses go unrecognized early in pregnancy (e.g., before implantation). This incomplete ascertainment, referred to as left truncation, can result in decreased study sensitivity and loss of precision. Often, this type of error can result in bias towards the null if ascertainment of fetal loss is not associated with PFOS exposures (i.e., non-differential). In some situations, differential loss is possible and bias away from the null and can manifest as an apparent protective effect. Fetal and childhood growth restriction were examined using several endpoints including low birth weight, small for gestational age (SGA), ponderal index (i.e., birth weight grams/birth length (cm^3) x 100), abdominal and head circumference, as well as upper arm/thigh length, mean height/length, and mean weight either at birth or later during childhood. The developmental effects synthesis is largely focused on the higher quality endpoints (i.e., classified as good in the Outcome domain) that were available in multiple studies to allow for an evaluation of consistency and other considerations across studies. However, even when databases were more limited, such as for spontaneous abortions, the evidence was evaluated for its ability to inform developmental toxicity more broadly, even if available in only one study.

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

Gestational duration measures were presented as either continuous (i.e., per each gestational week) or binary endpoints such as preterm birth (typically defined as gestational age < 37 weeks). Although changes in mean gestational age may lack some sensitivity, especially given the potential for measurement error, many of the studies were based on ultrasound measures early in pregnancy, which should increase the accuracy of estimated gestational age and the ability to detect associations that may be present. Any sources of error in the classification of these endpoints would also be anticipated to be non-differential with respect to PFOS exposure.

While they could impact precision and study sensitivity, they were not be considered a major concern for risk of bias.

3.4.4.1.3 Study Inclusion

There are 78 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and developmental effects. Although every study is included in the study evaluation heat maps for comprehensiveness, eight developmental epidemiological studies identified in the literature search were excluded for consideration in this synthesis because other studies report results for the same health outcomes and from the same study cohorts (i.e., were considered duplicative). For example, the Rokoff et al. (2018, 4238310) study overlapped with the Project Viva study by Sagiv et al. (2018, 4238410). The Gennings et al. (2020, 7643497) study is also not further considered here as it is a smaller subset of the Aarhus Birth Cohort described in Wikström et al. (2020, 6311677). Similarly, the Li et al. (2017, 3981358) Guangzhou Birth Cohort Study overlapped with a more recent study by Chu et al. (2020, 6315711). Four studies {Kishi, 2015, 2850268; Kobayashi, 2017, 3981430; Minatoya, 2017, 3981691; Kobayashi, 2022, 10176408} were also not considered in this synthesis, because they provided overlapping data from the same Hokkaido Study on Environment and Children's Health birth cohort population as Kashino et al. (2020, 6311632). For those Japanese studies with the same endpoints such as mean birthweight (BWT), the analysis with the largest sample size was used in forest plots and tables (e.g., Kashino et al., (2020, 6311632)). Although the Kobayashi et al. (2017, 3981430) study included a unique endpoint called ponderal index, this measure is more prone to measurement error and was not considered in any study given the wealth of other fetal growth restriction data. Similarly, the Costa et al., (2019, 5388081) study that examined a less accurate in utero growth estimate was not considered in lieu of their more accurate birth outcomes measures reported in the same cohort {Manzano-Salgado, 2017, 4238465}. One additional study by Bae et al. (2015, 2850239) was the only study to examine sex ratio and was not further considered here.

In general, to best gauge consistency and magnitude of reported associations U.S. EPA largely focused on the most accurate and most prevalent measures within each fetal growth endpoint. Two other studies with overlapping cohorts were included in the synthesis, as each study provided some unique data for different endpoints. For example, the Woods et al. (2017, 4183148) publication on the Health Outcomes and Measures of the Environment (HOME) cohort overlaps with Shoaff et al. (2018, 4619944) but has additional mean BWT data (communication with author). The mean BWT results for singleton and twin births from Bell et al. (2018, 5041287) are included in forest plots here as are the postnatal growth trajectory data in the same UPSTATE KIDS cohort by Yeung et al. (2019, 5080619) as they target different developmental windows. The Bjerregaard-Olesen et al. (2019, 5083648) study from the Aarhus birth cohort also overlaps with Bach et al. (2016, 3981534). The main effect results are comparable for head circumference and birth length in both studies despite a smaller sample size in the Aarhus birth cohort subset examined in Bjerregaard-Olesen et al. (2019, 5083648). Given that additional sex-specific data are available in the Bjerregaard-Olesen et al. (2019, 5083648) study, the synthesis for head circumference and birth length are based on this subset alone. Chen et al., (2021, 7263985) reported an implausibly large effect estimate for head circumference. After correspondence with study authors, an error was identified, and the study was not considered for head circumference.

Following exclusion of the nine studies noted above, 69 developmental epidemiological studies were included in the synthesis that were not included in the 2016 HESD report. Six additional studies {Alkhalawi, 2016, 3859818; Gundacker, 2021, 10176483; Jin, 2020, 6315720; Lee, 2013, 3859850; Lee, 2016, 3981528; Maekawa, 2017, 4238291} were considered *uninformative* due to critical study deficiencies in some risk of bias domains (e.g., confounding) or multiple domain deficiencies and are not further examined here. Thus, 63 studies were included across various developmental endpoints for further examination and synthesis.

Forty-three of the 63 different studies examined PFOS in relation to fetal growth restriction measured by the following endpoints: small for gestational age (SGA), low BWT, head circumference, as well as mean and standardized BWT and birth length measures. Twenty-two studies examined gestation duration, twelve examined post-natal growth, five each examined fetal loss, and birth defects.

3.4.4.1.4 Growth Restriction: Fetal Growth

3.4.4.1.4.1 Birth Weight

Of the 40 informative and non-overlapping studies that examined BWT measures in relation to PFOS exposures, 34 studies examined mean BWT differences. Fifteen studies examined standardized BWT measures (e.g., z-scores) with nine of these reporting results for mean and standardized BWT {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Eick, 2020, 7102797; Gyllenhammar, 2018, 4238300; Meng, 2018, 4829851; Sagiv, 2018, 4238410; Wang, 2019, 5080598; Wikström, 2020, 6311677; Workman, 2019, 5387046}. Twenty-five of the 34 mean BWT studies shown in Figure 3-46 and Figure 3-47 provided results based on a prospective birth cohort study design, and the remaining nine were cross-sectional analyses defined here as if biomarker samples were collected at birth or post-partum {Bell, 2018, 5041287; Callan, 2016, 3858524; de Cock, 2016, 3045435; Gao, 2019, 5387135; Gyllenhammar, 2018, 4238300; Kwon, 2016, 3858531; Shi, 2017, 3827535; Wang, 2019, 5080598; Xu, 2019, 5381338}.

Overall, eight of the PFOS studies relied on umbilical cord measures {Cao, 2018, 5080197; de Cock, 2016, 3045435; Govarts, 2016, 3230364; Kwon, 2016, 3858531; Shi, 2017, 3827535; Wang, 2019, 5080598; Workman, 2019, 5387046; Xu, 2019, 5381338}, and one collected blood samples in infants 3 weeks following delivery {Gyllenhammar, 2018, 4238300}. Results from the Bell et al. (2018, 5041287) study were based on infant whole blood taken from a heel stick and captured onto filter paper cards at 24 hours or more following delivery, and one study used both maternal serum samples collected 1–2 days before delivery and cord blood samples collected immediately after delivery {Gao, 2019, 5387135}. One study examined pre-conception maternal serum samples {Robledo, 2015, 2851197}. Twenty-one studies had maternal serum or plasma PFOS measures that were sampled during trimesters one {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Sagiv, 2018, 4238410}, two {Lauritzen, 2017, 3981410}, or three {Callan, 2016, 3858524; Chu, 2020, 6315711; Kashino, 2020, 6311632; Luo, 2021, 9959610; Valvi, 2017, 3983872; Yao, 2021, 9960202}, or across multiple trimesters {Chang, 2022, 9959688; Chen, 2021, 7263985; Eick, 2020, 7102797; Hjermitslev, 2020, 5880849; Lenters, 2016, 5617416; Marks, 2019, 5081319; Starling, 2017, 3858473; Wikström, 2020, 6311677; Woods, 2017, 4183148}. The study by Meng et al. (2018, 4829851) pooled exposure data from two study populations, one which measured PFOS in umbilical cord blood and one which measured PFOS in maternal blood samples collected in trimesters 1 and 2. For comparability with other studies of mean BWT, only

one biomarker measure was used here (e.g., preferably maternal samples when collected in conjunction with umbilical cord samples or maternal only when more than parent provided samples). In addition, other related publications (e.g., Gyllenhammar et al., 2017, 7323676)) or additional information or data (e.g., Woods et al., 2017, 4183148)) provided by study authors (communication with author) were used.

Fifteen of the 34 mean BWT studies included in the synthesis were rated *high* in overall study confidence {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Bell, 2018, 5041287; Chu, 2020, 6315711; Eick, 2020, 7102797; Govarts, 2016, 3230364; Lauritzen, 2017, 3981410; Lind, 2017, 3858512; Luo, 2021, 9959610; Manzano-Salgado, 2017, 4238465; Sagiv, 2018, 4238410; Starling, 2017, 3858473; Valvi, 2017, 3983872; Wikström, 2020, 6311677; Yao, 2021, 9960202}, while twelve were rated *medium* {Chang, 2022, 9959688; Chen, 2021, 7263985; de Cock, 2016, 3045435; Gyllenhammar, 2018, 4238300; Hjermitslev, 2020, 5880849; Kashino, 2020, 6311632; Kwon, 2016, 3858531; Lenters, 2016, 5617416; Meng, 2018, 4829851; Robledo, 2015, 2851197; Wang, 2019, 5080598; Woods, 2017, 4183148}, and seven were classified as *low* {Callan, 2016, 3858524; Cao, 2018, 5080197; Gao, 2019, 5387135; Marks, 2019, 5081319; Shi, 2017, 3827535; Workman, 2019, 5387046; Xu, 2019, 5381338}. Twenty-three of the twenty-seven *high* or *medium* confidence studies detailed in this synthesis were classified as having good study sensitivity {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Chen, 2021, 7263985; Gyllenhammar, 2018, 4238300; Hjermitslev, 2020, 5880849; Kashino, 2020, 6311632; Lauritzen, 2017, 3981410; Lenters, 2016, 5617416; Lind, 2017, 385812; Manzano-Salgado, 2017, 4238465; Meng, 2018, 4829851; Robledo, 2015, 2851197; Sagiv, 2018, 4238410; Starling, 2017, 3858473; Wikström, 2020, 6311677; Valvi, 2017, 3983872; Woods, 2017, 4183148} or adequate study sensitivity {Chang, 2022, 9959688; Chu, 2020, 6315711; Eick, 2020, 7102797; Govarts, 2016, 3230364; Luo, 2021, 9959610; Yao, 2021, 9960202}, while four had deficient study sensitivity {Bell, 2018, 5041287; de Cock, 2016, 3045435; Kwon, 2016, 3858531; Wang, 2019, 5080598} as shown in Figure 3-46, Figure 3-47, and Figure 3-48. The median exposure values across all of the studies were quite variable and ranged from 0.38 ng/mL {Kwon, 2016, 3858531} to 30.1 ng/mL {Meng, 2018, 4829851}.

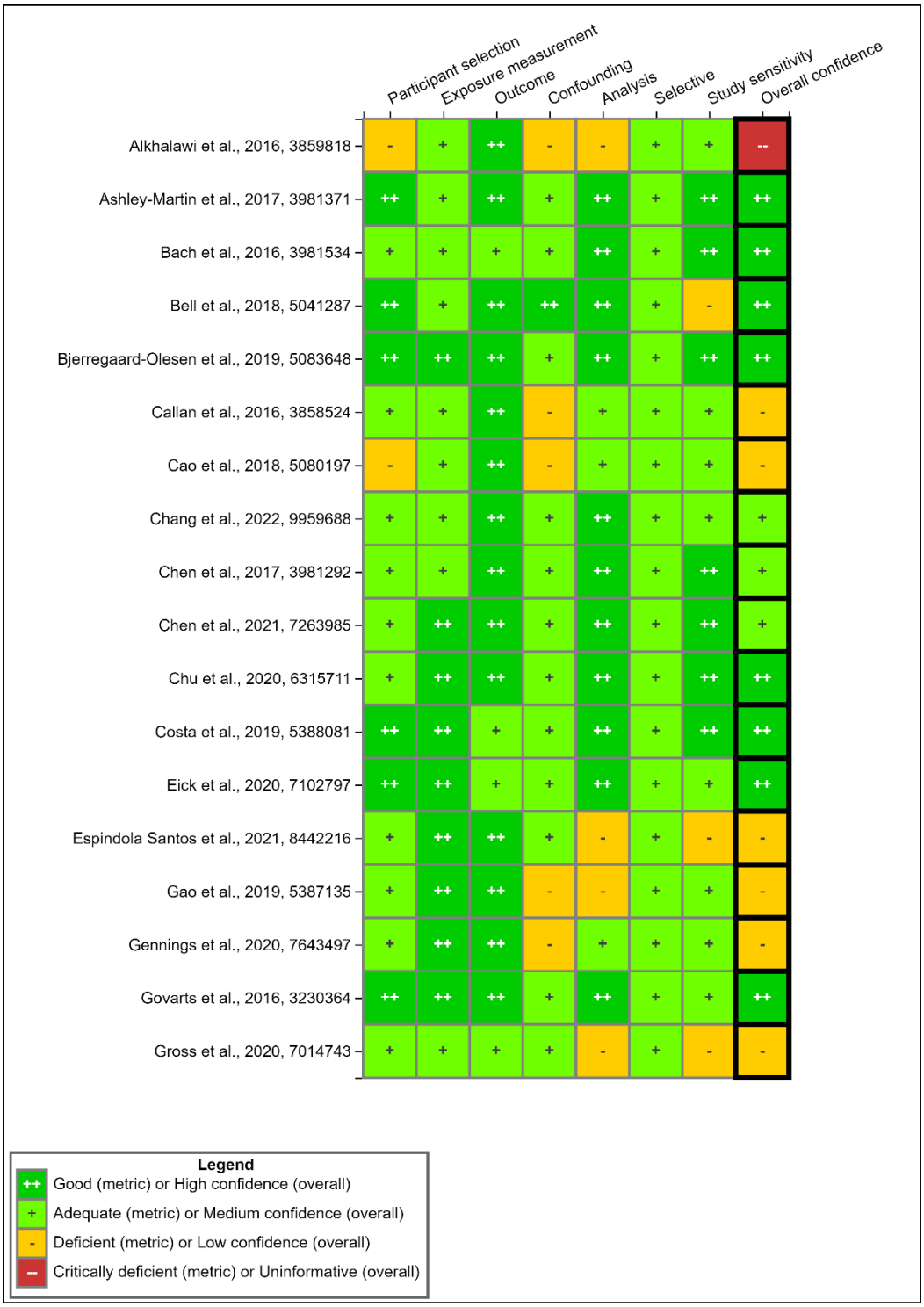


Figure 3-46. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Weight Effects[a]

Interactive figure and additional study details available on HAWC.

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

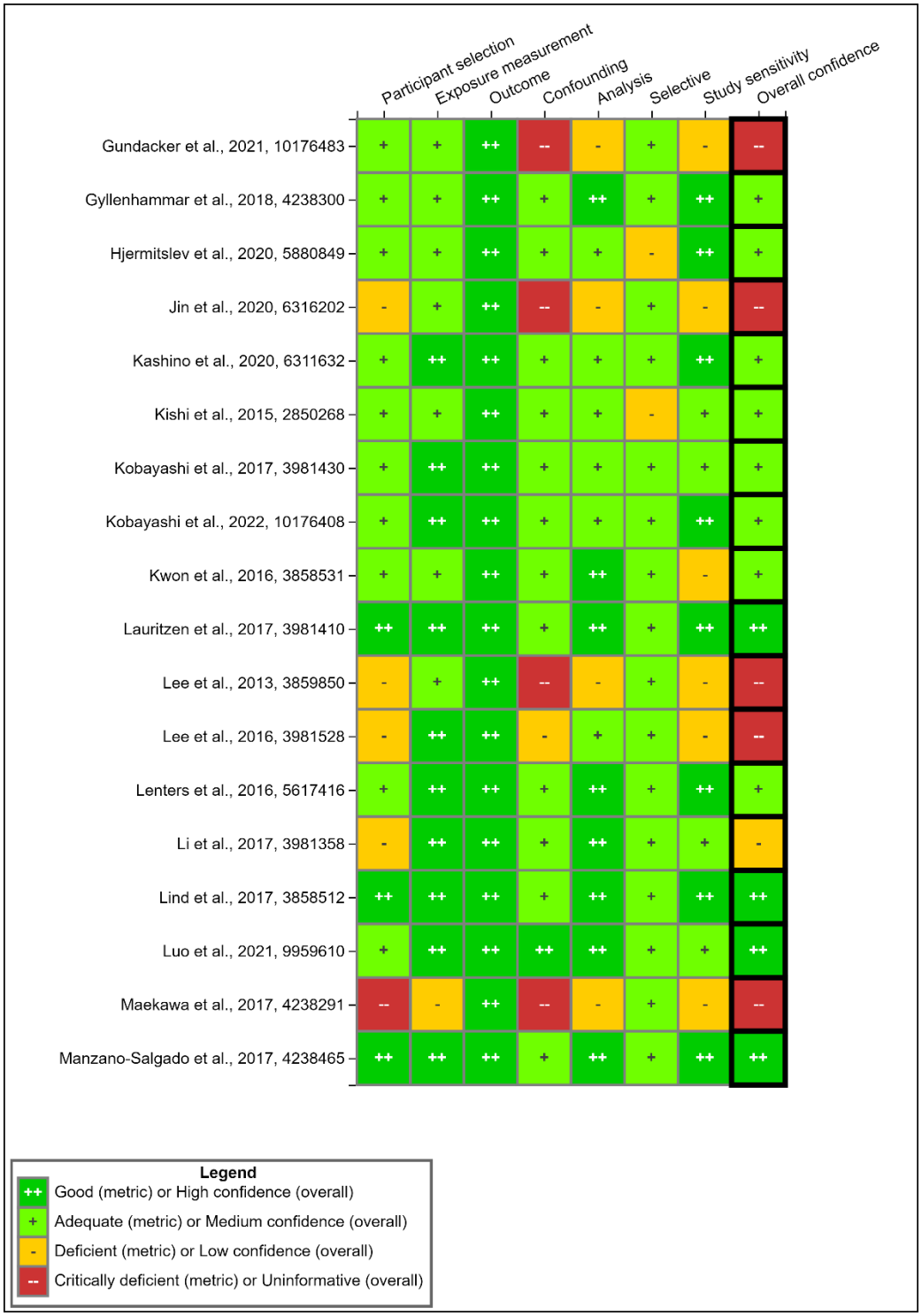


Figure 3-47. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Weight Effects (Continued)[a]

Interactive figure and additional study details available on HAWC.

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

Figure 3-48. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Weight Effects (Continued)[a]

Interactive figure and additional study details available on HAWC.

3.4.4.1.4.1.1 Mean Birth Weight Study Results: Overall Population Studies

Thirty of the 34 included studies that examined mean BWT data in the overall population {Bach, 2016, 3981534; Bell, 2018, 5041287; Callan, 2016, 3858524; Cao, 2018, 5080197; Chang, 2022, 9959688; Chen, 2021, 7263985; Chu, 2020, 6315711; de Cock, 2016, 3045435; Eick, 2020, 7102797; Gao, 2019, 5387135; Govarts, 2016, 3230364; Gyllenhammar, 2018, 4238300; Hjermitslev, 2020, 5880849; Kashino, 2020, 6311632; Kwon, 2016, 3858531; Lauritzen, 2017, 3981410; Lenters, 2016, 5617416; Luo, 2021, 9959610; Manzano-Salgado, 2017, 4238465; Marks, 2019, 5081319; Meng, 2018, 4829851; Robledo, 2015, 2851197; Shi, 2017, 3827535; Starling, 2017, 3858473; Valvi, 2017, 3983872; Wikström, 2020, 6311677; Woods, 2017, 4183148; Wu, 2012, 2919186; Xu, 2019, 5381338; Yao, 2021, 9960202}, while four only reported sex-specific data only {Ashley-Martin, 3981371; Lind, 2017, 3858512; Marks, 2019, 5081319; Robledo, 2015, 2851197}. Nineteen of the 30 PFOS studies with analyses based on an overall population reported some mean BWT deficits, albeit some of these were not statistically significant (Figure 3-49, Figure 3-50, Interactive figure and additional study details available on Tableau.

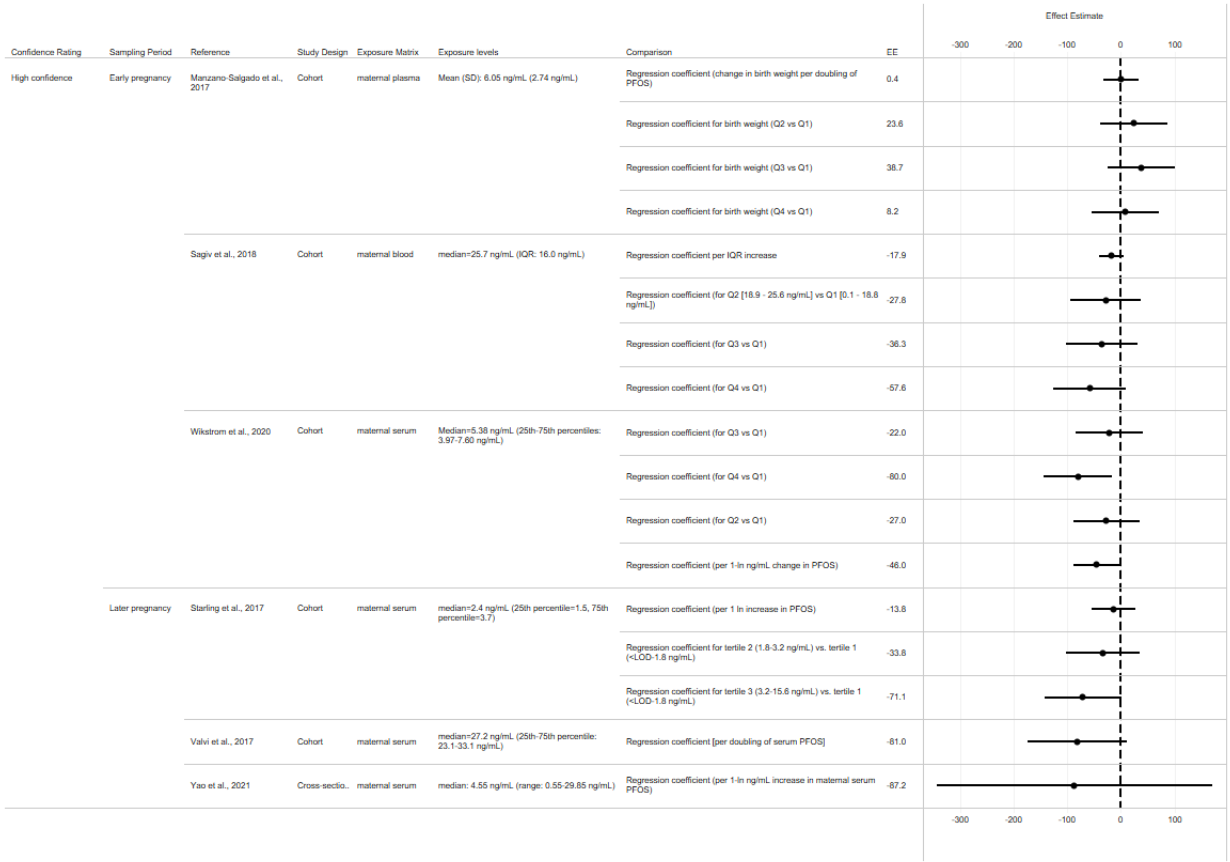


Figure 3-51, Interactive figure and additional study details available on Tableau.

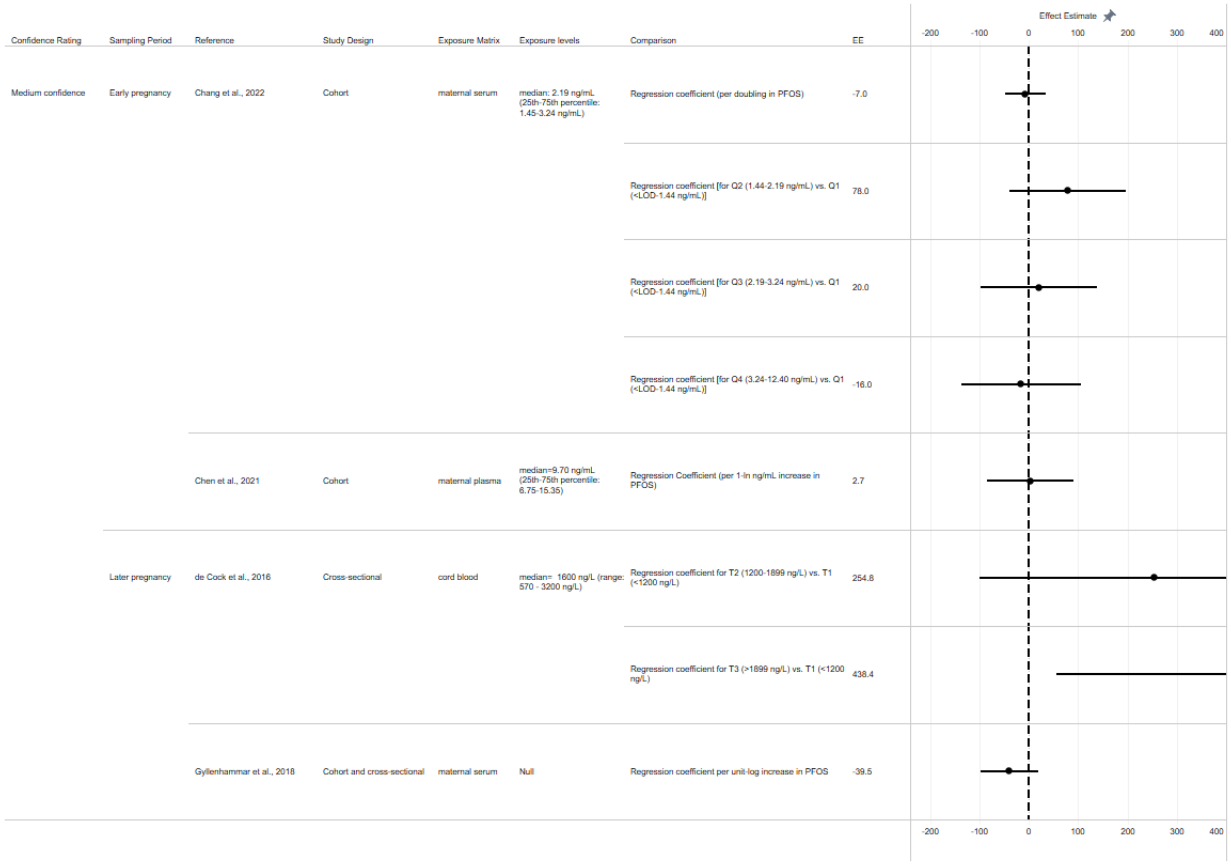


Figure 3-52, Figure 3-53**,** and Interactive figure and additional study details available on Tableau.

Figure 3-54). Nine mean BWT studies in the overall population reported null associations {Cao, 2018, 5080197; Chang, 2022, 9959688; Chen, 2021, 7263985; Eick, 2020, 7102797; Gao, 2019, 5387135; Govarts, 2016, 3230364; Hjermitslev, 2020, 5880849; Manzano-Salgado, 2017, 4238465; Woods, 2017, 4183148}, while two reported increased mean BWT deficits {de Cock, 2016, 3045435; Shi, 2017, 3827535}. Only two studies {Starling, 2017, 3858473; Sagiv, 2018, 4238410} out of ten studies which examined categorical data {Bach, 2016, 3981534, Cao, 2018, 5080197; Chang, 2022, 9959688; Eick, 2020, 7102797; Gao, 2019, 5387135; Govarts, 2016, 3230364; Manzano-Salgado, 2017, 4238465; Meng, 2018, 4829851; Sagiv, 2018, 4238410; Starling, 2017, 3858473; Wikström, 2020, 6311677} showed inverse monotonic exposure-response relationships. Although two studies {Bach, 2016, 3981534; Meng, 2018, 4829851} also showed large BWT deficits consistent in magnitude in the upper two quartiles (–50 to –62 g and –50 to –48 g relative to their quartile 1 referents, respectively).

Although there was a wide distribution of BWT deficits (range: –14 to –417 grams) in the overall population (i.e., both sexes combined) across both categorical and continuous exposure estimates, 18 of these ranged from –14 to –93 grams per each PFOS unit increase. This included all 10 *high* confidence studies with five of these reporting deficits ranging from 14 to 18 grams per each unit PFOS increase. The six *medium* confidence studies reporting deficits showed larger associations with an even narrower distribution ranging –35 to –69 grams per each unit PFOS increase. The three low confidence studies reporting deficits showed the largest associations ranging –0 to –417 grams per each unit PFOS increase including three studies ranging from –50 to –69 grams. Thus, there was some suggestion of larger and more variable BWT deficits in *low* confidence studies which have a higher potential for bias. There was also a preponderance of

inverse associations based on studies with later biomarker sampling timing (i.e., trimester two onward) including 15 of the overall 19 studies and 7 of the 10 *high* confidence studies only; this may be related to pregnancy hemodynamic influences on the PFOS biomarkers during pregnancy.

3.4.4.1.4.1.2 Mean BWT-Overall Population Summary

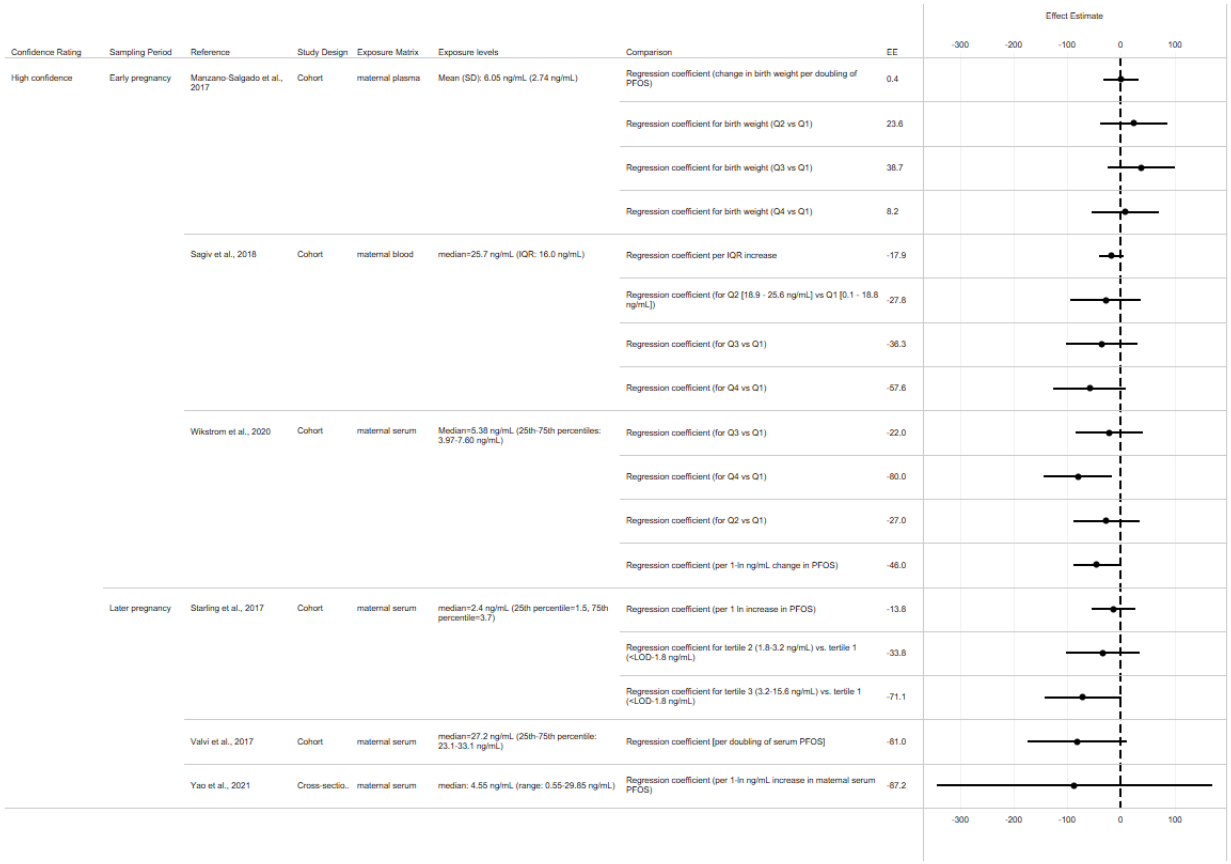
Eighteen of the nineteen studies that reported deficits based on either categorical or continuous expression ranged from –14 to –93 grams. A pattern of larger and more variable results was detected across study confidence with smaller and less variable BWT deficits among the higher confidence studies. Overall, there was evidence of an adverse monotonic exposure-response in two of ten studies, but an additional two studies showed large and consistent results in the upper two quartiles. Most of the evidence of mean birth weight difference was detected among the *medium* (6 of 12) or *high* (10 of 15) confidence studies. Study sensitivity was not an explanatory factor of the null BWT studies. There was some suggestion of a relationship between PFOS sample timing and magnitude of associations with the six of the largest deficits detected among studies that used maternal serum with some or all samples collected during trimester 3 or were based on umbilical cord samples. There was also a preponderance of inverse associations based on studies with later biomarker sampling timing (i.e., trimester two onward) that may be related to pregnancy hemodynamic influences on the PFOS biomarkers during pregnancy.

Figure 3-49. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.

Figure 3-50. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on Tableau.



Confidence Rating	Sampling Period	Reference	Study Design	Exposure Matrix	Exposure levels	Comparison	EE
High confidence	Early pregnancy	Manzano-Salgado et al., 2017	Cohort	maternal plasma	Mean (SD): 6.05 ng/mL (2.74 ng/mL)	Regression coefficient (change in birth weight per doubling of PFOS)	0.4
						Regression coefficient for birth weight (Q2 vs Q1)	23.6
						Regression coefficient for birth weight (Q3 vs Q1)	38.7
						Regression coefficient for birth weight (Q4 vs Q1)	8.2
		Sagiv et al., 2018	Cohort	maternal blood	median=25.7 ng/mL (IQR: 16.0 ng/mL)	Regression coefficient per IQR increase	-17.9
						Regression coefficient (for Q2 [18.9 - 25.6 ng/mL] vs Q1 [0.1 - 18.8 ng/mL])	-27.8
						Regression coefficient (for Q3 vs Q1)	-36.3
						Regression coefficient (for Q4 vs Q1)	-57.6
		Wikstrom et al., 2020	Cohort	maternal serum	Median=5.38 ng/mL (25th-75th percentiles: 3.97-7.60 ng/mL)	Regression coefficient (for Q3 vs Q1)	-22.0
						Regression coefficient (for Q4 vs Q1)	-80.0
						Regression coefficient (for Q2 vs Q1)	-27.0
						Regression coefficient (per 1-ln ng/mL change in PFOS)	-46.0
	Later pregnancy	Starling et al., 2017	Cohort	maternal serum	median=2.4 ng/mL (25th percentile=1.5, 75th percentile=3.7)	Regression coefficient (per 1 ln increase in PFOS)	-13.8
						Regression coefficient for tertile 2 (1.8-3.2 ng/mL) vs. tertile 1 (<LOD-1.8 ng/mL)	-33.8
						Regression coefficient for tertile 3 (3.2-15.6 ng/mL) vs. tertile 1 (<LOD-1.8 ng/mL)	-71.1
		Valvi et al., 2017	Cohort	maternal serum	median=27.2 ng/mL (25th-75th percentile: 23.1-33.1 ng/mL)	Regression coefficient [per doubling of serum PFOS]	-81.0
		Yao et al., 2021	Cross-sectio..	maternal serum	median: 4.55 ng/mL (range: 0.55-29.85 ng/mL)	Regression coefficient (per 1-ln ng/mL increase in maternal serum PFOS)	-87.2

Figure 3-51. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on Tableau.

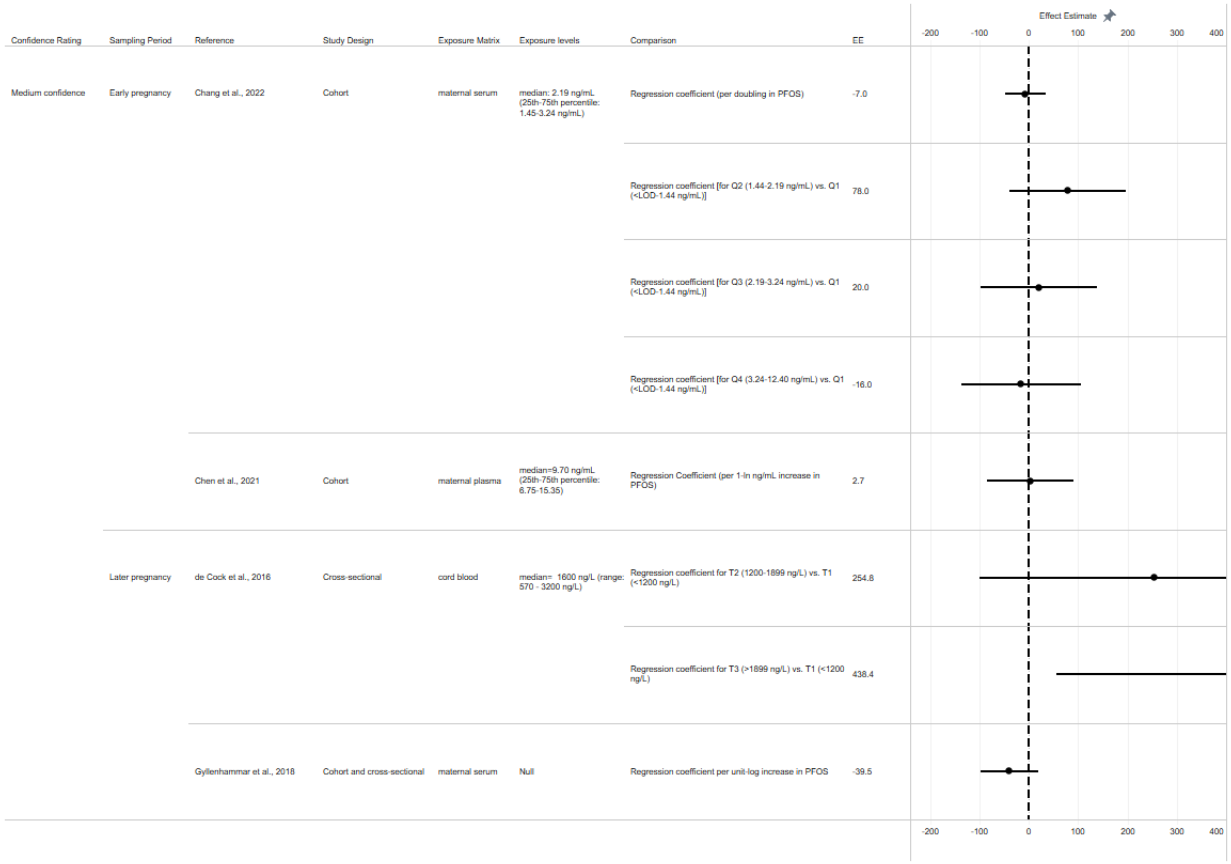


Figure 3-52. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on Tableau.

Figure 3-53. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on Tableau.

Figure 3-54. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on Tableau.

3.4.4.1.4.1.3 Mean Birth Weight Study Results: Sex Specific Studies

Ten of sixteen epidemiological studies examining sex-specific results in male neonates showed some BWT deficits. The remaining six studies {Ashley-Martin, 2017, 3981371; Cao, 2018, 5080197; de Cock, 2016, 3045435; Hjermitslev, 2020, 5880849; Robledo, 2015, 2851197; Shi, 2017, 3827535} in male neonates were either null or showed larger birth weights with increasing PFOS exposures. Six of fifteen epidemiological studies examining sex-specific results in female neonates showed some BWT deficits. The magnitude of associations was much more variable in boys (range: –9 to –150 grams) than in girls (range: –20 to –85 grams) per each unit PFOS increase. There was also little evidence of exposure-response relationships in either sex as only 1 out of 5 studies with categorical data showed monotonicity.

Six of the 15 studies examining mean BWT associations in both boys and girls detected some deficits in both sexes. Two of these six studies showed sex-specific deficits comparable in magnitude among boys and girls {Chu, 2010, 6315711; Wang, 2019, 5080598}. Three of these studies {Bach, 2016, 3981534; Meng, 2018, 4829851; Wikström, 2020, 6311677} showed larger deficits among girls and one showed larger deficits among boys {Kashino, 2020, 6311632}. The low confidence study by Marks et al. (2019, 5081319) of males only detected a small statistically significant association (−8.5 g; 95% CI: −15.9, −1.1) per each ln-unit PFOS increase and showed an exposure-response with reported large deficits in PFOS tertile 2 (−26.6 g; 95% CI: −147.3,

94.2) and tertile 3 (−83.9 g; 95% CI: −201.4, 33.7) compared to the tertile 1 referent. Four other studies reported mean BWT deficits only in boys {Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Valvi, 2017, 3983872}; no studies reported deficits in girls only.

Overall, there was more evidence of adverse associations detected in boys, but the magnitude of associations detected was more consistent in girls. There was an exposure-response relationship detected in only one of five studies with categorical data in both sexes. Study confidence and most other study characteristics did not seem to be explanatory patterns for the results, as, for example, nearly all (9 of 10 in boys) or all (6 of 6 girls) were either *high* or *medium* confidence. Definitive patterns by sample timing were also not evident in the male neonates across all study confidence levels but a larger proportion of the later sampled studies (60%) showed inverse associations in females compared to early sampled studies (38%). Study sensitivity was not an explanatory factor among the null studies in either sex.

3.4.4.1.4.1.4 Standardized Birth Weight Measures

Fifteen studies examined standardized BWT measures including fourteen studies reporting a change in BWT z-scores on a continuous scale per each PFOS comparison. Eight of the 15 studies were *high* confidence studies {Ashley-Martin, 2017, 3981371; Bach, 2016, 3981534; Eick, 2020, 7102797; Gardener, 2021, 7021199; Sagiv, 2018, 4238410; Shoaff, 2018, 4619944; Wikström, 2020, 6311677; Xiao, 2019, 5918609}, four were *medium* {Chen, 2017, 3981292; Gyllenhammar, 2018, 438300; Meng, 2018, 4829851; Wang, 2019, 5080598} and three were *low* confidence {Espindola-Santos, 2021, 8442216; Gross, 2020, 7014743; Workman, 2019, 5387046} (Figure 3-46, Figure 3-47, Figure 3-48).

Nine of the fifteen studies showed some evidence of adverse associations between PFOS exposures and BWT z-scores. Six of these were *high* confidence {Bach, 2016, 3981534; Gardener, 2021, 7021199; Sagiv, 2018, 4238410; Shoaff, 2018, 4619944; Wikström, 2020, 6311677; Xiao, 2019, 5918609}, two were *medium* confidence {Chen, 2017, 3981292; Wang, 2019, 5080598} and one was *low* confidence {Gross, 2020, 7014743}. None of the four studies reporting categorical data showed evidence of monotonicity across tertiles or quartiles. The *high* confidence study by Gardener et al. (2021, 7021199) reported that participants in the highest PFOS exposure quartile (relative to the lowest quartile) had a higher odds (OR = 1.41; 95% CI: 0.66, 2.03) of being in the lowest standardized birthweight category (vs. the top 3 BWT z-score quartiles). Four studies reporting associations in the overall population also reported standardized birth weight deficits in either or both male and female neonates. Two studies {Gardener, 2021, 7021199; Gyllenhammar, 2018, 4238300} also reported that there were no statistically significant interactions for their BWT-z measures by sex.

Among the fourteen studies examining continuous BWT z-score measures in the overall population, eight reported associations for different PFOS exposures. The *high* confidence study by Bach et al. (2016, 3981534) reported a statistically significant association between mean BWT z-score and PFOS quartiles 2 (−0.15; 95% CI: −0.29, −0.02) and quartile 4 (−0.11; 95% CI: −0.25, 0.02) only, with no exposure-response relationship detected. Although not statistically significant, both Wang et al. (2019, 5080598) (−0.15; 95% CI: −0.41, 0.11) and Shoaff et al. (2018, 4619944) reported associations similar in magnitude for their overall population (−0.12; 95% CI: −0.36, 0.13). The *medium* confidence study by Chen et al. {2017, 3981292} reported adverse associations in the overall population (−0.14; 95% CI: −0.26, −0.01) with comparable

results in both male and female neonates (BWT z-score range: −0.13 to −0.15). The *high* confidence study by Sagiv et al. (2018, 4238410) reported associations for PFOS quartile 4 in the overall population (–0.13; 95% CI: 0.26, 0.00); the largest association in this study was found for male neonates (−0.19; 95% CI: −0.33, −0.05) per each interquartile range (IQR) increase. The *high* confidence study by Wikström et al. {2020, 6311677} reported adverse associations per each ln-unit increase (–0.10; 95%CI: –0.20; -0.004) as well as in quartile 4 in the overall population (−0.17; 95% CI: −0.37, −0.03); these results appeared to be driven by associations detected in female neonates (–0.17; 95% CI: –0.30, –0.03 per each ln-unit increase; −0.30; 95% CI: −0.49, –0.10 for quartile 4). The *high* confidence study by Xiao et al. (2019, 5918609) reported z-scores fairly similar in magnitude for the overall population (−0.47; 95% CI: −0.85, −0.09), male neonates (−0.40; 95% CI: −0.89, 0.08), and female neonates (−0.56; 95% CI: −1.12, 0). Among the eight studies showing some deficits, the largest association was detected in the *low* confidence study by Gross et al. (2020, 7014743) for the overall population (–0.62; 95% CI: −0.96 to −0.29). The authors also reported large deficits for both males (–0.81; SE=0.24; p-value=0.001) and females (–0.46; SE=0.29; p-value=0.11) for PFOS levels greater than the mean level.

3.4.4.1.4.1.5 BWT z-score Summary

Nine out of 15 studies showed some associations between standardized BWT scores and PFOS exposures including eight *medium* or *high* confidence studies. None of the five studies with categorical data reported strong evidence of exposure-response relationships. No patterns by sample timing were evident as three of these studies had trimester one maternal samples; however, the strongest associations were seen in studies with later biomarker sampling. Study sensitivity did not seem to be an explanatory factor in the six null studies of standardized BWT most of these studies had moderate or large exposure contrasts and sufficient sample sizes. Although some studies may have been underpowered to detect associations small in magnitude relative to PFOS exposure, there was consistent lower BWT z-scores reported in these studies. There was no apparent pattern related to magnitude of deficits across study confidence, but more associations were evident across high confidence levels in general. Twice as many studies showing adverse associations were based on later (6 of 9) versus early (i.e., at least some trimester one maternal samples) pregnancy sampling (3 of 9); this might be reflective of some impact of pregnancy hemodynamics on biomarker concentrations over time. Few differences were seen across sexes including magnitude of associations as the majority of studies in both male (3 of 5 studies; 2 were *medium* or *high* confidence) and female (4 of 5 studies; 3 of 4 were *medium* or *high* confidence) neonates showed some associations between decreased standardized birth weights and increasing PFOS exposures. Overall, nine different studies out of fifteen showed some suggestion of inverse associations in the overall population or either or both sexes.

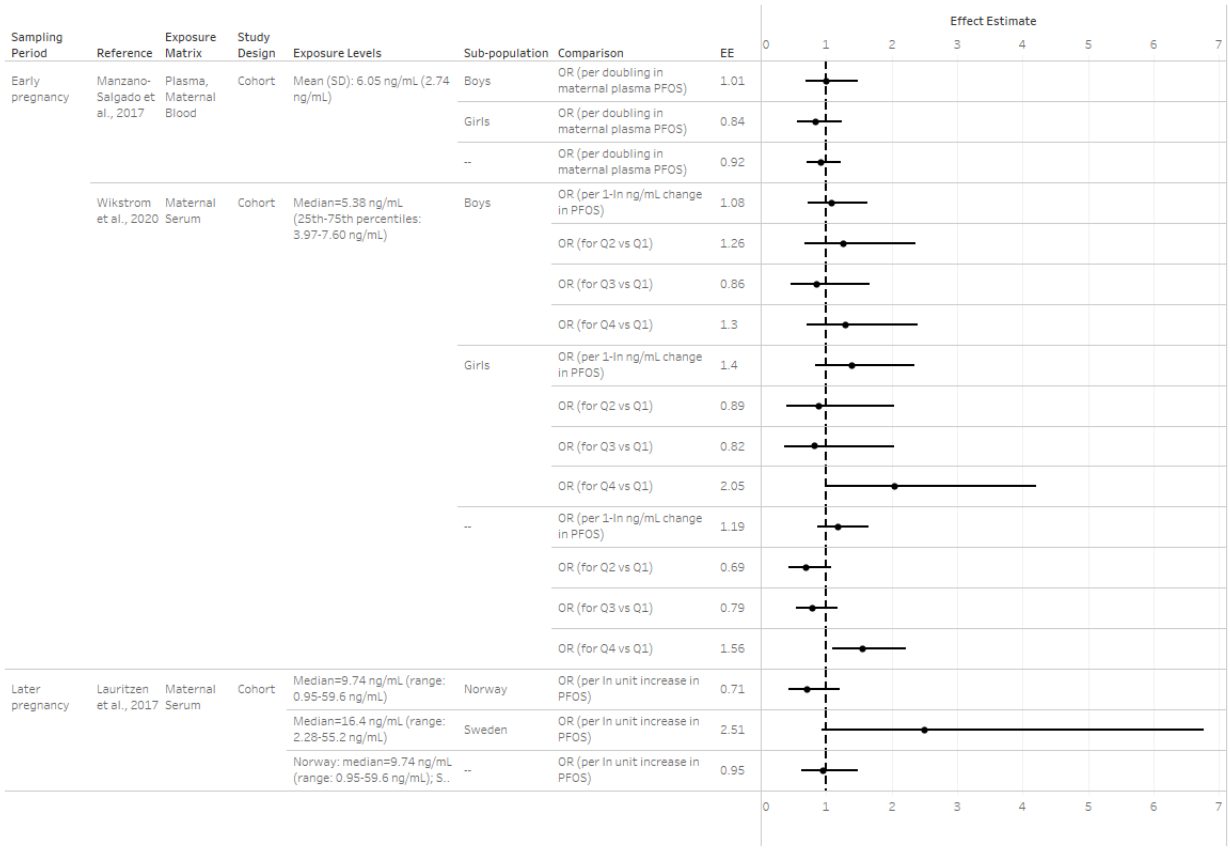
3.4.4.1.4.2 Small for Gestational Age/Low Birth Weight

Ten informative and non-overlapping epidemiological studies examined associations between PFOS exposure and different dichotomous fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints), LBW, or both (i.e., Manzano-Salgado et al. (2017, 4238465)). Overall, eleven studies examined either or both LBW or SGA in relation to PFOS exposure with four classified as *high* confidence {Chu, 2020, 6315711; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Wikström, 2020, 6311677}, three as *medium* confidence {Govarts, 2018, 4567442; Hjermitslev, 2020, 5880849; Meng, 2018, 4829851}, three as *low* {Chang, 2022, 9959688; Souza, 2020, 6833697; Xu, 2019, 5381338} and one as

uninformative {Arbuckle, 2013, 2152344}. Six of these studies had good sensitivity {Chu, 2020, 6315711; Hjermitslev, 2020, 5880849; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Meng, 2018, 4829851; Wikström, 2020, 6311677}, while five were considered adequate {Arbuckle, 2013, 2152344; Chang, 2022, 9959688; Govarts, 2018, 4567442; Souza, 2020, 6833697; Xu, 2019, 5381338).

Four {Lauritzen, 2017, 3981410; Wikström, 2020, 6311677; Souza, 2020, 6833697; Xu, 2019, 5381338} of the seven SGA studies reporting main effects showed some adverse associations, while three studies were null {Chang, 2022, 9959688; Govarts, 2018, 4567442; Manzano-Salgado, 2017, 4238465}. The magnitude of odds ratios (ORs) across the four studies showing adverse associations in the overall population (OR range: 1.19 to 4.14) was variable whether the effect estimates were based on either categorical or continuous exposures (per each unit increase) (Figure 3-58) with the two low confidence studies showing the largest risks. For example, Xu et al. (2019, 5381338) reported an OR of 4.14 (95% CI: 1.07, 16.0) for each log10 unit increase in PFOS. Souza et al (2020, 6833697) reported an OR of 3.67 (1.38–9.74) in quartile 4 relative to quartile 1. The *high* confidence Lauritzen et al. (2017, 3981410) study did not show an increased risk in the overall population per each ln-unit PFOS increase, but they did show a larger association among participants from Sweden (OR = 2.51; 95% CI: 0.93, 6.77). The *high* confidence study by Wikström et al. (2020, 6311677) reported an OR of 1.56 (95%CI: 1.09; 2.22 per each ln-unit increase) with a larger OR in girls (OR = 2.05; 95% CI: 1.00, 4.21) than boys (OR = 1.30; 95% CI: 0.70, 2.40). Similarly, a slight increased risk in their overall population (OR= 1.19; 95% CI: 0.87, 1.64) expressed per each ln-unit change was largely driven by results in girls (OR = 1.40; 95% CI: 0.83, 2.35).

Overall, four (2 *high* and 2 *low* confidence studies) reported increased risks for SGA with increasing PFOS exposures. The magnitude in risk across many of these studies were relatively large, but neither of two studies examining categorical exposures showed any evidence of an exposure-response relationship. Although the number of studies was small, few patterns were discernible across study characteristics or overall confidence for these SGA findings.



Sampling Period	Reference	Exposure Matrix	Study Design	Exposure Levels	Sub-population	Comparison	EE
Early pregnancy	Manzano-Salgado et al., 2017	Plasma, Maternal Blood	Cohort	Mean (SD): 6.05 ng/mL (2.74 ng/mL)	Boys	OR (per doubling in maternal plasma PFOS)	1.01
					Girls	OR (per doubling in maternal plasma PFOS)	0.84
					--	OR (per doubling in maternal plasma PFOS)	0.92
	Wikstrom et al., 2020	Maternal Serum	Cohort	Median=5.38 ng/mL (25th-75th percentiles: 3.97-7.60 ng/mL)	Boys	OR (per 1-ln ng/mL change in PFOS)	1.08
						OR (for Q2 vs Q1)	1.26
						OR (for Q3 vs Q1)	0.86
						OR (for Q4 vs Q1)	1.3
					Girls	OR (per 1-ln ng/mL change in PFOS)	1.4
						OR (for Q2 vs Q1)	0.89
						OR (for Q3 vs Q1)	0.82
						OR (for Q4 vs Q1)	2.05
					--	OR (per 1-ln ng/mL change in PFOS)	1.19
						OR (for Q2 vs Q1)	0.69
						OR (for Q3 vs Q1)	0.79
						OR (for Q4 vs Q1)	1.56
Later pregnancy	Lauritzen et al., 2017	Maternal Serum	Cohort	Median=9.74 ng/mL (range: 0.95-59.6 ng/mL)	Norway	OR (per ln unit increase in PFOS)	0.71
				Median=16.4 ng/mL (range: 2.28-55.2 ng/mL)	Sweden	OR (per ln unit increase in PFOS)	2.51
				Norway: median=9.74 ng/mL (range: 0.95-59.6 ng/mL); S..	--	OR (per ln unit increase in PFOS)	0.95

Figure 3-55. Odds of Small-for-gestational-age in Children from High Confidence Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.
Small-for-gestational-age defined as birthweight below the 10th percentile for the reference population.

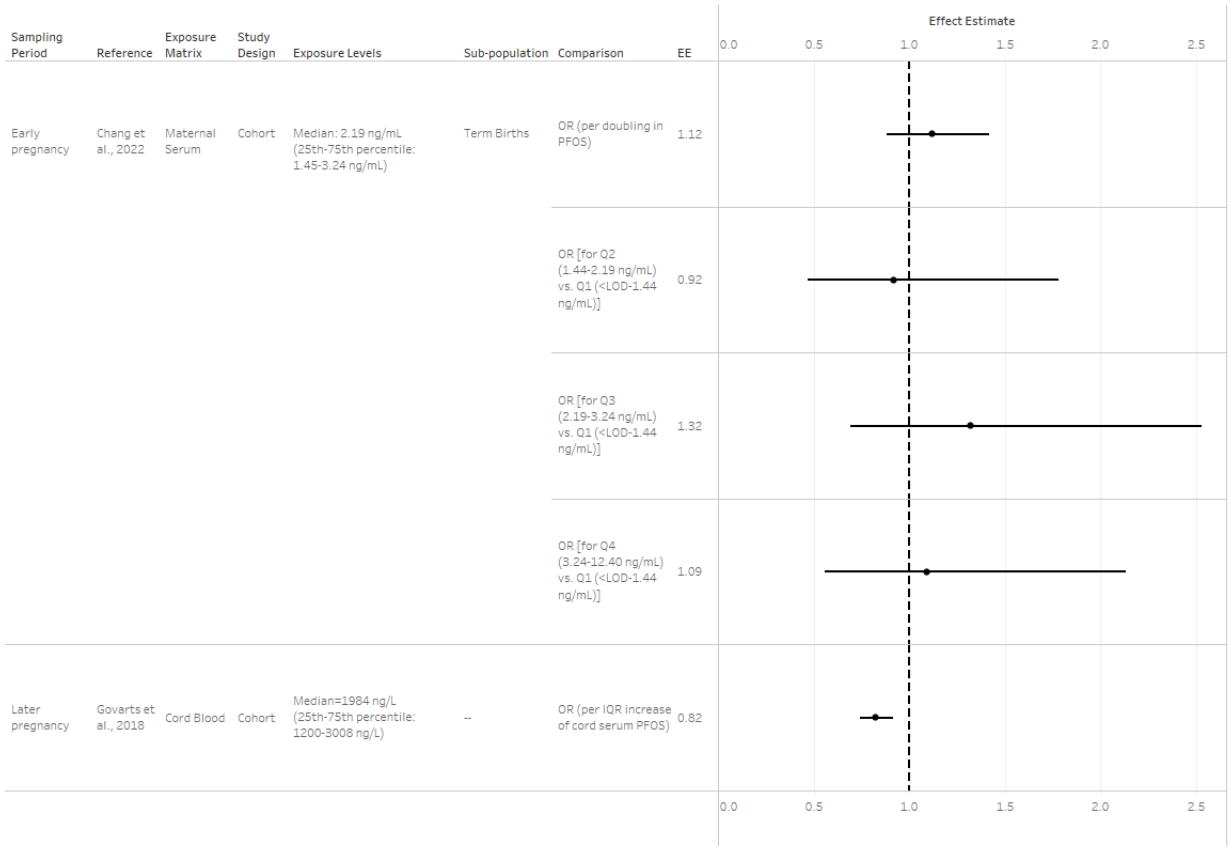


Figure 3-56. Odds of Small-for-gestational-age in Children from Medium Confidence Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.
Small-for-gestational-age defined as birthweight below the 10th percentile for the reference population.

Five studies examined LBW in relation to PFOS including one considered *uninformative* {Arbuckle, 2013, 2152344} and two each that were either *high* {Chu, 2020, 6315711; Manzano-Salgado, 2017, 4238465} or *medium* confidence { Hjermitslev, 2020, 5880849; Meng, 2018, 4829851}. All but two {Arbuckle, 2013, 2152344; Hjermitslev, 2020, 5880849} of the five LBW studies reported some associations with either the overall population, or in either boys or girls (Figure 3-58) although no evidence of exposure-response relationships were reported in those studies analyzing categorical exposures.

Although the number of studies was small, few discernible patterns by study characteristics or confidence levels were evident across these LBW findings. The three LBW studies that showed increased risks were all either *medium* or *high* confidence with two of these showing fairly small ORs. The *high* confidence study by Manzano-Salgado et al. (2017, 4238465) did not detect associations in the overall population but showed an increased risk for term LBW among boys only (OR = 1.68; 95% CI: 0.62, 4.54). The *medium* confidence study by Meng et al. (2018, 4829851) reported non-significant increased ORs (range 1.2–1.8) in the overall population across all quartiles but no evidence of an exposure-response relationship. The *high* confidence study by Chu et al. (2020, 6315711) reported limited evidence of an exposure-response relationship in the

overall population with imprecise increased risks shown for PFOS exposure quartile 3 (OR = 1.41; 95% CI: 0.23, 8.82) and quartile 4 (OR = 3.70; 95% CI: 0.61, 22.6) compared to the quartile one referent.

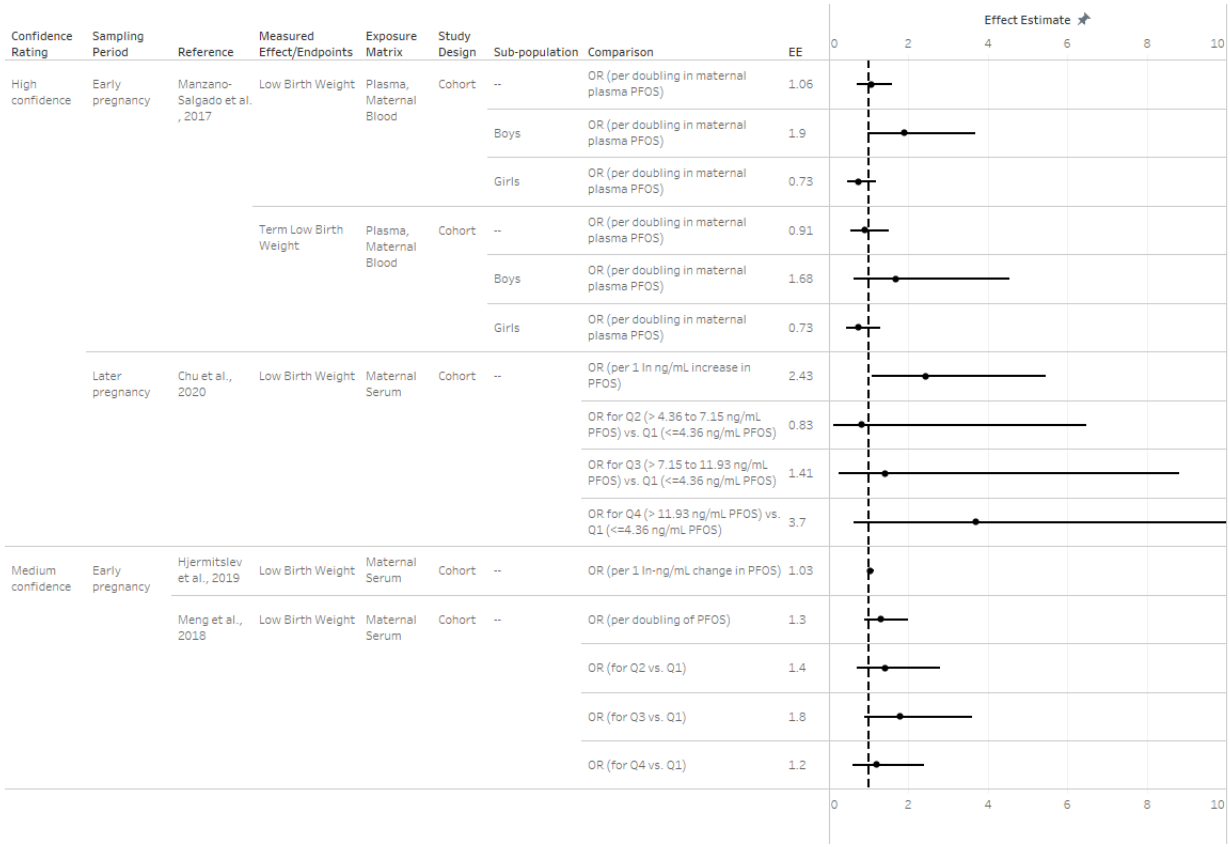


Figure 3-57. Odds of Low Birthweight in Children from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on Tableau.
Low birthweight defined as birthweight < 2,500 g.

Collectively, the majority (7 of 10) of SGA and LBW studies were supportive of an increased risk with increasing PFOS exposures. The increased odds ranged from 1.19 to 4.14 although evidence of exposure-response relationships was lacking. There was no evidence of differences by study confidence as five of these seven were either *high* (n=4) or *medium* (n=1) confidence. There was also no evidence of sample timing differences as the majority of studies with associations were reported in studies based on early sampling periods.

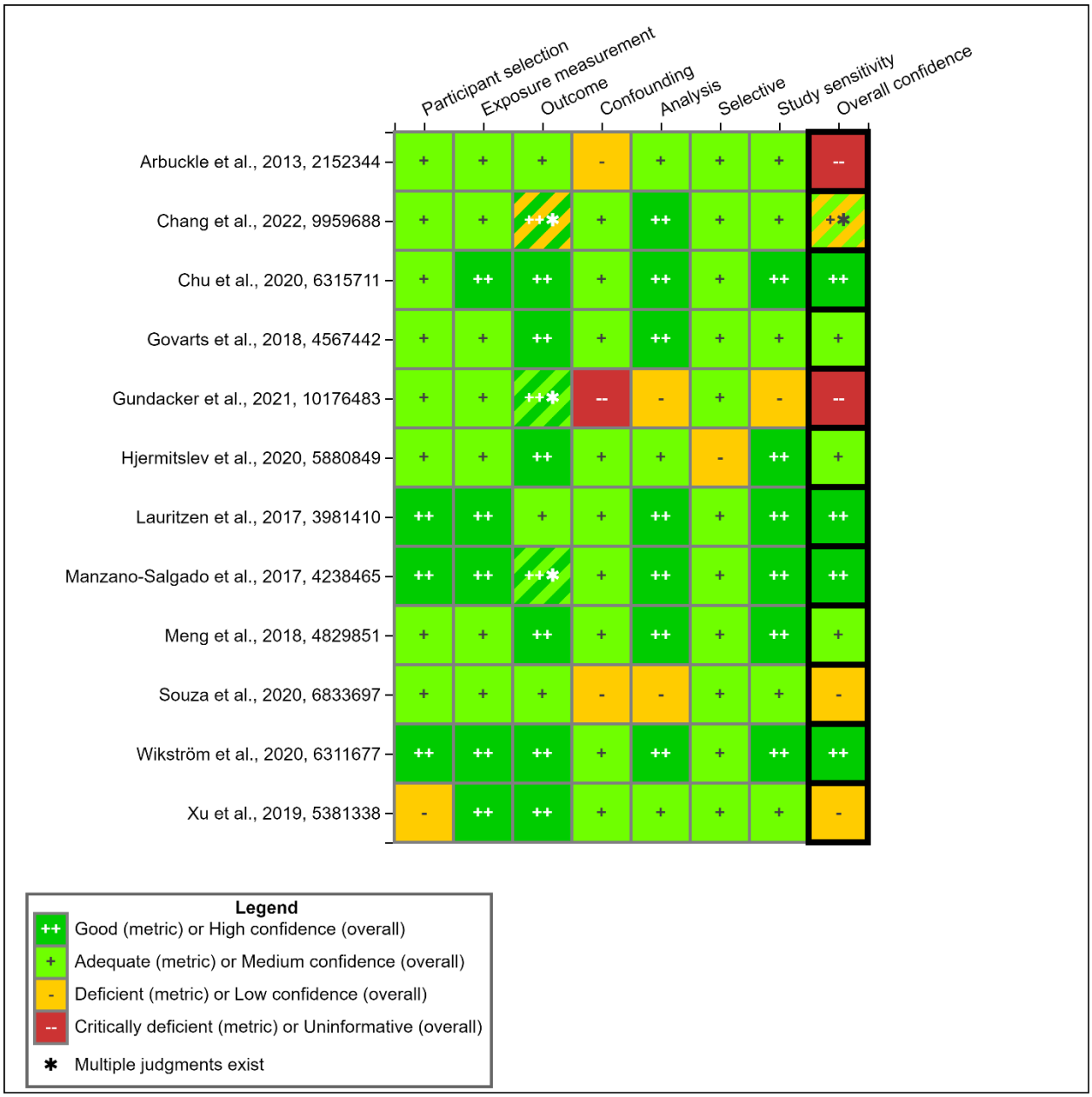


Figure 3-58. Summary of Study Evaluation for Epidemiology Studies of PFOS and Low Birth Weight or Small for Gestational Age Effects

Interactive figure and additional study details available on HAWC.

3.4.4.1.4.3 Birth Length

Thirty-one birth length studies were considered as part of the study evaluation as shown in Figure 3-59 and Figure 3-60. Four studies were considered *uninformative* {Alkhalawi, 2016, 3859818; Gundacker, 2021, 10176483; Jin, 2020, 6315720; Lee, 2013, 3859850} and four more studies noted above {Bach, 2016, 3981534; Kishi, 2015, 2850268, Kobayashi, 2017, 3981430;

Kobayashi, 2022, 10176408} were not further considered for multiple publications from the same cohort studies. Twenty-three non-overlapping and informative studies examined birth length in relation to PFOS with five of these examining standardized birth length measures only {Chen, 2017, 3981292; Espindola-Santos, 2021, 8442216; Gyllenhammar, 2018, 4238300; Shoaff, 2018, 4619944; Xiao, 2019, 5918609}, and one evaluating both measures {Workman, 2019, 5387046}. Twelve studies examined sex-specific data with two studies {Marks, 2019, 5081319; Robledo, 2015, 2851197} reporting only sex-specific results. Eighteen studies examined mean birth length differences in the overall study population.

Seven of these 23 included studies were *high* confidence {Bell, 2018, 5041287; Bjerregaard-Olesen, 2019, 5083648; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Shoaff, 2018, 4619944; Valvi, 2017, 3983872; Xiao, 2019, 5918609}, eight were *medium* confidence {Chen, 2017, 3981292; Chen, 2021, 7263985; Gyllenhammar, 2018, 4238300; Hjermitslev, 2020, 5880849; Kashino, 2020, 6311632; Luo, 2021, 9959610; Robledo, 2015, 2851197; Wang, 2019, 5080598} and eight were *low* confidence studies {Callan, 2016, 3858524; Cao, 2018, 5080197; Espindola-Santos, 2021, 8442216; Gao, 2018, 5387135; Marks, 2019, 5081319; Shi, 2017, 3827535; Workman, 2019, 5387046; Xu, 2019, 5381338}. Twelve PFOS studies had good study sensitivity {Bjerregaard-Olesen, 2019, 5083648; Chen, 2017, 3981292; Chen, 2021, 7263985; Gyllenhammar, 2018, 4238300; Hjermitslev, 2020, 5880849; Kashino, 2020, 6311632; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Robledo, 2015, 2851197; Shoaff, 2018, 4619944; Valvi, 2017, 3983872; Xiao, 2019, 5918609}, while eight had adequate sensitivity {Callan, 2016, 3858524; Cao, 2018, 5080197; Gao, 2018, 5387135; Luo, 2021, 9959610; Marks, 2019, 5081319; Shi, 2017, 3827535; Workman, 2019, 5387046; Xu, 2019, 5381338} and three {Bell, 2018, 5041287; Espindola-Santos, 2021, 8442216; Wang, 2019, 5080598} were considered deficient.

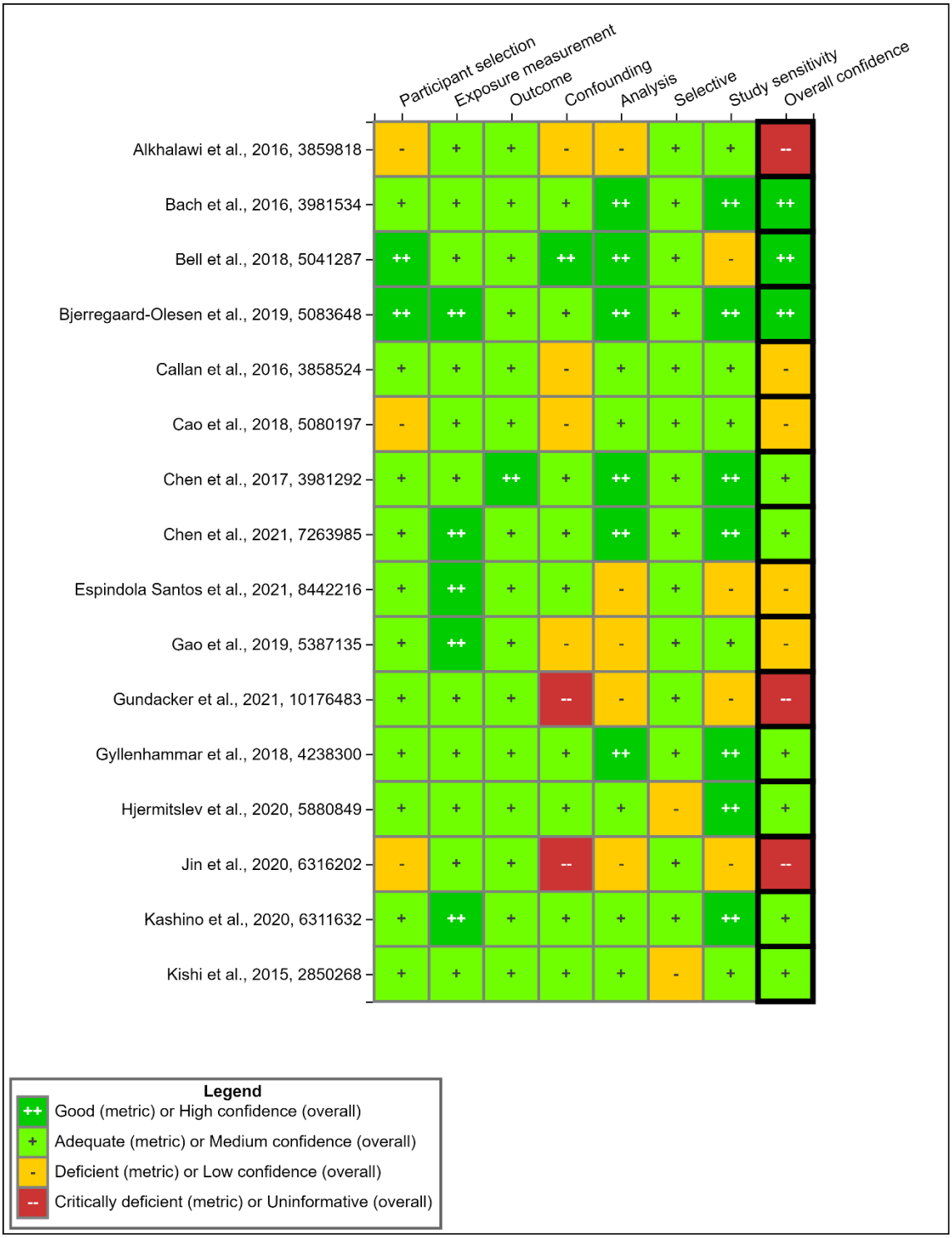


Figure 3-59. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Length Effects[a]

Interactive figure and additional study details available on HAWC.

[a] Includes three overlapping studies: Bjerregaard-Olsen et al. (2019, 5083648); Kishi et al. (2015, 2850268); Kobayashi et al. (2017, 3981430).

Figure 3-60. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Length Effects (Continued)a

Interactive figure and additional study details available on HAWC.

[a] Includes three overlapping studies: Bjerregaard-Olsen et al. (2019, 5083648); Kishi et al. (2015, 2850268); Kobayashi et al. (2017, 3981430).

Of the 23 studies examining either standardized birth length or mean birth length measures, seven studies showed some adverse associations based on the overall population. This included three of the six {Chen, 2017, 3981292; Espindola-Santos, 2021, 8442216; Gyllenhammar, 2018, 4238300; Shoaff, 2018, 461994; Workman, 2019, 5387046; Xiao, 2019, 5918609} studies that reported standardized birth length data. The *high* confidence study by Xiao et al. (2019, 5918609) reported reduced birth length z-scores (−0.33; 95% CI: −0.69, 0.03) in the overall population, as well as for both male (−0.41; 95% CI: −0.87, 0.05) and female neonates (−0.23; 95% CI: −0.75, 0.30) per each log2 increase in PFOS. Although smaller in magnitude, the *medium* confidence study by Chen et al. (2017, 3981292) also reported a birth length deficit of −0.16 (95% CI: −0.31, −0.02) in the overall population as well as male (−0.15; 95% CI: −0.33, 0.03) and female neonates (−0.20; 95% CI: −0.44, 0.05 per each ln unit PFOS increase). The other *high* confidence study by Shoaff et al. (2018, 4619944) of standardized birth length measures showed a deficit only for tertile 3 (−0.24; 95% CI: −0.64, 0.15) compared to tertile 1.

Four {Callan, 2017, 3858524; Chen, 2021, 7263985: Lauritzen, 2017, 3981410; Workman et al., 2019, 5387046} of the sixteen studies examining mean birth length in the overall population in relation to PFOS showed some evidence of reductions. The *high* confidence study by Lauritzen et al. (2017, 3981410) showed a small deficit in the overall population (–0.3 cm; 95% CI: −0.7, 0.1), but detected the strongest association when restricted to the Swedish population (−1.2 cm; 95% CI: −2.1, −0.3). The *medium* confidence study by Chen et al. (2021, 7263985) reported birth length deficits in the overall population (–0.27 cm; 95% CI: –0.51, –0.02), males (–0.14 cm; 95% CI: –0.55, 0.26), and females (–0.40 cm; 95% CI: –0.74, –0.06) per each PFOS ln-unit increase. The *low* confidence study by Workman et al. (2019, 5387046) reported a non-statistically significant birth length reduction of –0.16 cm (95% CI: –0.92, 0.60) per each ln-unit PFOS increase. The *low* confidence study by Callan et al. (2017, 3858524) reported a slightly larger birth length reduction of –0.22 cm (95% CI: –1.0, 0.57) per each ln-unit PFOS increase.

Five different sex-specific studies reported some birth length deficits in either or both male (4 of 11) and female (2 of 10) neonates including the Chen et al. (2021, 7263985) results noted above. Among the two sex-specific only studies {Robledo, 2015, 2851197; Marks, 2019, 5081319}, the Marks et al. {2019, 5081319} *low* confidence study of boys only showed adverse associations (−0.52 cm; 95% CI: −1.05, 0.01 for tertile 3 vs. tertile 1). The *high* confidence study by Valvi et al. (2017, 3983872) reported no associations in the overall population but did detect a non-significant birth length deficit in male neonates (−0.18 cm; 95% CI: −0.60, 0.23 per each PFOS log2 exposure increase). The *low* confidence study Wang et al. (2019, 5080598) study also reported a non-significant birth length deficit in males that was similar in magnitude (−0.17 cm; 95% CI: –0.71, 0.37). Although it was not statistically significant, the *high* confidence study by Bjerregaard-Olesen et al. (2019, 5083648) detected a difference in mean birth length among girls only (−0.3 cm; 95%CI: −0.7, 0.0 per each IQR PFOS increase). One study not reporting sex-specific differences did report that there were no statistically significant interactions by sex for their birth length and PFOS measures {Gyllenhammar, 2018, 4238300}.

In summary, of the 23 birth length studies, 11 different ones showed some adverse associations either in the overall population, or in either or both sexes. Two of 10 studies in females and four of 11 studies in males reported some birth length deficits. Although there were more studies in males that reported decreased birth length, there was little consistency across sex or even compared to the overall population. None of the five studies examining categorical data in either

sex or the overall population showed any evidence of an adverse exposure-response relationship. Few patterns were evident across study characteristics or confidence levels, although the database may be prone to bias due to pregnancy hemodynamics as eight of the studies that showed associations relied on later biomarker samples.

3.4.4.1.4.4 Head Circumference at Birth

Nineteen informative studies that examined head circumference were considered in the synthesis. Seven studies were rated as *medium* {Chen, 2021, 7263985; Gyllenhammar, 2018, 4238300; Hjermitslev, 2020, 5880849; Kashino, 2020, 6311632; Lind, 2017, 3858512; Robledo, 2015, 2851197; Wang, 2019, 5080598} confidence, while six were *high* confidence {Bell, 2018, 5041287; Bjerregaard-Olesen, 2019, 5083648; Lauritzen, 2017, 3981410; Manzano-Salgado, 2017, 4238465; Valvi, 2017, 3983872; Xiao, 2019, 5918609} and six were *low* {Callan, 2016, 3858524; Cao, 2018, 5080197; Espindola-Santos, 2021, 8442216; Marks, 2019, 5081319; Workman, 2019, 5387046; Xu, 2019, 5381338}. Three studies were deficient in study sensitivity {Bell, 2018, 5041287; Espindola-Santos, 2021, 8442216; Wang, 2019, 5080598}, while eleven had good {Bjerregaard-Olesen, 2019, 5083648; Chen, 2021, 7263985; Gyllenhammar, 2018, 4238300; Hjermitslev, 2020, 5880849; Kashino, 2020, 6311632; Lauritzen, 2017, 3981410; Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Robledo, 2015, 2851197; Valvi, 2017, 3983872; Xiao, 2019, 5918609} and five had adequate study sensitivity {Callan, 2016, 3858524; Cao, 2018, 5080197; Marks, 2019, 5081319; Workman, 2019, 5387046; Xu, 2019, 5381338}.

Figure 3-61. Summary of Study Evaluation for Epidemiology Studies of PFOS and Head Circumference Effects

Interactive figure and additional study details available on HAWC.

Sixteen of the 19 included studies examined PFOS in relation to mean head circumference differences including 13 studies with results in the overall population and 11 different studies with sex-specific data. Three of the mean head circumference studies {Lind, 2017, 3858512; Marks, 2019, 5081319; Robledo, 2015, 2851197} only reported sex-specific data, including the low confidence study by Marks et al. (2019, 5081319) which only examined male neonates. The three remaining studies {Espindola-Santos, 2021, 8442216; Gyllenhammar, 2018, 4238300; Xiao, 2019, 5918609} examined unitless standardized measures.

Five of the 16 studies with data based on the overall population reported some associations between PFOS and different head circumference measures. This included one study based on standardized head circumference and four studies examining mean head circumference. The *high* confidence study by Xiao et al. (2019, 5918609) showed consistent head circumference z-score deficits across their overall population (−0.26; 95% CI: −0.68, 0.16), as well as male (−0.15; 95% CI: −0.68, 0.39) and female neonates (−0.42; 95% CI: −1.05, 0.21) per each log2 increase in PFOS. Although the *high* confidence study by Lauritzen et al. (2017, 3981410) reported a null association in the combined Norwegian and Swedish population, they did detect a large head circumference reduction amongst their Swedish population only (−0.4 cm; 95% CI: −0.9, 0.04) per each ln-unit PFOS change.

Only three of the 14 studies examining mean head circumference differences in the overall population reported any evidence of associations with none of these reaching statistical significance. The *high* confidence study by Bach et al. (2016, 3981534) showed a small, non-significant head circumference differences (-0.1 cm; 95% CI: −0.2, 0.1 per each PFOS IQR increase). In their *low* confidence study, Cao et al. (2018, 5080197) reported a non-significant inverse association in the overall population (−0.23 cm; 95% CI: −1.19, 0.73 per each ln-unit PFOS) as did the *low* confidence study by Callan et al. (2016, 3858524) (−0.39 cm; 95% CI: −0.98, 0.20 per each ln unit PFOS).

Two of ten studies examining female neonates and four of 11 examining male neonates reported some inverse associations between increasing PFOS and mean head circumference. One study not reporting sex-specific differences did report that there were no statistically significant interactions by sex for their head circumference and PFOS measures {Gyllenhammar, 2018, 4238300}. The head circumference reductions were consistently around –0.3 cm in males in three (one each *low, medium,* and *high* confidence) of four studies. The *medium* confidence study by Lind et al. (2017, 3858512) reported deficits across all quartiles (range: –0.3 to –0.4 cm) but only in males. The *high* confidence study by Valvi et al. (2017, 3983872) also reported deficits only in male neonates (−0.28 cm; 95%CI: −0.65, 0.09 per each doubling of serum PFOS exposures), while head circumference increases were found for female neonates (0.48 cm; 95%CI: 0.05, 0.90). The *low* confidence study of boys only by Marks et al. (2019, 5081319) reported monotonic deficits across PFOS tertiles 2 (−0.13 cm; 95% CI: −0.45, 0.19) and 3 (−0.31 cm; 95% CI: −0.62, 0.01) compared to tertile 1. The *medium* confidence study by Kashino et al. (2020, 6311632) reported smaller deficits only in male neonates (−0.14 cm; 95% CI: −0.61, 0.32 per each log10 PFOS). Although it was not statistically significant, the *high* confidence study by Bjerregaard-Olesen et al. (2019, 5083648) detected a small difference in mean head circumference among girls only (−0.1 cm; 95% CI: −0.3, 0.1 per each IQR PFOS increase). The low confidence study by Cao et al. (2018, 5080197) found a large head circumference difference

(–1.22 cm; 95% CI: −2.70, 0.25 for tertile 3 vs. 1) among females with some evidence of an exposure-response relationship.

Although there were nine different studies that showed some evidence of associations between PFOS and head circumference in the overall population or different subsets by countries or sex, there was limited epidemiological evidence of associations among the overall population with only four of 13 studies showing any inverse associations. Mean sex-specific head circumference deficits were detected in six different studies including four in male neonates and two others in females only. An additional study with standardized head circumference measures showed deficits in both sexes, but larger deficits were noted among females. One of two studies in each sex showed some evidence of an exposure-response relationship. A very large association was seen in one low confidence study among females, but more consistent results were seen across four studies in males (two *high*, one *medium* and one *low* confidence). Although limited numbers across different study characteristic or overall confidence level sub-groups precluded a detailed assessment, few patterns were evident across the ten different studies that showed some adverse associations with head circumference. Only two {Bjerregaard-Olesen, 2019, 5083648; Lind, 2017, 3858512} of these nine studies had any early pregnancy (i.e., trimester 1) samples, with seven studies {Callan, 2016, 3858524; Cao, 2018, 5080197; Kashino, 2020, 6311632; Lauritzen, 2017, 3981410; Marks, 2019, 5081319; Valvi, 2017, 3983872; Xiao, 2019, 5918609} based on either second and/or third trimester maternal samples or later. Overall, nine of 19 studies showing some evidence of adverse associations with some uncertainty as to what degree these results may be influenced by pregnancy hemodynamics due to later sample timing. There was considerable heterogeneity of results within and across both sexes and different studies.

3.4.4.1.4.5 Fetal Growth Restriction Summary

The majority of studies examining fetal growth restriction showed some evidence of associations with PFOS exposures especially those that included BWT data (i.e., SGA, low BWT, as well as mean and standardized BWT measures). The evidence for two fetal growth measures such as head circumference and birth length were less consistent. For many of these endpoints, there was a preponderance of associations amongst studies with later biomarker samples that may be more prone to potential biases from pregnancy hemodynamic impacts. There was limited evidence of exposure-response relationships in either analyses specific to the overall population or different sexes, although the categorical data generally supported the linearly expressed associations that were detected.

Among the most accurate fetal growth restriction endpoints examined here, there was generally consistent evidence for BWT deficits across different measures and types of PFOS exposure metrics considered. BWT deficits were detected in the roughly two-thirds of included studies whether measured as mean BWT or standardized z-scores. This included 19 out of 30 mean BWT studies in the overall population and 16 of 27 *medium* or *high* confidence studies. Most of the sex-specific mean BWT studies showed some adverse associations in either male or female neonates, and although it was not consistent across studies, more deficits were found in male neonates. As noted above, many of the individual study results lacked precision and were not statistically significant especially the sex-stratified results which may have been largely underpowered to detect sex-specific differences.

The magnitude of some fetal growth measures were at times considered large especially when considering the per unit PFOS increases across the exposure distributions. Although some of the other endpoints were fairly small in magnitude, the birth weight deficits and odds ratios for birthweight-related measures were more sizeable especially when considering most were expressed on a per-unit increase basis. For example, for all but one of the 19 studies showing mean BWT deficits in the overall population, reported deficits ranging from –14 to –93 grams per each PFOS unit increase. Associations were also seen for the majority of studies examining small for gestational age and low birth weight measures.

The current database (since the 2016 HESD) is fairly robust given the wealth of studies included here, with most studies considered *high* or *medium* confidence (e.g., 23 out of 30 mean BWT) and most having adequate or good study sensitivity. As noted earlier, one source of uncertainty is that previous meta-analyses of PFOS by Dzierlenga et al. (2020, 7643488) and PFOA by Steenland et al. (2018, 5079861) have shown that some measures like mean BWT may be prone to bias from pregnancy hemodynamics especially in studies with sampling later in pregnancy. Although a limited number of studies across some strata does not fully lend itself to differentiating patterns across different study characteristics, like study confidence and sample timing, some patterns emerged across the study results. For many of these endpoints, there was a preponderance of associations, such as birth weight measures, amongst studies with later biomarker samples (i.e., either exclusive trimester 2 maternal sample or later, such as umbilical cord or post-partum maternal samples) that may be more prone to pregnancy hemodynamic impacts. This would seem to comport with the PFOS meta-analysis by Dzierlenga et al. (2020 7643488) that suggested that results for mean BWT may be impacted by some bias due to pregnancy hemodynamics. Therefore, despite some consistency in evidence across these fetal growth endpoints, some important uncertainties remain mainly around the degree that some of the results examined here may be influenced by sample timing.

3.4.4.1.5 Postnatal growth

Eleven studies examined PFOS exposure in relation to postnatal growth measures (Figure 3-62). The synthesis here is focused on postnatal growth measures including mean and standardized weight {Cao, 2018, 5080197; Chen, 2017, 3981292; de Cock, 2014, 2713590; Gyllenhammar, 2018, 4238300; Lee, 2018, 4238394; Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944; Starling, 2019, 5412449; Yeung, 2019, 5080619} and height {Cao, 2018, 5080197; Chen, 2017, 3981292; de Cock, 2014, 2713590; Gyllenhammar, 2018, 4238300; Lee, 2018, 4238394; Shoaff, 2018, 4619944; Yeung, 2019, 5080619}, as well as body mass index (BMI)/adiposity measures {Chen, 2017, 3981292; de Cock, 2014, 2713590; Gross, 2020, 7014743; Jensen, 2020, 6833719; Shoaff, 2018, 4619944; Starling, 2019, 5412449; Yeung, 2019, 5080619} and estimates of rapid growth during infancy {Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944; Starling, 2019, 5412449; Yeung, 2019, 5080619}.

Four postnatal growth studies were *high* confidence {Jensen, 2020, 6833719; Shoaff, 2018, 4619944; Starling, 2019, 5412449; Yeung, 2019, 5080619}, four were *medium* confidence {Chen, 2017, 3981292; de Cock, 2014, 2713590; Gyllenhammar, 2018, 4238300; Manzano-Salgado, 2017, 4238509}, and three were *low* confidence {Cao, 2018, 5080197; Gross, 2020, 7014743; Lee, 2018, 4238394}. As shown in Figure 3-62 seven postnatal growth studies had good study sensitivity {Chen, 2017, 3981292; Gyllenhammar, 2018, 4238300; Jensen, 2020, 6833719; Lee, 2018, 4238394; Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944;

Starling, 2019, 5412449}, two each were adequate {Cao, 2018, 5080197; Yeung, 2019, 5080619} or deficient {de Cock, 2014, 2713590; Gross, 2020, 7014743}. The *medium* confidence study by de Cock et al. (2014, 2713590) did not report effect estimates but indicated that there were no statistically significant associations between PFOS quartiles and infant BMI (p-value=0.59), infant weight (p-value=0.80), and infant height (p-value=0.98) measures up to 11 months of age. But their lack of reporting of effect estimates precluded consideration of magnitude and direction of any associations and are not further examined below in the summaries.

The medium confidence study by Manzano-Salgado et al. (2017, 4238509) reported null *associations* for their overall population, female, and male neonates for weight gain z-score measured at 6 months per each log2 PFOS increase. The low confidence study by Lee et al. (2018, 4238394) reported statistically significant inverse associations per each PFOS ln unit increase for height at age 2 years (−0.77 cm; 95% CI: −1.27, −0.15) as well as height change from birth to 2 years (−0.71 cm; 95% CI: −1.27, −0.15). Small differences were seen for mean weight differences at age 2 years (−0.17 cm; 95% CI: −0.38, 0.04) but not for weight change from birth to 2 years. Although no exposure-response relationships were detected when examined across PFOS categories those with the highest exposure saw smaller statistically significant height increases at age 2 compared to lower exposures. Although a statistically significant birth length association was detected, the *medium* confidence study by Chen et al. (2017, 3981292) reported no association with infant height z-score up to 24 months. They did report statistically significant lower infant weight z-scores among female neonates comparable in magnitude for 6 to 12 months (−0.25; 95% CI: −0.47, −0.04) or 12 to 24 months (−0.25; 95% CI: −0.41, −0.06) per each ln unit PFOS increase. Females seemed to drive the deficit detected in the overall population (−0.13; 95% CI: −0.32, 0.07 per each ln unit PFOS increase) for the 6-to-12-month window. The *medium* confidence study by Gyllenhammar et al. (2018, 4238300) did not detect standardized BWT deficits per each IQR PFOS change, but they showed slight weight deficits (~ –0.2) at 3 months that persisted throughout 60 months of age. In contrast, standardized birth length measures were null for increasing PFOS exposures regardless of the time windows examined. Compared to the tertile 1 referent, the low confidence study of infants followed up to a median age of 19.7 months by Cao et al. (2018, 5080197) reported slight increases in postnatal length (i.e., height) (1.37 cm; 95% CI: −0.5, 3.28), while large postnatal weight deficits were reported for PFOS tertiles 2 (−138 g; 95% CI: −574, 298) and 3 (−78 g; 95%CI: −532, 375).

Associations at five months of age in the overall population (-0.28; 95% CI: –0.51, –0.05) and females (–0.56; 95% CI: –0.87, –0.26) from the *high* confidence study by Starling et al. (2019, 5412449) were detected for weight-for-age z-scores, as well as weight-for-length z-scores (overall: –0.26; 95% CI: –0.53, 0.00; females; –0.52; 95% CI: –0.88, –0.17). Exposure-response relationships were observed across tertiles for both of these 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 and exposure-response relationships for infant weight-for-age z-score (−0.33; 95% CI: −0.65, −0.01) and weight-for-length z-score (−0.34; 95% CI: −0.59, −0.08) in PFOS tertile 3 compared to tertile 1. Small deficits that were not statistically significant were observed in tertile 3 for length for age z-score (−0.22; 95% CI: −0.49, 0.04). In their *high* confidence study, Yeung et al. (2019, 5080619) reported statistically significant negative growth trajectories weight-for-length z-scores in relation to each log SD increase in PFOS exposures among singletons followed for three years. No associations were detected for

infant length (i.e., height) measures. Some sex-specific results were detected with larger associations seen in singleton females for weight for length z-score (−0.10; 95%CI: −0.16, -0.05) and weight z-score (−0.07; 95%CI: −0.13, −0.01). An infant weight deficit of –22.0 g (95% CI: −59.5, 15.6 per each 1 log SD PFOS increase) was also observed that was driven by results in females (–51.6 g; 95% CI: –102.3, –0.8).

Overall, seven of 8 studies with quantitative estimates (including 5 *high* and *medium* confidence studies) showed some associations between PFOS exposures and different measures of infant weight. Two of four studies with categorical data showed some evidence of inverse monotonic exposure-response relationships. Two of six studies with quantitative estimates examining different infant height measures showed some evidence of adverse associations with PFOS. Study quality ratings, including study sensitivity and overall confidence, did not appear to be explanatory factors for heterogeneous results across studies.

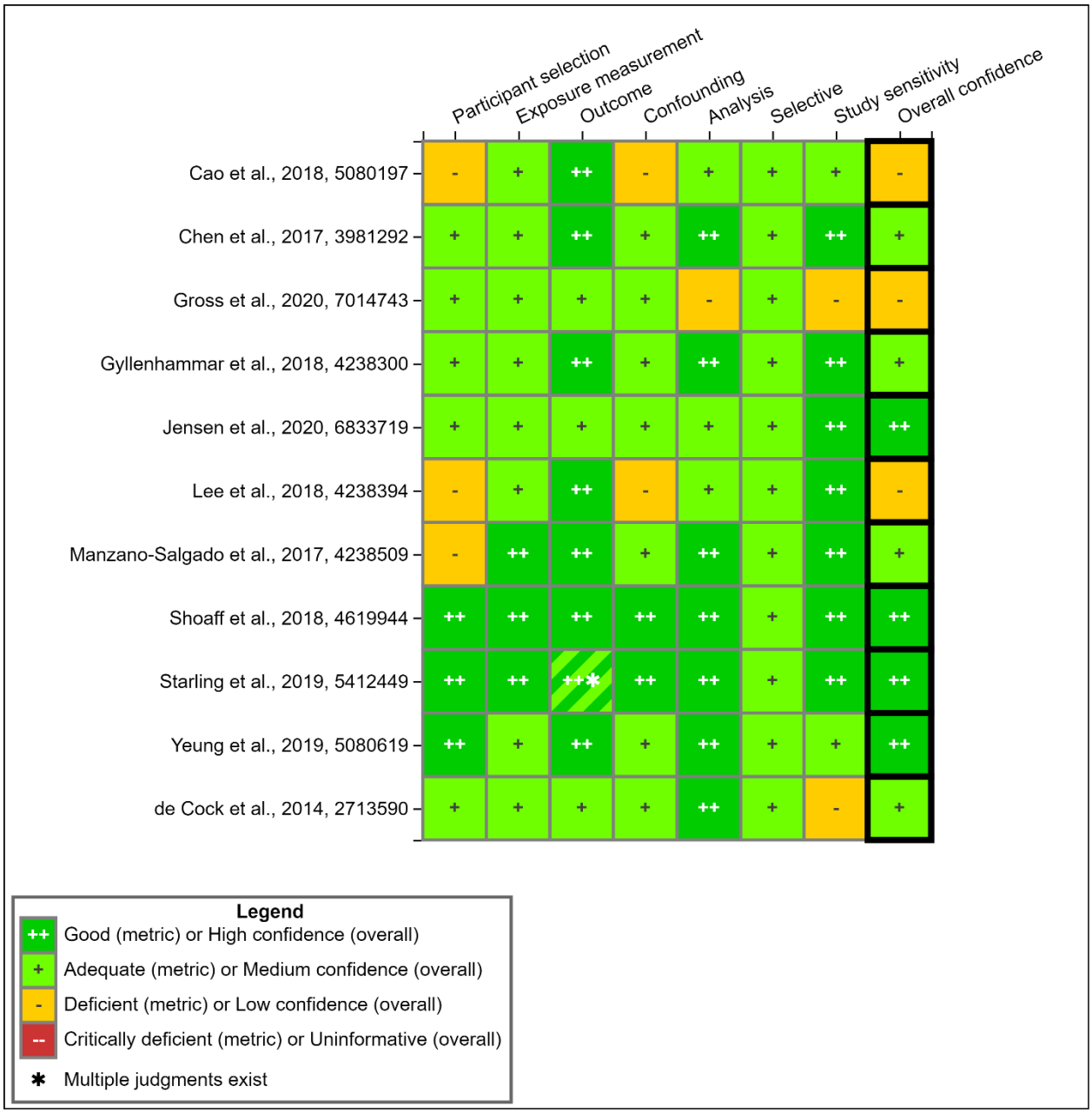


Figure 3-62. Summary of Study Evaluation for Epidemiology Studies of PFOS and Postnatal Growth Effects

Interactive figure and additional study details available on HAWC.

3.4.4.1.5.1 Adiposity/BMI

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 decreases in infant BMI z-score (−0.36; 95% CI: −0.60, −0.12). Although they were not statistically significant, the *medium* confidence Chen et al. (2017, 3981292) reported consistently small BMI z-scores across infant developmental windows (range: −0.08 to −0.10) per each ln unit PFOS. These results seem to be

driven by results in females especially for the 6 to 12 months (−0.33; 95% CI: −0.59, −0.08) and 12 to 24 months (−0.25; 95% CI: −0.45, −0.05) developmental periods. In their *high* confidence study, Yeung et al. (2019, 5080619) reported statistically significant negative growth trajectories for BMI and BMI z-score in relation to each log SD increase in PFOS exposures among singletons followed for three years. No exposure-response relationship was detected for BMI z-scores. Some sex-specific results were detected with larger associations seen in singleton females BMI z-score (−0.11; 95% CI: –0.17, –0.05) and BMI (–0.16 kg/m2; 95% CI: –0.24, –0.08). In the *high* confidence study by Starling et al. (2019, 5412449), decreased adiposity (–2.08; 95% CI: –3.81, –0.35) among girls were detected in PFOS tertile 3 compared to the tertile 1 referent. The *high* confidence study by Jensen et al. (2020, 6833719) reported null associations between adiposity and per each 1-unit increase in PFOS measured at 3 and 18 months. The low confidence study by Gross et al. (2020, 7014743) reported an inverse association (OR = 0.43; 95% CI: 0.17 to 1.09) of being overweight at 18 months for PFOS levels greater than the mean level. They also reported a lower odds ratio of being overweight at 18 months in males (OR = 0.19; p-value=0.04) than females (OR = 0.85; p-value=0.85). Mixed results were seen for measures of adiposity and increased BMI with increasing PFOS exposures.

3.4.4.1.5.2 Rapid Weight Gain

Four *high* confidence studies {Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944; Starling et al. 2019, 5412449; Yeung, 2019, 5080619} examined rapid infant growth. Limited evidence of associations was reported, as only one {Starling et al., 2019, 5412449} of four studies {Manzano-Salgado, 2017, 4238509; Shoaff, 2018, 4619944; Starling et al. 2019, 5412449; Yeung, 2019, 5080619} showed increased odds or rapid weight gain with increasing PFOS. For example, Starling et al. (2019, 5412449) reported a small OR of 1.36 for rapid growth in the overall population based on either weight for length-based z-scores. Study sensitivity was not an explanatory factor for the null studies.

3.4.4.1.5.3 Postnatal Growth Summary

Seven (3 *high*, 2 *medium*, and 2 *low* confidence) of the 8 studies with quantitative estimates examining different infant weight measures showed some evidence of adverse associations with PFOS exposures either in the overall population or either/or both male or female neonates. There was some evidence of exposure-response relationships as two of the four studies on infant weight showed adverse monotonic relationships across PFOS categories. No patterns by study characteristics or study confidence were evident. Only two (one *low* and one *high* confidence) of the seven studies with quantitative estimates examining different infant height measures showed some evidence of adverse associations with PFOS exposures. Two of the six postnatal growth studies with quantitative estimates showed increased infant BMI or adiposity with increasing PFOS exposures, while three showed decreased risk of higher BMI or adiposity. Only one out of four *high* confidence studies showed any evidence of rapid growth among infants following PFOS exposures. Although the data for some endpoints was less consistent, the majority of infant weight studies indicated that PFOS may be associated with post-natal growth measures up to two years of age.

3.4.4.1.6 Gestational Duration

Twenty-two different studies examined gestational duration measures (i.e., PTB or gestational age measures) in relation to PFOS exposures. Nine of these studies examined both PTB and

gestational age measures, while two studies only examined PTB {Liu, 2020, 6833609; Gardener, 2021, 7021199}.

3.4.4.1.6.1 Gestational Age

Seventeen of the 20 studies reporting gestational age estimates in relation to PFOS exposures were considered informative and included here including two that were uninformative {Gundacker, 2021, 10176483; Lee, 2013, 3859850} and one excluded study based on an overlapping cohort {Li, 2017, 3981358}. Sixteen non-overlapping and informative studies examined mean gestational age (in weeks) in relation to PFOS exposures including one study reporting sex-specific results only {Lind, 2017, 3858512}.

Among the 16 different studies included here, nine were *high* confidence {Bach, 2016, 3981534; Bell, 2018, 5041287; Chu, 2020, 6315711; Eick, 2020, 7102797; Huo, 2020, 6835452; Lauritzen, 2017, 3981410; Lind, 2017, 3858512; Manzano-Salgado, 2017, 4238465; Sagiv et al. 2018, 4238410}, four were *medium* {Gyllenhammar, 2018, 4238300; Hjermitslev, 2020, 5880849; Meng, 2018, 4829851; Yang, 2022, 10176806} and four were *low* confidence {Bangma, 2020, 6833725; Gao, 2019, 5387135; Workman, 2019, 5387046; Xu, 2019, 5381338}. Ten of these studies had good study sensitivity, six were adequate {Bangma, 2020, 6833725; Eick, 2020, 7102797; Gao, 2019, 5387135; Workman, 2019, 5387046; Xu, 2019, 5381338; Yang, 2022, 10176806} and one was deficient {Bell et al., 2018, 5041287}.

Nine of the 15 studies examining mean gestational age change in the overall population reported some deficits. Among these, four were *high* confidence, and three were *medium* and two were *low* confidence. The *medium* confidence study by Gyllenhammar et al. (2018, 4238300) reported a deficit of –0.29 weeks (95% CI: –0.59, 0.01) per each IQR PFOS change; they also reported that there were no statistically significant interactions by sex for their PFOS measures. The *high* confidence study by Sagiv et al. (2018, 4238410) reported a similar gestational age reduction in the overall population (–0.36 weeks; 95% CI: −0.64, −0.09) for PFOS quartile 4 versus quartile 1; this seemed to be driven by associations among boys only (z-score: −0.19; 95% CI: −0.33, −0.05) per each IQR increase). The *high* confidence study by Chu et al. (2020, 6315711) reported similar deficits in the overall population (–0.32 weeks; 95% CI: –0.53, –0.11) which was driven by female neonates (–0.61 weeks; 95% CI: –0.90, –0.32). The *high* confidence study by Lauritzen et al. (2017, 3981410) only showed deficits among their Swedish population (–0.4 weeks; 95%CI: –0.9, 0.2). Compared to tertile 1, the *low* confidence study by Gao et al. (2019, 5387135) reported deficits in tertile 2 (–0.40 weeks; 95% CI: –0.92, 0.12) and tertile 3 (–0.20; 95%CI: –0.61, 0.20). The *high* confidence study by Manzano-Salgado et al. (2017, 4238465) reported deficits in quartile 4 among the overall population (–0.31 weeks; 95% CI: –0.55, –0.06) compared to quartile 1. Despite low overall PFOS concentrations, the *medium* confidence study by Yang et al. {2022, 10176806} showed reduced gestational age only among pre-term births for both total PFOS (−1.26 weeks; 95%CI: − 2.46, -0.05) and linear PFOS (−1.80 weeks; 95%CI: −3.24, −0.37) per each IQR increase, with results larger results in female (−1.06 weeks; 95%CI: −2.87, 0.74) than male neonates (−0.41 weeks; 95%CI: −2.20, 1.37). The *medium* confidence study by Meng et al. (2018, 4829851) reported statistically significant gestational age deficits (range: –0.16 to –0.29 weeks) across all quartiles but no evidence of an exposure-response relationship. The low confidence study by Workman et al. (2019, 5387046) reported a non-significant decrease (–0.17 weeks; 95% CI: –0.52, 0.18) per each ln-unit PFOS change.

Overall, nine of the 15 studies based on the overall population showed some evidence of inverse associations between PFOS and gestational age. This included seven *medium* or *high* confidence studies. The four *high* confidence studies showed deficits in the overall population consistent in magnitude (range: –0.30 to –0.40 weeks). Apart from one study with very large deficits, the remaining two *medium* and *two* low confidence studies all ranged from –0.17–0.30 weeks for different PFOS contrasts). No exposure-response relationships were detected in any study, and no definitive patterns were seen based on other study characteristics or in the other few studies with sex-specific data. For example, 3 of 7 studies showed decreased gestational ages in relation to PFOS exposures among both male or female neonates. Study sensitivity did not seem to be an explanatory factor as five of six studies that did not show adverse associations had good or adequate study sensitivity. Lastly, sample timing did not seem to be an explanatory factor of the results as an equal proportion (60%) of studies showing inverse associations between PFOS and gestational age deficits were based on earlier and later biomarker sampling.

3.4.4.1.6.2 Preterm Birth

As shown in Figure 3-63, eleven studies examined the relationship between PFOS and preterm birth (PTB); all of the studies were either *medium* {Hjermitslev, 2020, 5880849; Liu, 2020, 6833609; Meng, 2018, 4829851; Yang 2022, 10176806} or *high* confidence {Bach, 2016, 3981534; Chu, 2020, 6315711; Eick, 2020, 7102797; Gardener, 2021, 7021199; Huo, 2020, 6835452; Manzano-Salgado, 2017, 4238465; Sagiv, 2018, 4238410}. Nine of the eleven studies were prospective birth cohort studies, while the two studies by Liu et al. (2020, 6833609) and Yang et al. (2022, 10176806) were case-control studies nested with prospective birth cohorts. Four studies had maternal exposure measures that were sampled during trimester one {Bach, 2016, 3981534; Manzano-Salgado, 2017, 4238465; Sagiv, 2018, 4238410}, or trimester three {Gardener, 2021, 7021199}. The *high* confidence study by Chu et al. {2020, 6315711} sampled during the late third trimester or within three days of delivery. Four studies collected samples across multiple trimesters {Eick, 2020, 7102797; Hjermitslev, 2020, 5880849; Huo, 2020, 6835452; Liu, 2020, 6833609}. One study used umbilical cord serum samples {Yang 2022, 10176806}. The *medium* confidence study by Meng et al. (2018, 4829851) pooled umbilical cord blood and maternal serum (trimester 1 and 2) exposure data from two study populations. Seven studies had good study sensitivity, while four others were considered adequate {Eick, 2020, 7102797; Liu, 2020, 6833609; Gardener, 2021, 7021199; Yang 2022, 10176806} with the median exposure values in the overall population ranging from 1.79 ng/mL {Liu et al. 2020, 6833609} to 30.1 ng/mL {Meng, 2018, 4829851}. Lower levels were also seen for a total PFOS measure in Yang et al. {2022, 10176806} for both cases (median (IQR) = 0.27 (0.30) ng/mL) and controls (0.21 (0.37) ng/mL).

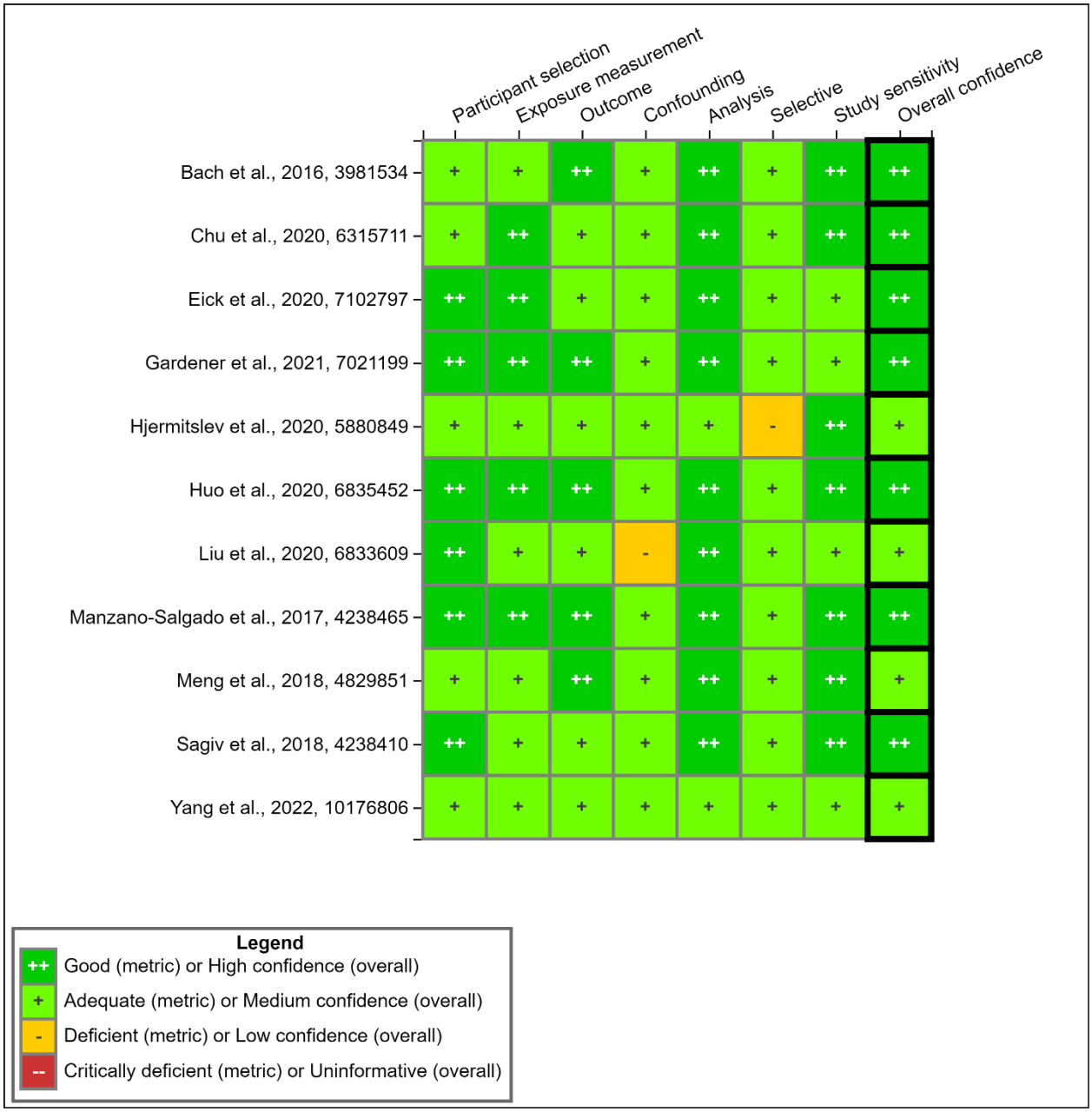


Figure 3-63. Summary of Study Evaluation for Epidemiology Studies of PFOS and Preterm Birth Effects

Interactive figure and additional study details available on HAWC.

Adverse associations were reported in seven of the 11 PTB studies with ORs from 1.5- to 5-fold higher for elevated PFOS exposures. The *medium* confidence study by Meng et al. (2018, 4829851) study reported statistically significant non-monotonic increased ORs for PTB in the upper three PFOS quartiles (OR range: 1.9–3.3), as well as per each doubling of PFOS exposures (OR = 1.5; 95% CI: 1.1, 2.2). The *high* confidence study by Chu et al. (2020, 6315711) reported some statistically significant increased ORs per each ln unit increase (OR = 2.03; 95% CI: 1.24,

3.32) as well as an exposure-response relationship across upper three quartiles (OR range: 2.22–4.99) exposures when compared to the referent. The *high* confidence study by Eick et al. (2020, 7102797) reported an exposure-response relationship as well (tertile 2 OR = 1.21; 95% CI: 0.50, 2.91; tertile3 OR = 1.87; 95% CI: 0.72, 4.88, compared to tertile 1). Although they were not statistically significant, the *medium* confidence study by Liu et al. (2020, 6833609) reported increased ORs of similar magnitude per each $\log_{10}$ unit increase (OR = 1.30; 95% CI: 0.76, 2.21) or when quartile 3 (OR = 1.51; 95% CI: 0.85, 2.69) and quartile 4 (OR = 1.35; 95% CI: 0.74, 2.45) exposures were compared to the referent. The *high* confidence study by Sagiv et al. (2018, 4238410) study reported consistently elevated non-monotonic ORs for PTB in the upper three PFOS quartiles (OR range: 2.0–2.4), but smaller ORs when examined per each IQR PFOS increase (OR = 1.1; 95% CI: 1.0, 1.3). The *high* confidence study by Gardener et al. (2021, 7021199) reported that participants in the PFOS exposure quartiles 2 (OR = 1.94; 95% CI: 0.66, 5.68) and 4 (OR = 1.41; 95% CI: 0.46, 4.33) had higher odds of preterm birth (relative to the lowest quartile). Despite low overall PFOS concentrations, the *medium* confidence study by Yang et al. (2022, 10176806) showed statistically significant increased odds of preterm birth per each IQR increase in total PFOS (OR = 1.44; 95% CI: 1.18, 1.79), linear PFOS (OR = 1.41; 95% CI: 1.19, 1.73), and branched PFOS (OR = 1.11; 95% CI: 1.01, 1.29). No differences were observed for male or female stratified results (OR range: 1.40-1.45). Null or inverse associations were reported by Bach et al. (2016, 3981534), Huo et al. (2020, 6835452), Manzano-Salgado et al. (2017, 4238465) and Hjermitslev et al. (2019, 5880849). Overall, only two {Chu, 2020, 6315711; Eick, 2020, 7102797} out of eight studies showed evidence of exposure-response relationships.

Overall, 7 of 11 studies reported increased odds of preterm birth in relation to PFOS with some sizeable relative risks reported. There was some limited evidence of exposure-response relationships as well. Although small numbers limited the confidence in many of the sub-strata comparisons, few patterns in the PTB results emerged based on study confidence (all 11 studies were *medium* or *high* confidence), sample timing or other study characteristics. For example, three of the four null studies were considered to have good sensitivity to detect associations that may be present. The results for preterm birth are robust with respect to adverse associations detected with increasing PFOS exposures.

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

3.4.4.1.6.3 Gestational Duration Summary

Overall, there is robust evidence of an impact of PFOS exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) as most of studies showed some adverse associations. This was strengthened by consistency in the reported magnitude of gestational age deficits despite different exposure levels and metrics examined. Although they were not as consistent in magnitude (60% of the PTB studies showed some adverse associations), some of the effect estimates were large for preterm birth in relation to PFOS exposures with limited evidence of exposure-response relationships. Few patterns were evident as explanatory factors for heterogeneous results based on our qualitative analysis.

3.4.4.1.7 Fetal Loss

As shown in Figure 3-64, five (2 *high*, 2 *medium* and 1 *low* confidence) studies examined PFOS exposure and fetal loss. All of these studies had good study sensitivity owing largely to very large sample size and sufficient sample sizes {Buck Louis, 2016, 3858527; Jensen, 2015, 2850253; Liew, 2020, 6387285; Wang, 2021, 10176703; Wikström, 2021, 7413606}.

The *high* confidence study by Wikström et al. (2021, 7413606) showed little evidence of association between PFOS and miscarriages (OR = 1.13; 95% CI: 0.82, 1.52 per doubling of PFOS exposures). The authors did not report an exposure-response relationship across PFOS quartiles but did show elevated non-significant ORs of approximately 1.2 and 1.3 for the upper two quartiles. Although the ORs were not statistically significant in the *medium* confidence study by Liew et al. (2020, 6387285), there was some suggestion of an exposure-response for miscarriages across PFOS quartiles (OR range: 1.1–1.4). Similarly, the low confidence study by Jensen et al. (2015, 2850253) reported increased non-significant risks across tertiles 2 and 3 (OR range: 1.15–1.33). No association was detected in the *high* confidence study by Wang et al. (2021, 10176703) (OR = 0.95; 95%CI: 0.87, 1.04) or the *medium* confidence study by Buck Louis et al. (2016, 3858527) (hazard ratio (HR) = 0.81; 95% CI: 0.65, 1.00 per each SD PFOS increase).

Overall, there was positive evidence for fetal loss with increased relative risk estimates in three out of five studies. In those three studies, the magnitude of associations detected were low but consistently reported in the range of 1.1 of 1.4 with an exposure-response relationship detected in one study. No patterns in the results were detected by study confidence ratings including sensitivity.

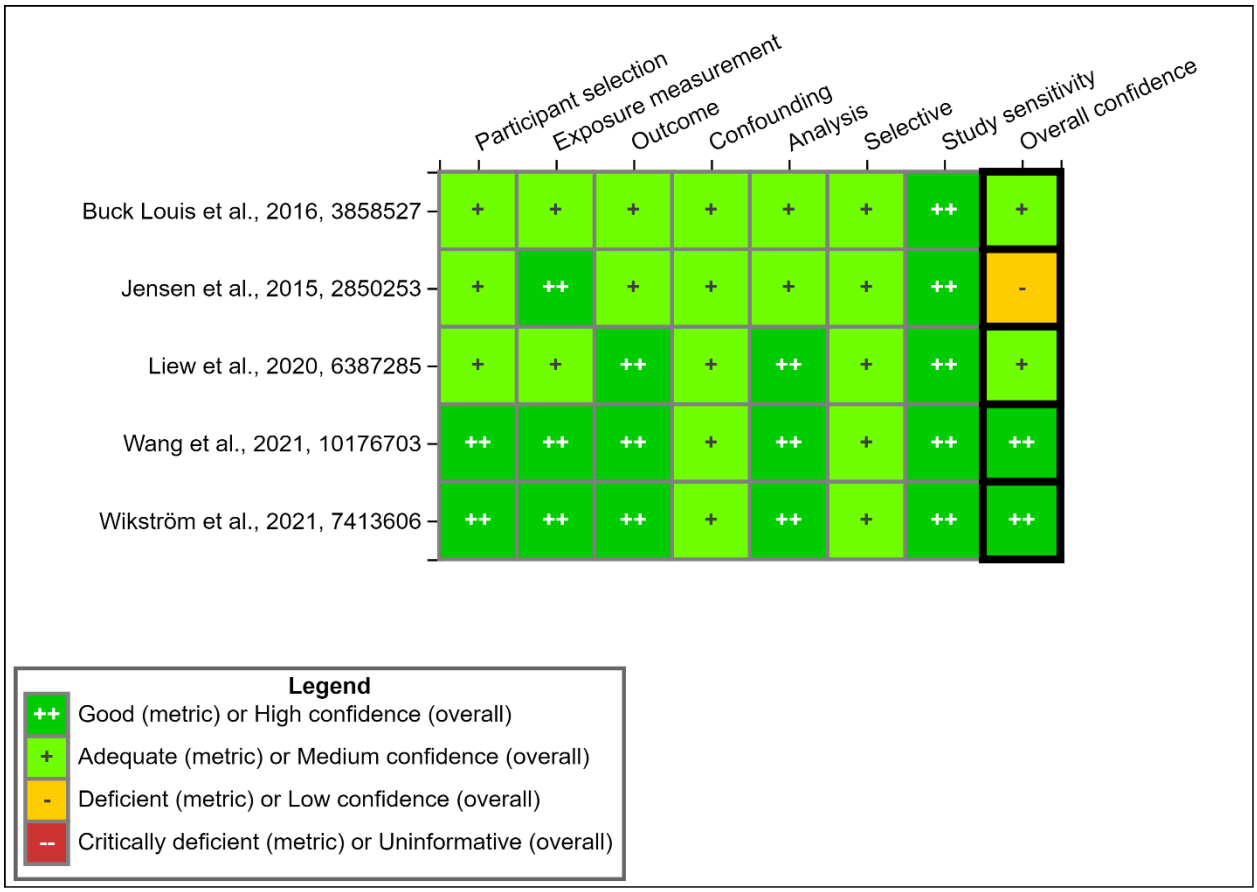


Figure 3-64. Summary of Study Evaluation for Epidemiology Studies of PFOS and Fetal Loss Effects

Interactive figure and additional study details available on HAWC.

3.4.4.1.8 Birth Defects

As shown in Figure 3-65, five (3 *medium* and 2 *low* confidence) studies examined PFOS exposure in relation to birth defects. Four of the five studies had adequate sensitivity. This included a *medium* confidence study by Ou et al. (2021, 7493134) that reported increased risks for septal defects (OR = 1.92; 95% CI: 0.80, 4.60), conotruncal defects (OR = 1.65; 95% CI: 0.59, 4.63) and total congenital heart defects (OR=1.61; 95%CI: 0.91, 2.84) among participants with maternal serum levels over >75th PFOS percentile (relative to those <75th percentile). A low confidence study of a non-specific grouping of all birth defects {Cao, 2018, 5080197} reported a small but imprecise increased risk (OR = 1.27; 95% CI: 0.59, 2.73). Interpretation of all birth defect groupings is challenging given that etiological heterogeneity may occur across individual defects.

Three studies examined PFOS exposures in relation to cryptorchidism. The *medium* confidence study by Vesterholm Jensen et al. (2014, 2850926) detected an inverse association for cryptorchidism (OR = 0.51; 95% CI: 0.21–1.20) per each ln-unit increase in PFOS exposures. This risk seemed to be largely driven by boys from Finland. The *medium* confidence study by

Toft et al. (2016, 3102984) reported null associations per each ln-unit increase in PFOS exposures and both cryptorchidism (OR = 0.99; 95% CI: 0.75, 1.30) and hypospadias (OR = 0.87; 95% CI: 0.57, 1.34). The *low* confidence study by Anand-Ivell et al. (2018, 4728675) did not find statistically significant PFOS exposure differences among cryptorchidism or hypospadia cases compared to controls, but they did not examine this in a multivariate fashion adjusting for confounders.

Overall, there was very limited evidence of associations between PFOS and birth defects based on the available epidemiological studies. This was based on cryptorchidism, hypospadias or all birth defect groupings. As noted previously, there is considerable uncertainty in interpreting results for broad any defect groupings which are anticipated to have decreased sensitivity to detect associations.

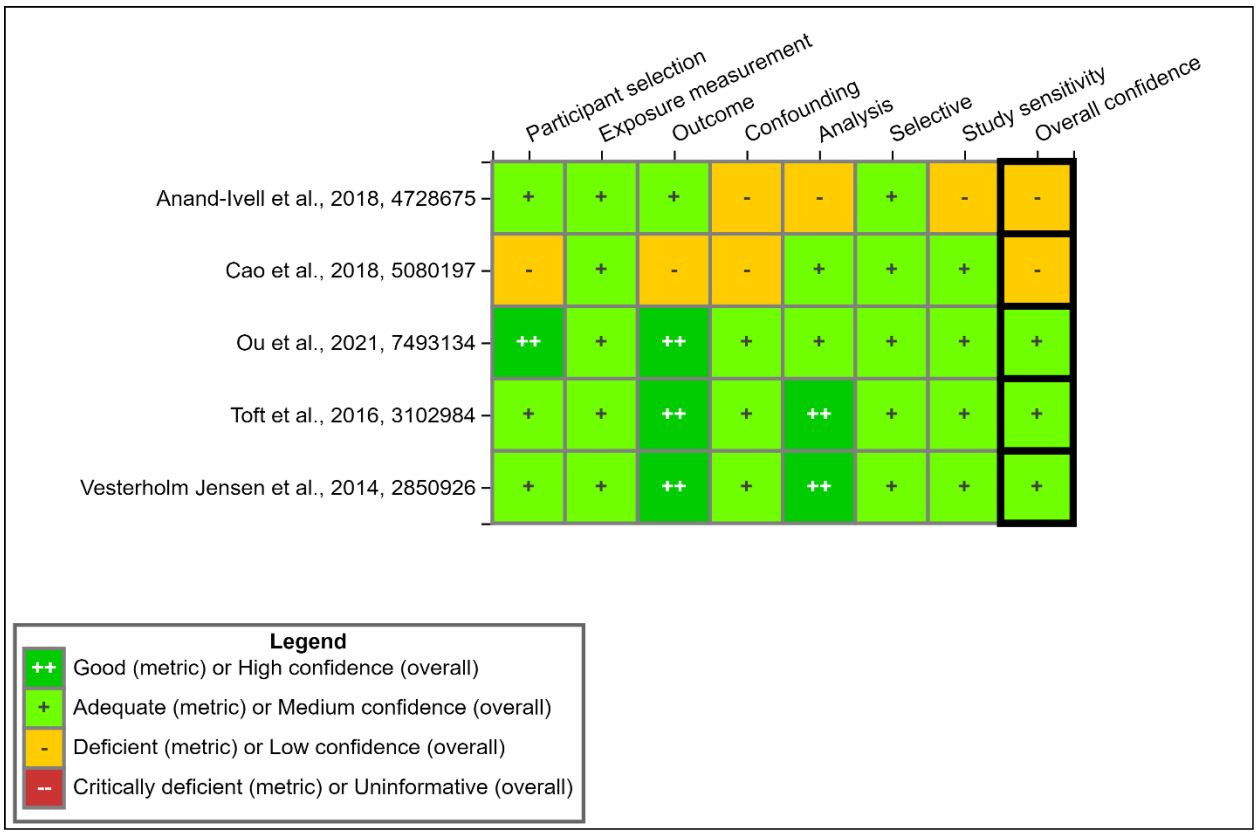


Figure 3-65. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Defect Effects

Interactive figure and additional study details available on HAWC.

3.4.4.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 4 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and 15 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and developmental effects. Study quality evaluations for these 19 studies are shown in Figure 3-66.

Figure 3-66. Summary of Study Evaluation for Toxicology Studies of PFOS and Developmental Effects

Interactive figure and additional study details available on HAWC.

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

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

3.4.4.2.1 Maternal Effects

Multiple developmental studies evaluated maternal weight outcomes in rats, mice, and rabbits (Figure 3-67). Yahia et al. (2008, 2919381) observed a decrease in body weight in ICR mouse dams administered 20 mg/kg/day PFOS from gestational day 1 to 17 (GD 1 to GD 17) or GD 18. The dams exhibited no clinical signs of toxicity. Thibodeaux et al. (2003, 757855) observed significantly decreased maternal body weight gain in CD-1 mice at exposed to 20 mg/kg/day PFOS (highest dose tested in the study); food and water consumption were not affected by treatment. Lee et al. (2015, 2851075) also reported reduced maternal body weight gain in CD-1 mice treated with 2 or 8 mg/kg/day PFOS (not 0.5 mg/kg/day) compared to controls. Dams in the 2 and 8 mg/kg/day dose groups had significantly lower mean body weights on GD 14–GD 17. In contrast, Lai et al. (2017, 3981773) did not observe a significant difference in maternal body weight in CD-1 mouse dams orally exposed to 0, 0.3, or 3 mg/kg/day throughout gestation (GD 1–GD 20). The authors determined that there were no observable maternal effects related to PFOS exposure at the relatively low doses evaluated. Wan et al. (2020, 7174720) found no effect of PFOS on maternal body weight in CD-1 mouse dams orally dosed with 0, 1, or 3 mg/kg/day from GD 4.5 to GD 17.5. Likewise, Fuentes et al. (2006, 757859) found no treatment-related effects on maternal body weight, maternal body weight gain, or maternal food consumption in CD-1 mouse dams orally exposed to 0, 1.5, 3, or 6 mg/kg/day PFOS from GD 6 to GD 18. Mshaty et al. (2020, 6833692) orally administered PFOS to C57BL/6J mice from postnatal day 1 (PND 1) to PND 14, resulting in lactational exposure to pups. Mean maternal body weights were evaluated at PND 21 and determined to be comparable between the control and the 1 mg/kg/day dose groups.

Thibodeaux et al. (2003, 757855) observed significant, dose-dependent decreases in maternal body weight, food consumption, and water consumption in Sprague Dawley rats dosed with ≥ 2 mg/kg/day PFOS from GD 2 to GD 20. Xia et al. (2011, 2919267) also observed reduced body weight on GD 21 in Sprague Dawley rats dosed with 2 mg/kg/day from GD 2 to GD 21. In a 2-generation reproductive toxicity study in rats, Luebker et al. (2005, 1276160) similarly observed dose-dependent decreases in maternal body weight in the 3.2 mg/kg/day dose group of the parental generation (P_0) from day 15 of the premating exposure through lactation day 1 (LD 1), the last recorded weight; this dose group also had significantly decreased maternal weight gain from GD 0 to GD 20. The 1.6 mg/kg/day dams experienced transient decreases in maternal weight compared to controls in the window between GD 3 and GD 11. There were no reported differences in the maternal weight of adult first generation (F_1) females during precohabitation until the end of lactation, though the highest dose tested in these females was only 0.4 mg/kg/day. Following the 2-generation study, Luebker et al. (2005, 757857) conducted a follow up 1-generation study that examined additional PFOS doses during development. Crl:Cd(Sd)Igs Vaf/Plus rat dams were gavaged with 0, 0.4, 0.8, 1, 1.2, 1.6, or 2 mg/kg/day PFOS. Dosing started 6 weeks prior to mating and continued through mating and gestation with the final dose on LD 4. The authors observed no treatment-related effects on body weight change during gestation, but body weight gain was reduced in the 0.8, 1, 1.6, and 2 mg/kg/day groups relative to controls during lactation. They also reported a general trend for reduced food

consumption with increasing dose during gestation and lactation {Luebker, 2005, 757857}. In another study with Sprague Dawley rats dosed with 0, 5, or 20 mg/kg/day PFOS from GD 12 to GD 18, Li et al. (2016, 3981495) also reported reduced mean maternal body weights in the 20 mg/kg/day dose group. In another study, Conley et al. (2022, 10176381) reported a significant 43% weight gain reduction relative to controls in Sprague-Dawley (Crl:CD(SD)) rat dams dosed with 30 mg/kg/day PFOS from GD 14 to GD 18; no significant effects were observed for the 0.1, 0.3, 1, 3, or 10 mg/kg/day PFOS groups. Zhang et al. (2021, 6988534) also reported no significant treatment-related effects on maternal body weight in Sprague-Dawley rat dams dosed with 0, 1, or 5 mg/kg/day PFOS from GD 12 to GD 18. Butenhoff et al. (2009, 757873) observed comparable maternal body weight and body weight gain during gestation in Sprague Dawley rat dams dosed with 0, 0.1, 0.3, or 1 mg/kg/day PFOS from GD 0 to LD 20 but observed significantly lower absolute body weights during lactation (PND 4–PND 20) in dams treated with 1 mg/kg/day PFOS. Transient decreases in food consumption were observed in the 0.3 and 1.0 mg/kg/day groups throughout the study, though these findings were not considered treatment-related or adverse.

In a single rabbit study, Argus Research Laboratories (2000, 5080012) reported significantly decreased maternal body weight gain from GD 7 to GD 21 at PFOS doses ≥ 1 mg/kg/day (mean body weight change of 0.38, 0.3, 0.2, and −0.01 kg with 0, 1, 2.5, and 3.75 mg/kg/day PFOS, respectively); no significant effect was observed from GD 21 to GD 29. There were observations of scant or no feces for some does in the 1.0, 2.5, and 3.75 mg/kg/day groups. Observations of scant feces were significant relative to control at 3.75 mg/kg/day. Significant reductions in absolute (g/day) and relative (g/kg/day) feed consumption was also observed in the 2.5 and 3.75 mg/kg/day dose groups.

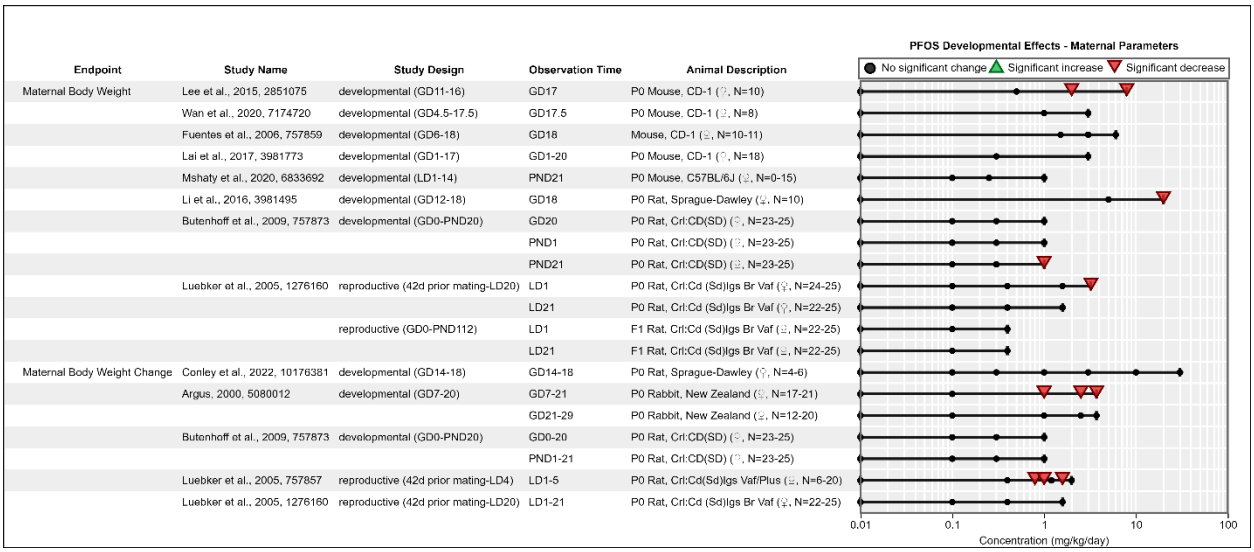


Figure 3-67. Maternal Body Weight in Mice, Rats, and Rabbits Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; LD = lactational day; P_0 = parental generation; F_1 = first generation; d = day.

3.4.4.2.2 Viability

Decreases in both fetal and pup survival and viability with perinatal PFOS exposure were observed in multiple studies (Figure 3-68). Lee et al. (2015, 2851075) reported a significantly higher incidence of resorptions, post-implantation loss, and dead fetuses at GD 17 after dosing pregnant CD-1 mice by gavage with 0.5, 2, or 8 mg/kg/day from GD 11 to GD 16; however, there was no significant difference in the mean number of implantations. A significant decrease in mean number of live fetuses was also observed in the 2.0 and 8.0 mg/kg/day dose groups vs. controls. A decrease in the mean number of live fetuses was also reported in the 0.5 mg/kg/day dose group but this difference was not significant relative to control. Administration of 0, 1, 5, 10, 15, or 20 mg/kg/day PFOS to CD-1 mice from GD 1 to GD 17 did not affect the number of implantation sites but resulted in a significant increase in post-implantation loss, as measured by decrease in mean percentage of live fetuses, in dams administered 20 mg/kg/day {Thibodeaux, 2003, 757855}. In another study, CD-1 mouse dams were dosed with 0, 3, or 6 mg/kg/day PFOS from GD 6 to GD 18. The authors found no treatment-related effects on the number of litters with dead fetuses, the total number of dead fetuses, dead fetuses per litter, or live fetuses per litter, and there were no effects of PFOS on the number of implantation sites, the percentage of post-implantation loss, the number of early or late resorptions, or fetal sex ratio {Fuentes, 2006, 757859}.

Mice appear to be more sensitive to alterations in fetal viability than rats. Thibodeaux et al. (2003, 757855) dosed pregnant Sprague-Dawley rats with 0, 1, 2, 3, 5, or 10 mg/kg PFOS daily by gavage from GD 2 to GD 20. The number of implantations was not affected by treatment and there were no treatment-related effects observed on the live rat fetuses at term. Likewise, Zhang et al. {2021, 6988534} dosed Sprague-Dawley rat dams with 0, 1, or 5 mg/kg/day PFOS from GD 12 to GD 18 and found no treatment-related effects on liveborn pups per litter, pup survival, or pup sex ratio. Butenhoff et al. (2009, 757873) also observed no treatment-related effects on the number of implantation sites or resorptions in pregnant Sprague-Dawley rats exposed to 0.1, 0.3, or 1.0 mg/kg/day by gavage from GD 0 to PND 20. Similarly, Conley et al. (2022, 10176381) found no effects of PFOS on the number of live fetuses per litter or total resorptions in a study wherein Sprague-Dawley (Crl:CD(SD)) rat dams were dosed with 0, 0.1, 0.3, 1, 3, 10, or 30 mg/kg/day PFOS from GD 14 to GD 18.

In pregnant New Zealand white rabbits cesarean sectioned on GD 29 after gestational exposure to PFOS, Argus Research Laboratories (2000, 5080012) reported no significant effects on implantations or resorptions. However, Argus Research Laboratories (2000, 5080012) did report abortions among New Zealand white rabbits orally dosed with 2.5 mg/kg/day (1/17 does, 5.9%) or 3.75 mg/kg/day (9/21 does, 42.8%) from GD 7 to GD 20. The abortion rate was significantly greater relative to control for the 3.75 mg/kg/day dose group. Argus Research Laboratories (2000, 5080012) reported no significant effects on the mean number of live fetuses/doe, number of dead fetuses/doe, mean litter size, and offspring viability.

Altered pup viability was observed in studies of both rats and mice. In one- and two-generation reproductive toxicity studies in Sprague Dawley rats, Luebker et al. (2005, 757857; 2005, 1276160) observed reduced pup viability index (ratio of the number of pups alive at PND 5 to the number of live pups born) at higher maternal PFOS doses. A significant decrease in pup viability for the one-generation study was associated with a dose of 1.6 mg/kg/day {Luebker, 2005, 757857}; the number of dams with all pups dying between PND 1 and PND 5 was also

significantly increased in the 2 mg/kg/day dose group. The dose associated with a decreased viability index in F_1 pups was also 1.6 mg/kg/day in the two-generation study {Luebker, 2005, 1276160}; between PND 1 and PND 4, 100% of dams had all pups dying in the 3.2 mg/kg/day dose group. Following gestational exposure to PFOS on GD 19–GD 20, Grasty et al. (2003, 5085464) observed survival of 98%, 66%, and 3% of rat pups in the control, 25, and 50 mg/kg/day groups, respectively, on PND 5. Similarly, Xia et al. (2011, 2919267) found decreased number of delivered pups per litter and increased pup mortality between birth and PND 3 for rats treated with 2 mg/kg/day on GD 2 to GD 21. Chen et al. (2012, 1276152) also observed decreased pup survival through PND 3 in rat pups exposed to 2 mg/kg/day PFOS from GD 1 to GD 21. Thibodeaux et al. (2003, 757855) and Lau et al. (2003, 757854) similarly observed decreased pup survival in rats exposed to ≥ 2.0 mg/kg/day PFOS from GD 2 to GD 21.

Lau et al. (2003, 757854) also reported PFOS-related effects on survival in mice following gestational exposure to PFOS. Briefly, most mouse pups from dams administered 15 or 20 mg/kg/day did not survive for 24 hours after birth. Fifty percent mortality was observed at 10 mg/kg/day. Survival of pups in the 1 and 5 mg/kg/day treated dams was similar to controls. Yahia et al. (2008, 2919381) also observed significant effects on pup survival. In this study, pregnant ICR mice/group were administered 0, 1, 10, or 20 mg/kg of PFOS daily by gavage from GD 1 to GD 17 or GD 18. All neonates in the 20 mg/kg/day dose group were born pale, weak, and inactive, and all died within a few hours of birth. At 10 mg/kg/day, 45% of those born died within 24 hours. Survival of the 1 mg/kg/day group was similar to that of controls. Of the developmental studies identified in the most recent literature search, only Mshaty et al. (2020, 6833692) evaluated the impact of lactational (PND 1–PND 14) PFOS exposure on pup survival. Mshaty et al. (2020, 6833692) observed no difference in C57BL/6J mouse pup survival through PND 21 between control group pups and pups exposed to 1 mg/kg/day PFOS (quantitative data not provided).

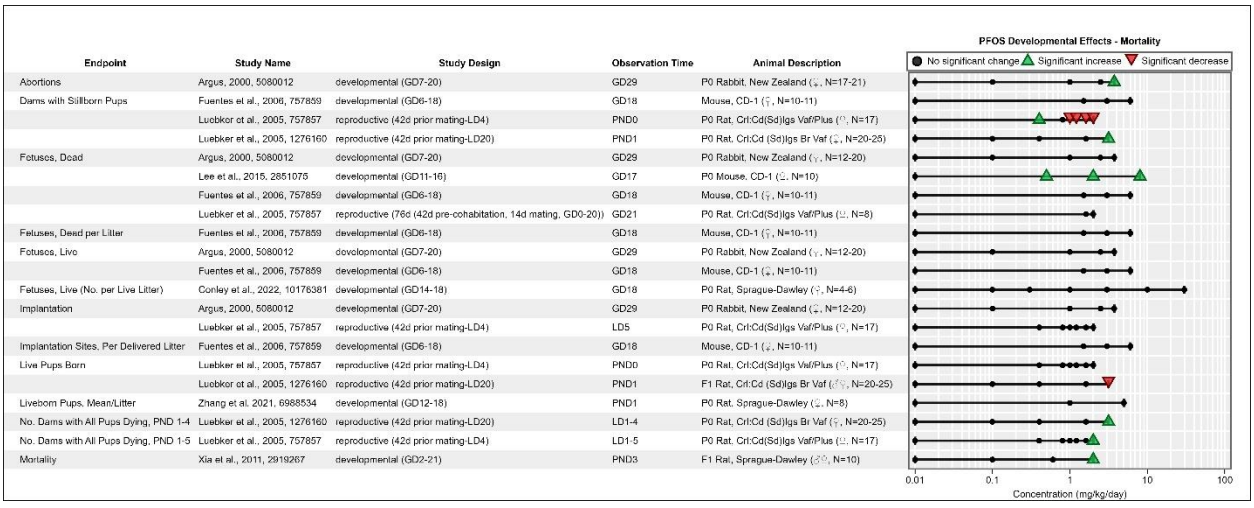
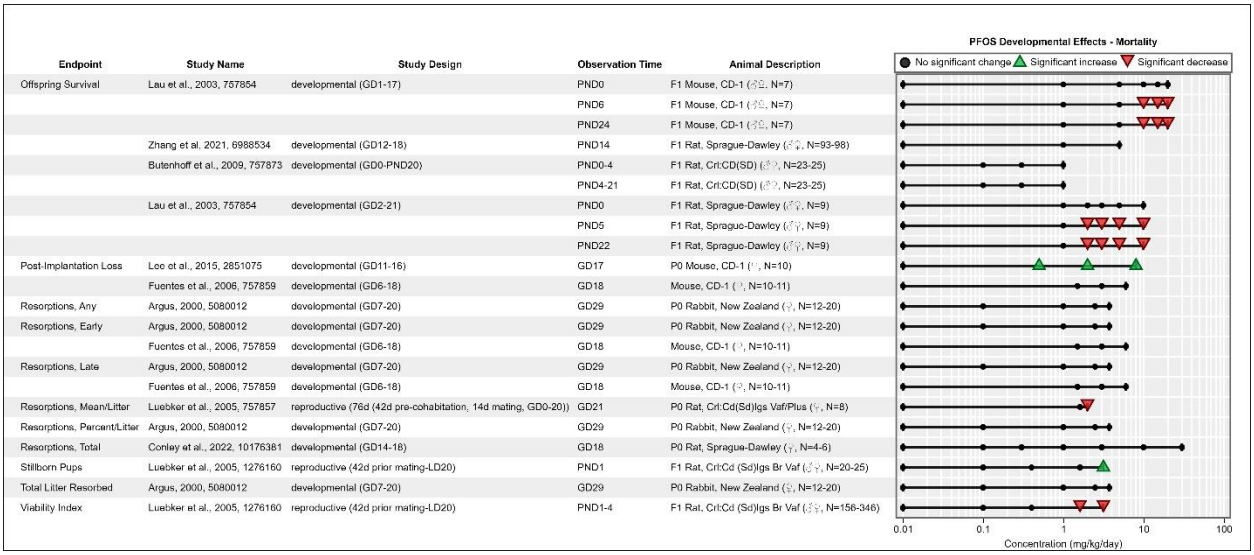


Figure 3-68. Mortality and Viability in Mice, Rats, and Rabbits Following Exposure to PFOS (logarithmic scale)

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data.
Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; LD = lactational day; P_0 = parental generation; F_1 = first generation; d = day.



Endpoint	Study Name	Study Design	Observation Time	Animal Description
Offspring Survival	Lau et al., 2003, 757854	developmental (GD1-17)	PND0	F1 Mouse, CD-1 (♂♀, N=7)
			PND6	F1 Mouse, CD-1 (♂♀, N=7)
			PND24	F1 Mouse, CD-1 (♂♀, N=7)
	Zhang et al. 2021, 6988534	developmental (GD12-18)	PND14	F1 Rat, Sprague-Dawley (♂♀, N=93-98)
	Butenhoff et al., 2009, 757873	developmental (GD0-PND20)	PND0-4	F1 Rat, Crl:CD(SD) (♂♀, N=23-25)
			PND4-21	F1 Rat, Crl:CD(SD) (♂♀, N=23-25)
	Lau et al., 2003, 757854	developmental (GD2-21)	PND0	F1 Rat, Sprague-Dawley (♂♀, N=9)
			PND5	F1 Rat, Sprague-Dawley (♂♀, N=9)
			PND22	F1 Rat, Sprague-Dawley (♂♀, N=9)
Post-Implantation Loss	Lee et al., 2015, 2851075	developmental (GD11-16)	GD17	P0 Mouse, CD-1 (♀, N=10)
	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Resorptions, Any	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
Resorptions, Early	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Resorptions, Late	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Resorptions, Mean/Litter	Luebker et al., 2005, 757857	reproductive (76d (42d pre-cohabitation, 14d mating, GD0-20))	GD21	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=8)
Resorptions, Percent/Litter	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
Resorptions, Total	Conley et al., 2022, 10176381	developmental (GD14-18)	GD18	P0 Rat, Sprague-Dawley (♀, N=4-6)
Stillborn Pups	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	PND1	F1 Rat, Crl:Cd (Sd)Igs Br Vaf (♂♀, N=20-25)
Total Litter Resorbed	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
Viability Index	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	PND1-4	F1 Rat, Crl:Cd (Sd)Igs Br Vaf (♂♀, N=156-346)

Figure 3-69. Mortality and Viability in Mice, Rats, and Rabbits Following Exposure to PFOS (Continued, logarithmic scale)

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data.
Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; LD = lactational day; P_0 = parental generation; F_1 = first generation; d = day.

3.4.4.2.3 Skeletal, Soft Tissue, and Gross Effects

Skeletal defects in offspring, including bone ossification, are a known effect of gestational PFOS exposure. In one study, 0, 1, 10, or 20 mg/kg of PFOS was administered daily by gavage to pregnant ICR mice from GD 1 to GD 17 or GD 18 {Yahia, 2008, 2919381}. Five dams/group were sacrificed on GD 18 for fetal external and skeletal effects. In the fetuses from dams treated with 20 mg/kg/day, there were significant increases in the numbers of fetuses with cleft palates (98.56%), sternal defects (100%), delayed ossification of phalanges (57.23%), wavy ribs (84.09%), spina bifida occulta (100%), and curved fetus (68.47%). In mice, Thibodeaux et al. (2003, 757855) observed significantly increased incidences of cleft palate at 15 and 20 mg/kg/day PFOS, sternal defects at 5, 10, 15, and 20 mg/kg/day PFOS, and ventricular septal defects at 20 mg/kg/day PFOS. Thibodeaux et al. (2003, 757855) also observed significantly increased incidences of these deformities in rats. The authors reported incidences of cleft palate at 10 mg/kg/day PFOS and sternal defects at 2 and 10 mg/kg/day PFOS. In another study, CD-1 mouse dams were exposed to 0, 1.5, 3, or 6 mg/kg/day PFOS from GD 6 to GD 18 {Fuentes, 2006, 757859}. The authors reported a lower incidence of incomplete calcaneus ossification in the 3 mg/kg/day group (6% fetal incidence, 20% litter incidence) relative to controls (46% fetal incidence, 80% litter incidence). The same study observed no treatment-related effects on fetal or litter incidence of the following skeletal development outcomes: supernumerary ribs, asymmetric sternebra, incomplete ossification of vertebra, or total skeletal malformations {Fuentes, 2006, 757859}.

Skeletal malformations in fetal and neonatal rabbits were reported in Argus Research Laboratories (2000, 5080012) at comparatively lower PFOS doses than those described in rat and mouse studies. A significant decrease in the mean number of isolated ossification sites of the metacarpal per fetus per litter was observed in the 3.75 mg/kg/day dose group vs. control (4.82

vs. 4.98, respectively); no significant change in mean number of ossification sites per fetus per litter was reported in the 0.1 (4.97), 1 (4.99), or 2.5 mg/kg/day (4.97) dose groups. A significant decrease in the mean number of sternal center ossification sites per fetus per litter was observed in the 2.5 and 3.75 mg/kg/day dose groups relative to control (3.81 and 3.82, respectively, relative to 3.98 for the control group); no significant change in the mean number of sternal center ossification sites per fetus per litter was detected in the 0.1 (3.92) and 1mg/kg/day (3.95) dose groups. A significant difference in fetal incidence of irregular ossification of the skull was reported in both the 2.5 and 3.75 mg/kg/day dose groups relative to control (0.8% and 9.2% incidence respectively, relative to 4% in the control); no significant difference was observed in the 0.1 (5.6%) and 1 mg/kg/day (2%) dose groups. There were no significant differences in litter incidence of irregular ossification of the skull in the 0.1, 1, 2.5, and 3.75 dose groups vs. control (38.9%, 15.8%, 6.2%, and 25%, respectively, vs. 30%). A significant decrease in mean number of ossification sites in the hyoid body per fetus per litter was reported in the 3.75 mg/kg/day dose group (0.92) vs. Control (1); no change in mean number of hyoid ossification sites was reported in other dose groups (mean of 1 for the 0.1, 1, and 2.5 mg/kg/day dose groups). A significant increase in fetal incidence of a hole in the parietal bone was observed in the 3.75 mg/kg/day dose group vs. Control (6.5% vs. 0%); no holes were detected in the 0.1, 1, and 2.5 mg/kg/day dose groups. Litter incidence of a hole in the parietal was 1 (8.3%) in the 3.75 mg/kg/day dose group and 0 (0%) in the 0, 0.1, 1, and 2.5 mg/kg/day dose groups. Fetal incidence of unossified pubis was also significantly increased in the 3.75 mg/kg/day group vs. Control (3.7% vs. 0%). No other dose groups exhibited unossified pubis. A significant increase in litter incidence of unossified pubis was observed in the 3.75 mg/kg/day group vs. Control (16.7% vs. 0%). The rest of the dose groups exhibited 0% litter incidence of unossified pubis. However, fetal alterations were observed in a similar percentage of litters across all dose groups (70%, 61.1%, 47.4%, 25%, and 66.7% in the 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively). No significant difference was seen in the mean percentage of fetuses per litter with any alteration (14.1%, 17%, 9.5%, 3.6%, and 17.4% in the 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively).

3.4.4.2.4 Fetal or Pup Body Weight

Several studies in different species reported data on fetal body weight (Figure 3-70). In a study in CD-1 mice with gestational PFOS exposure from GD 11 to GD 16, Lee et al. (2015, 2851075) reported mean fetal body weights on GD 17 of 1.72, 1.54, 1.3, and 1.12 g in the 0, 0.5, 2, and 8 mg/kg/day dose groups, respectively. The mean fetal weights reported for the 2 and 8 mg/kg/day groups were significantly lower than those reported for the control dose group. In another study with CD-1 mice that were exposed to 0, 1, or 3 mg/kg/day PFOS from GD 4.5 to GD 17.5, Wan et al. (2020, 7174720) reported a significant reduction in fetal body weight in the 3 mg/kg/day group compared to controls. In contrast, Fuentes et al. (2006, 757859) found no treatment-related effects on mean fetal weight per litter on GD 18 in CD-1 mice exposed to 0, 1.5, 3, or 6 mg/kg/day PFOS from GD 6 to GD 18. Li et al. (2021, 9959491) observed a dose-dependent decrease in fetal body weight in mice (strain not specified) exposed to 0, 0.5, 2.5, or 12.5 mg/kg/day PFOS from GD 1 to GD 17, whereby the mean fetal weights in the 2.5 and 12.5 mg/kg/day groups were decreased by approximately 17% and 24%, respectively, relative to controls. However, the reduction in weight did not reach significance, though it should be noted that the sample size was small (n = 3 litters/group). Li et al. (2016, 3981495) reported mean GD 18.5 fetal body weights of 2.73, 2.68, and 2.48 g in the 0, 5, and 20 mg/kg/day dose groups (sexes combined) following exposure of Sprague-Dawley rat to PFOS from GD 12 to GD 18.

Mean fetal body weight for the 20 mg/kg/day dose group was significantly different from that of the control group. Mean fetal body weight in males alone was also significantly decreased at 20 mg/kg/day (2.79, 2.74, and 2.43 g for the 0, 5, and 20 mg/kg/day dose groups, respectively). Thibodeaux et al. (2003, 757855) similarly observed a decrease in rat fetal weight following gestational exposure to 10 mg/kg/day PFOS. In a one-generation reproductive study in Sprague Dawley rats, Luebker et al. (2005, 757857) reported no effect on pooled fetal body weights with PFOS doses up to 2 mg/kg/day. Similarly, Conley et al. (2022, 10176381) found no effects of PFOS on fetal body weight on GD 18 in Sprague-Dawley rats (Crl:CD(SD)) exposed to 0, 0.1, 0.3, 1, 3, 10, or 30 mg/kg/day from GD 14 to GD 18. In a study in New Zealand white rabbits, Argus Research Laboratories (2000, 5080012) reported mean live fetal body weights of 44.15, 41.67, 42.37, 39.89, and 33.41 g/litter in 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively. Fetal body weights for the 2.5 and 3.75 mg/kg/day dose groups were significantly lower than fetal body weight reported in the control group.

Several other studies measured body weights of pups after birth (Figure 3-70). Zhang et al. (2021, 6988534) found no PFOS-related effects on pup body weight on PND 1, 3, 7, and 14 in Sprague-Dawley rat pups exposed to 0, 1, or 5 mg/kg/day from GD 12 to GD 18. The most sensitive endpoint in the one- and two-generation reproductive studies in Sprague Dawley rats (dams treated with PFOS pre-conception through gestation for 63 or 84 days, respectively) was decreased pup body weight {Luebker, 2005, 757857; Luebker, 2005, 1276160}. The NOAEL and LOAEL for pup body weight effects was 0.1 and 0.4 mg/kg/day, respectively, in the two-generation study {Luebker, 2005, 1276160}; the lowest dose of 0.1 mg/kg/day was not tested (NT) in the one-generation study {Luebker, 2005, 757857} where the LOAEL was 0.4 mg/kg/day for decreased pup body weight, decreased maternal body weight, and decreased gestation length. Lau et al. (2003, 757854) also reported significant weight deficits in Sprague Dawley rat pups on PND 0 after gestational PFOS exposures of 2, 3, or 5 mg/kg/day, but not 1 mg/kg/day. Similarly, Xia et al. (2011, 2919267) observed significantly reduced pup body weights in Sprague Dawley rats on PND 0 and PND 21 following gestational exposure to 2 mg/kg/day PFOS.

For this endpoint, rats appear to be more sensitive than mice. Yahia et al. (2008, 2919381) reported significant decreases in ICR mouse neonatal weight at relatively high doses of 10 and 20 mg/kg/day. Lau et al. (2003, 757854) did not report statistically significant reductions in pup body weights of CD-1 mice gestationally exposed to PFOS doses up to 20 mg/kg/day. Zhong et al. (2016, 3748828) measured body weights of C57BL/6 mouse pups that had been exposed to 0, 0.1, 1, or 5 mg/kg/day PFOS *in utero* from GD 1 to GD 17. They did not see significant differences in body weight measurements of male or female mice at 4 and 8 weeks of age. Mshaty et al. (2020, 6833692) also reported no effects on C57BL/6J mouse pup body weight at PND 21 following lactational exposure to 1 mg/kg/day PFOS from PND 1 to PND 14.

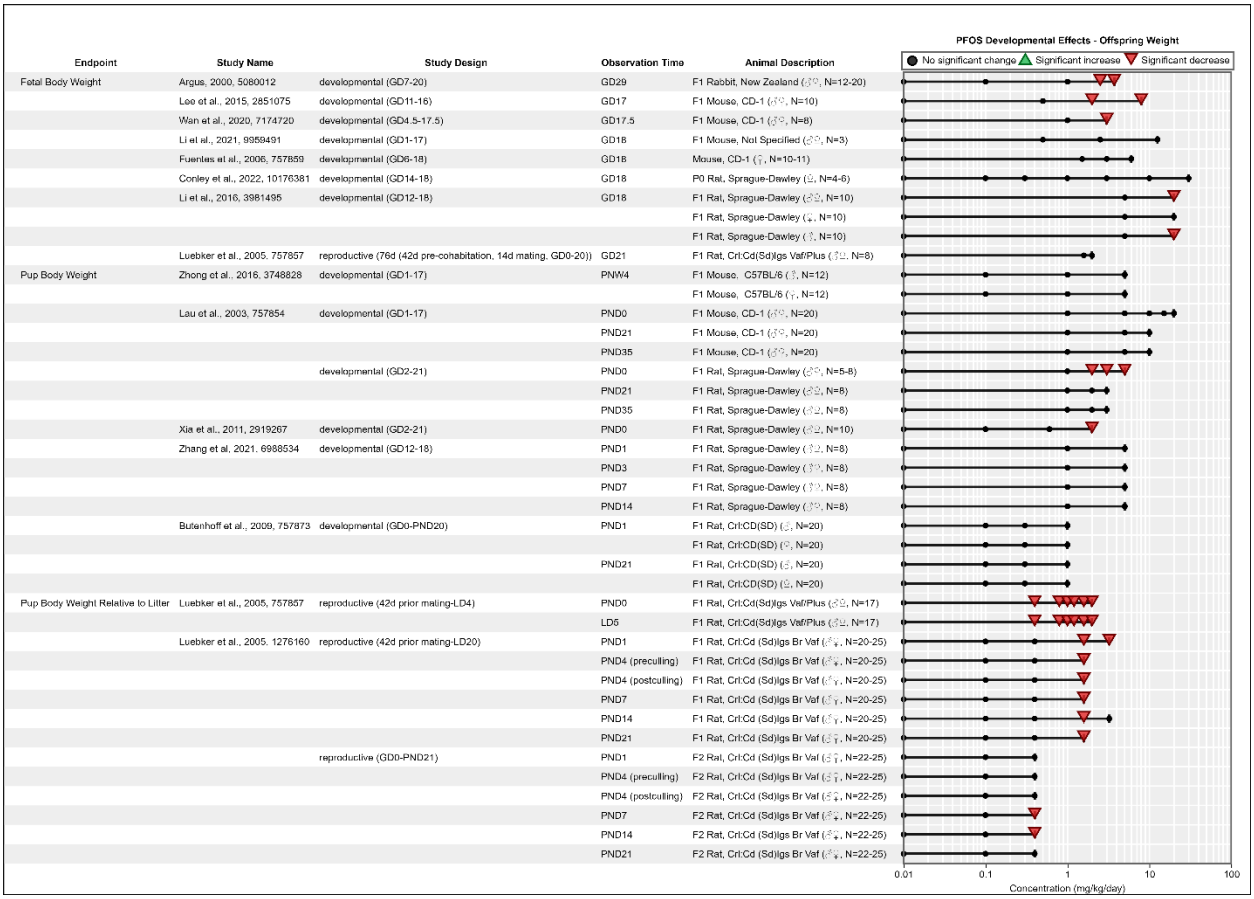


Figure 3-70. Offspring Weight in Mice, Rats, and Rabbits Following Exposure to PFOS (logarithmic scale, sorted by observation time)

PFOS concentration is presented in logarithmic scale to optimize the spatial presentation of data.
Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; LD = lactational day; F_1 = first generation; F_2 = second generation; d = day.

3.4.4.2.5 Placenta

Placental endpoints were reported in six studies with rats, mice, or rabbits. Li et al. (2016, 3981495) reported a significant decrease in mean placental weight in Sprague-Dawley rat dams exposed to 20 mg/kg/day PFOS from GD 12 to GD 18 relative to control (442.8 mg vs. 480.4 mg). No significant difference in placental weights was detected in dams exposed to 5 mg/kg/day PFOS relative to control (455.1 mg vs. 480.4 mg). At ≥ 0.5 mg/kg/day, Lee et al. (2015, 2851075) observed significant decreases in mean absolute placental weight (185.63, 177.32, 163.22, and 151.54 mg at 0, 0.5, 2, and 8 mg/kg/day, respectively) and placental capacity (ratio of fetal weight/placental weight; 9.3, 8.68, 7.96, and 7.39 at 0, 0.5, 2, and 8 mg/kg/day, respectively) in mice exposed to PFOS from GD 11 to GD 16 and sacrificed at GD 17. In the same study, microscopic evaluation revealed necrotic changes and dose-dependent decreases in the frequency of glycogen trophoblast cells and sinusoidal trophoblast cells at dose levels ≥ 2.0 and ≥ 0.5 mg/kg/day, respectively {Lee, 2015, 2851075}. Li et al. (2021, 9959491) dosed mouse dams (strain not specified) with 0, 0.5, 2.5, or 12.5 mg/kg/day PFOS from GD 1 to GD 17 and observed smaller placental diameter in the 12.5 mg/kg/day group compared to controls, though the statistical significance of that effects is unclear. Wan et al. (2020, 7174720) found no effects

on absolute or relative placenta weight, junctional zone area, labyrinth zone area, or the ratio of labyrinth to junctional zone area in CD-1 mice exposed to 0, 1, or 3 mg/kg/day PFOS from GD 4.5 to GD 17.5. Argus Research Laboratories (2000, 5080012) did not observe any placental effects in exposed rabbits and Luebker et al. (2005, 757857) observed no changes in placental size, color, or shape in exposed rats.

3.4.4.2.6 Postnatal Development

Gestational PFOS exposure is associated with effects on postnatal development. Lau et al. (2003, 757854) observed delayed eye opening in rats and mice following developmental exposure to PFOS. A significant, treatment-related delay in eye opening was reported in mice following gestational exposure to PFOS (eye opening at PND 14.8 in control vs. Eye opening at PND 15.1, PND 15.5, and PND 15.6 at 1, 5, and 10 mg/kg/day, respectively). The NOAEL for delays in eye opening in rats was 1 mg/kg/day PFOS. Mshaty et al. (2020, 6833692) evaluated age at eye opening in mice exposed to 1 mg/kg/day from PND 1 through PND 14 and found no significant effects. A two-generation reproduction study in rats {Luebker, 2005, 1276160} evaluated various developmental landmarks in the F_1 offspring and observed significant delays in pups attaining pinna unfolding, eye opening, surface righting, and air righting in the 1.6 mg/kg/day dose group. Eye opening was also slightly, but significantly, delayed in pups exposed to 0.4 mg/kg/day.

Developmental PFOS exposure also had adverse effects on lung development, further described in the Respiratory Section of the PFOS Appendix (Section C.7).

3.4.4.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse developmental outcomes is discussed in Section 3.3.4 of the 2016 PFOS HESD (EPA, 2016, 3603365). There are 33 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to developmental effects. A summary of these studies is shown in Figure 3-71.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	1	0	0	1
Big Data, Non-Targeted Analysis	5	6	4	14
Cell Growth, Differentiation, Proliferation, Or Viability	7	0	15	19
Cell Signaling Or Signal Transduction	5	1	5	10
Extracellular Matrix Or Molecules	0	0	1	1
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	3	1	2	6
Hormone Function	2	0	1	2
Inflammation And Immune Response	0	1	1	2
Oxidative Stress	1	1	3	5
Xenobiotic Metabolism	1	0	2	3
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	13	7	16	33

Figure 3-71. Summary of Mechanistic Studies of PFOS and Developmental Effects

Interactive figure and additional study details available on Tableau.

Mechanistic data available from *in vitro*, *in vivo*, and epidemiological studies were evaluated to inform the mode of action of developmental effects of PFOS. Outcomes included early survival, general development, and gross morphology; fetal growth and placental effects; metabolism; lung development; hepatic development; testes development; cardiac development; and neurological development.

3.4.4.3.1 Early Survival, General Development, Gross Morphology

Mechanisms through which PFOS exposure may alter survival and development were studied in several zebrafish embryo bioassay studies. Several of these studies identified in the current assessment were included in a recent review of developmental effects of PFOS in zebrafish models {Lee, 2020, 6323794}. In general, PFOS can lead to embryo and/or larva malformation, delays in hatching, and decreases in body length. Wang et al. (2017, 3981383) exposed embryos to 0.2, 0.4, 0.8, or 1.6 mg/L PFOS and observed significant and dose-dependent reductions in hatching rate and heart rate as well as significant increases in mortality and malformations in the spine and swim bladder. The overt effects in general development and gross morphology coincided with dose-dependent increases in reactive oxygen species (ROS), lipid peroxidation, and antioxidant enzyme activity (including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px)). Interestingly, co-exposure of the embryos with PFOS and multi-walled carbon nanotubes (MWCNTs) reduced toxicity in several of these endpoints and attenuated the increase in oxidative stress biomarkers caused by PFOS, suggesting that oxidative stress is a key event that mediates alterations in development and gross morphology following exposure to PFOS. Another zebrafish embryo bioassay conducted by Dang et al. (2018,

4651759) reported that exposure to 0.1, 1, or 10 μM PFOS did not affect hatching and survival rates, but did increase malformation rates by 7%, possibly due to downregulation of the growth hormone/insulin-like growth factors (GH/IGFs) axis. Blanc et al. (2019, 5413062) determined the lethal/effect concentrations (LC/ECs) for zebrafish embryos at 96-hours post-fertilization (hpf). The 50% lethal effect concentration (LC_{50}) was 88 μM, which is lower than the previously determined value of 109 μM by Hagenaars et al. (2011, 1279113). The 10% lethal effect concentration (LC_{10}) was 35 μM and was used in subsequent experiments to explore mechanisms that may contribute to the developmental toxicity at the transcriptional and epigenetic level, which are described in the Section below {Blanc, 2019, 5413062}. Lastly, Chen et al. (2014, 2540874) found that PFOS exposure of zebrafish embryos led to several malformations, including uninflated swim bladder, underdeveloped gut, and curved spine, which paralleled histological alterations in the swim bladder and gut. To complement the functional data, the authors examined differential gene expression by microarray analysis, which revealed upregulated genes involved in nucleic and macromolecule metabolism, cell differentiation and proliferation, neuron differentiation and development, and voltage-gated channels. Genes that were downregulated were associated with cellular protein metabolic processes, macromolecular complex assembly, protein-DNA complex assembly, and positive regulation of translation and multicellular organism growth. The authors also used the genomic data to identify the top predicted developmental toxicity pathways initiated by PFOS exposure, including Peroxisome Proliferator-Activated Receptor alpha (PPARα)-mediated pathways, decreases of transmembrane potential of mitochondria and mitochondrial membrane, and cardiac necrosis/cell death.

Two *in vitro* studies by Xu et al. (2013, 2968325; 2015, 2850066) examined the effects of PFOS on changes in mouse embryonic stem cell (mESC) pluripotency markers, which control normal cell differentiation and development. Xu et al. (2013, 2968325) found that PFOS exposure did not affect cell viability. However, PFOS exposure decreased mRNA and protein levels of the pluripotency markers Sox2 and Nanog, but not Oct4. They also measured several miRNAs, including miR-145 and miR-490-3p, which can regulate Sox2 and Nanog, and found them to be increased, supporting the epigenetic mechanisms of control of these markers. In Xu et al. (2015, 2850066), cell differentiation effects on mouse embryoid bodies (mEBs) were examined. eBs are formed when embryonic stem cells spontaneously differentiate into the three germ cell layers, mimicking early gastrulation. The authors found that mEB formation was unaffected by PFOS, but that PFOS exposure increased the mRNA and protein levels of the previously studied pluripotency markers (Oct4, Sox2, and Nanog); this is notably a reversal of the findings from their previous study in mESCs {xu, 2013, 2968325}. Xu et al. (2015, 2850066) found that PFOS exposure in mEBs decreased differentiation markers (Sox17, FOXA2, SMA, Brachyury, Nestin, Fgf5), as well as Polycomb group (PcG) proteins and several miRNAs also involved in differentiation. These alterations could disturb the dynamic equilibrium of embryonic differentiation and induce developmental toxicity. Altogether, the results suggest that PFOS exposure can disturb the expression of pluripotency factors that are essential during early embryonic development, potentially via miRNA dysregulation, which may reflect mechanisms of toxicity that are relevant during a critical window of embryonic development.

Global epigenetic changes in response to PFOS exposure were measured in several studies, including in one zebrafish study and two epidemiological studies. Blanc et al. (2019, 5413062) found that PFOS induced global DNA hypermethylation, minor alterations in gene expression of several epigenetic factors (including DNA methylation, histone deacetylation, and histone

demethylation factors) following PFOS exposure. Moreover, the genes encoding the DNA methyltransferase *dnmt3ab* and the H3K4 histone demethylase *kdm5ba* were significantly downregulated. H3K4 methylation is associated with open, transcriptionally active regions and depleted of DNA methylation. The authors did not measure methylation patterns on H3K4 or other histones; to confirm alterations to H3K4 methylation status, additional studies are required.

In cord blood samples from a Japanese birth cohort study, Miura et al. (2018, 5080353) measured PFOS levels in tandem with epigenetic modifications during fetal development. The authors found significant associations between global hypermethylation and PFOS exposure. The top differentially methylated regions (DMRs) of the genome that were associated with PFOS exposure included hypermethylation of CpG sites of *CYP2E1*, *SMAD*, and *SLC17A9*; however, the authors did not measure the expression level of these genes to confirm the effect of the epigenetic alterations. In contrast, another study of human cord blood samples conducted by Liu et al. (2018, 4926233) found that PFOS exposure was associated with low methylation of Alu retrotransposon family in cord blood DNA samples, indicating global hypomethylation. Demethylation of Alu elements has been proposed to induce insertion and/or homologous recombination and cause alterations to genomic stability and, subsequently, gene transcription. In another study of human cord blood samples, PFOS exposure was associated with DNA methylation changes at key CpG sites associated with genes in pathways important for several physiological functions and diseases, including nervous system development, tissue morphology, digestive system development, embryonic development, endocrine system development, cancer, eye disease, organ abnormalities, cardiovascular disease, and connective tissue disorders {Leung, 2018, 4633577}.

Lastly, in a study of human cord blood in a prospective cohort in China, PFOS exposure was associated with significantly shorter leukocyte telomere lengths and increased ROS in female newborns. Interestingly, the effects were not observed in male newborns, suggesting sex-specific effects in early-life sensitivity to PFOS exposure at the molecular level. The authors determined that the effect of PFOS on shortened leukocyte telomere length was partially mediated through ROS in females, indicating a programing role of PFOS on telomere length during gestation {Liu, 2018, 4239494}.

3.4.4.3.2 Fetal Growth and Placental Development

Growth was measured in developing zebrafish larvae in three studies. Wang et al. (2017, 3981383), reported a dose-dependent reduction in body length that coincided with dose-dependent increases in ROS generation, lipid peroxidation, and the activities of antioxidant enzymes in larvae exposed to 0.2, 0.4, 0.8, or 1.6 mg/L PFOS. Reduction in body length was likely due to PFOS-related increased oxidative stress and lipid peroxidation. In Jantzen et al. (2016, 3860114), the morphometric endpoints of interocular distance, total body length, and yolk sac area were measured in zebrafish embryos. PFOS exposure significantly decreased all three parameters relative to controls, indicating slowed embryonic development, at values 5- to 25-fold below previously calculated LC_{50} values. The authors found alterations in the expression of several genes involved in development, including calcium ion binding (*calm3a*), cell cycle regulation (*cdkn1a*), aromatic compound metabolism (*cyp1a*), and angiogenesis (*flk1*), as well as increased *tfc3a* (muscle development) expression and decreased *ap1s* (protein transport). Lastly, Dang et al. (2018, 4651759) found that PFOS significantly inhibited body length and growth of larvae. This appeared to be mediated through the growth hormone/insulin-like growth factor

(GH/IGF) axis, as several GH/IGF axis genes had decreased expression, including the genes *gro*wth hormone releasing hormone (*ghrh*), growth hormone receptors a and b (*ghra* and *ghrb*), insulin-like growth factor 1 receptor a and b (*igf1ra* and *igf1rb*), insulin-like growth factor 2 receptor (*igf2r*), insulin-like growth factor 2a (*igf2a*), and insulin-like growth factor binding protein 2a and 2b (*igfbp2a* and i*gfbp2b*).

In three *in vivo* rodent studies, fetal growth and placental disruption in response to maternal PFOS exposure were measured. In a mouse study, Lee et al. (2015, 2851075) reported a relationship between gene expression of prolactin-family hormones and placental and fetal outcomes following maternal exposure to 0, 0.5, 2.0, or 8.0 mg/kg/day PFOS from GD 11–16 via gavage. Dose-dependent increases in placental histopathological lesions and reductions in placental weights, fetal weights, and number of live fetuses were significantly correlated with reductions in gene expression of mouse placental lactogen (*mPL-II*), prolactin-like protein Cα (*mPLP-Cα*), and prolactin-like protein K (*mPLP-K*). Given the alterations in prolactin-family gene expression, the authors propose that this placental disruption is related to endocrine (i.e., prolactin) dysfunction. Li et al. (2016, 3981495) also found that maternal PFOS exposure reduced fetal and placental weight, which coincided with increased corticosterone in fetal serum. In the placenta, activity of 11b-hydroxysteroid dehydrogenase 2, and expression of several genes involved in development (i.e., extracellular matrix, growth factors and hormones, ion transporters, signal transducers, and structural constituents) were downregulated, suggesting intrauterine growth restriction was related to altered placental development and functionality. Li et al. (2020, 6833703) also found that PFOS exposure was associated with reduced placental size in mice and proposed that the disruption was mediated by the dysregulation of a long non-coding RNA, H19 which plays a role in regulation of embryonic growth {Monnier, 2013, 10439067}, which was altered in placental tissues (i.e., hypomethylation of the H19 promoter and increased expression of the gene). *In vitro* experiments in human placental trophoblast cells (HTR-8/sVneo) provided further support for a mechanism involving H19; cell growth that was inhibited by PFOS was partially alleviated following suppression of H19 via transfection with si-H19 {Li, 2020, 6833703}.

Sonkar et al. (2019, 5918797) also used HTR-8/sVneo cells to evaluate the epigenetic mechanisms through which PFOS exposure adversely effects the placenta. The authors reported increased ROS production, possibly due to alterations of several DNA methyltransferases and sirtuins, which consequently led to a reduction in global DNA methylation and increased protein lysine acetylation. The authors propose that ROS production could lead to pregnancy complications, such as preeclampsia and intrauterine growth restrictions.

In a human placental choriocarcinoma cell line (JEG-3), PFOS exposure was found to induce placental cell cytotoxicity and inhibition of aromatase activity {Gorrochategui, 2014, 2324895}. In Yang et al. (2016, 3981458), 0.1 μM PFOS inhibited decidualization of the first trimester human decidual stromal cells (collected from the uterine lining). PFOS also downregulated 11-hydroxysteroid dehydrogenase 1 (11β-HSD1), an enzyme that converts the inactive form of cortisol to the active form of cortisol, and inhibited the glucocorticoid-driven reduction of the proinflammatory cytokines IL-6 and IL1-β, which could result in a reduced immune-tolerance environment in early pregnancy. In human amnion and fetal lung cells exposed to PFOS *in vitro*, PFOS exposure upregulated the gene expression of Caspase3 and apoptotic peptidase activating

factor 1 (*APAF1)*, genes that initiate apoptosis. This effect was concentration (between 10^{-4} and 10^{-6} M PFOS) and time-dependent (between 24 and 48 hours) {Karakas-Celik, 2014, 2850400}.

Lastly, in humans, Ouidir et al. (2020, 6833759) recruited pregnant women and measured plasma PFOS levels during the first trimester of the pregnancy and examined global methylation in the placenta at birth. The authors found significant associations between PFOS exposure and DNA methylation changes in the placenta, and the associated downregulation of certain genes, particularly the reduced gene expression of several genes associated with anthropometry parameters such as shorter birth length, reduced birth weight, and reduced head circumference that were previously associated with PFAS exposure {Buck, 2018, 5016992}. These data suggest that the prenatal toxicity of PFOS might be driven by epigenetic changes in the placenta {Ouidir, 2020, 6833759}.

3.4.4.3.3 Metabolism

Metabolomic profiles in relation to PFOS exposure were analyzed in humans in two studies. In a cross-sectional study in 8-year-old children in Cincinnati, OH, the authors conducted untargeted, high-resolution metabolomic profiling in relation to serum PFOS concentrations. They found that PFOS exposure was associated with several lipid and dietary factors, including arginine, proline, aspartate, asparagine, and butanoate metabolism {Kingsley, 2019, 5405904}. In a study of mothers that were part of the Child Health and Development Studies (CHDS) cohort, maternal serum was analyzed for PFOS as well as underwent metabolomics profiling to determine if metabolic alterations reflected in measurements from maternal serum could possibly contribute to later health outcomes in their children {Hu, 2019, 5412445}. PFOS exposure was associated with a distinct metabolic profile, including a positive association with urea cycle metabolites and a positive association with carnitine shuttle metabolites. This profile indicates disruption of fatty acid metabolism, which could possibly cause developmental alterations in offspring {Hu, 2019, 5412445}.

3.4.4.3.4 Lung Development

In a human fetal lung fibroblast cell line (Hel299), PFOS exposure upregulated the expression of *Caspase3* and *Apaf1*, genes that initiate apoptosis. This effect was dose and time-dependent {Karakas-Celik, 2014, 2850400}. These results indicate that PFOS can cause *in vitro* toxicity (via apoptotic mechanisms) in embryonic cells, possibly affecting the development.

3.4.4.3.5 Hepatic Development

Liang et al. (2019, 5412467) studied the effects of developmental exposure to PFOS on metabolic liver function in Kunming mice, in post-natal day 1 offspring. They found that PFOS exposure during gestation increased liver triglycerides, total cholesterol, and low density lipoprotein (LDL), and decreased high density lipoprotein (HDL) in the offspring. The mRNA of several factors involved in fatty acid oxidation, update, and hepatic export of livers were altered, indicating developmental perturbation of lipid metabolic function. These *in vivo* results show that PFOS may disrupt hepatic lipid metabolism through negative effects on hepatocellular lipid trafficking in mice developmentally exposed to PFOS.

3.4.4.3.6 Cardiac Development

Several *in vitro* studies examined developmental toxicity of PFOS using embryonic stem cell-derived cardiomyocytes (ESC-CMs) as a model of the early stages of heart development {Cheng,

2013, 2850971; Zhou, 2017, 3981356; Zhang, 2016, 3981565; Tang, 2017, 3981359; Liu, 2020, 6833698; Yang, 2020, 6315676}. Most of the studies utilized mouse ESC-CMs but one study, Yang et al. (2020, 6315676), used a human ESC-CM model of cardiac differentiation. Cardiac differentiation was inhibited in PFOS-treated mouse ESC-CMs, shown by a concentration-dependent decrease in the contract positive rate (i.e., percentage of beating embryoid bodies) on differentiation days 8–10 {Cheng, 2013, 2850971; Zhou, 2017, 3981356; Zhang, 2016, 3981565; Tang, 2017, 3981359} and a decreased proportion of α-actinin-positive cells (a marker of cardiomyocytes) on differentiation day 10 {Zhang, 2016, 3981565; Tang, 2017, 3981359}. The median inhibition of differentiation (ID_{50}), defined as the concentration at which PFOS inhibited the development of contracting cardiomyocytes by 50%, ranged from 40 µM (Zhang et al. (2016, 3981565) to 73 µM {Zhou, 2017, 3981356}. Collectively, these results provide *in vitro* evidence of potential developmental cardiotoxicity following PFOS exposure.

Several *in vitro* studies have demonstrated that PFOS can significantly alter gene and protein expression at multiple time points during differentiation of cardiomyocytes from mouse or human ESCs, specifically for genes in the myosin heavy chain, myosin light chain, and cardiac troponin T families. In human ESC-CMs, 0.1–60 µM PFOS significantly inhibited the expression of cardiac-specific homeobox gene Nk2 homeobox 5 (*NKX2.5*), myosin heavy chain 6 (*MYH6)*, and myosin light chain 7 (*MYL7)*, and significantly reduced protein levels of NKX2.5 and cardiac troponin T2 (TNNT2) on day 8 and/or day 12 of differentiation {Yang, 2020, 6315676}. In mouse ESC-CMs, on differentiation day 5, PFOS (20–40 µM) reduced gene and protein levels of Brachyury (mesodermal marker), cardiac transcription factors GATA binding protein 4 (GATA4), and myocyte enhancer factor 2C (MEF2C) {Zhang, 2016, 3981565}. On differentiation days 9–10, PFOS reduced the expression of *Myh6* and *Tnnt2* (i.e., *cTnT*) in a dose-dependent manner from 2.5 to 160 µg/mL PFOS {Cheng, 2013, 2850971; Zhou, 2017, 3981356}. Cheng et al. (2013, 2850971) found that PFOS significantly altered the chronological order of gene expression during *in vitro* cardiogenesis. Expression of important cardiac genes were significantly lower in PFOS-treated cells compared to controls on day 9, but expression of *Nkx2.5* and *Mlc1a* were significantly higher in PFOS-treated cells by day 14 of differentiation {Cheng, 2013, 2850971}.

Proteomic analysis during cardiac differentiation of mouse ESCs revealed 176 differentially expressed proteins (67 upregulated and 109 downregulated) {Zhang, 2016, 3981565}. The differentially expressed proteins were mainly associated with catalytical activity, protein binding, nucleotide binding, and nucleic acid binding. PFOS significantly affected 32 signaling pathways, with metabolic pathways the most affected. The PPAR signaling pathway and mitogen-activated protein kinase (MAPK) signaling pathways were also significantly affected by PFOS.

Yang et al. (2020, 6315676) studied global gene expression during cardiac differentiation of human ESCs exposed to 60 µM PFOS. Their analysis revealed 584 differentially expressed genes (247 upregulated and 337 downregulated) on differentiation day 8, and 707 differentially expressed genes (389 upregulated and 318 downregulated) on differentiation day 12. In total, 199 genes were affected on both days 8 and 12. The majority of affected genes are related to extracellular matrix and cell membrane. Seven Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were affected by PFOS on both days (mostly neural-related pathways and a few general pathways), but cardiac pathways were not greatly affected. PFOS downregulated cardiac markers such as natriuretic peptide A (*NPPA)*, natriuretic peptide B (*NPPB)*, *NKX2.5*,

MYH6, *MYL2*, and *MYH7*, but upregulated epicardial markers WT1 transcription factor (*WT1*) and T-box transcription factor 18 (*TBX18*). Wingless-related integration site (WNT) signaling pathway-related genes (secreted frizzled related protein 2 (*SFRP2)* and frizzled-related protein (*FRZB*)) and IGF signaling pathway genes (*IGF2* and IGF binding protein 5 (*IGFBP5)*) were significantly upregulated in PFOS-treated cells. The authors postulated that PFOS stimulated differentiation to epicardial cells more than to cardiomyocytes by stimulating the WNT signaling pathway.

Mouse ESC cardiac differentiation assays have demonstrated that exposure to PFOS can cause mitochondrial toxicity in these cells. In contrast, one study in human ESCs-derived cardiomyocytes {Yang, 2020, 6315676} found that PFOS did not affect mitochondrial integrity on day 12 of differentiation.

Cheng et al. (2013, 2850971) found that PFOS reduced ATP production, increased accumulation of ROS, and stimulated apoptosis in mouse ESC-CMs. However, Tang et al. (2017, 3981359) demonstrated that PFOS decreased intracellular ATP and lowered mitochondrial membrane potential in mouse ESC-CMs without inducing apoptosis. Exposure to PFOS during cardiac differentiation also caused structural damage to mitochondria (e.g., swelling, vacuolar structure, loss of cristae) and the mitochondria-associated endoplasmic reticulum membrane (MAM). Furthermore, PFOS increased intracellular lactate production, fatty acid content, and disrupted calcium fluxes. Analysis of protein expression demonstrated that destruction of the MAM structure occurred along with activation of Rictor/mTORC2 signaling pathway via phosphorylation of epidermal growth factor receptor, which led to accumulation of intracellular fatty acid and resulted in blocking of the $[Ca^{2+}]_{mito}$ transient.

The mechanisms behind PFOS mitochondrial toxicity were further explored by Liu et al. (2020, 6833698) who found that PFOS-treated ESC-CMs displayed autophagosome accumulation accompanied by increased levels of p62 and ubiquitinated proteins, increased lysosomal pH, and decreased the levels of lysosome-associated membrane protein (Lamp2a) and the mature form of Cathepsin D (lysosomal protease), suggesting an impairment of autophagy-lysosome degradation. PFOS also blocked mitophagy, the removal of damaged mitochondria through autophagy, thereby disrupting the balance between mitophagy and biogenesis {Liu, 2020, 6833698}. The authors postulated that the mechanism of PFOS-induced toxicity to ESC-CMs involves reduced lysosomal acidification, inhibited maturation of cathepsin D, blocked fusion between lysosomes and autophagosomes, accumulation of autophagosomes, and dysfunctional mitochondria.

One study included in the prior 2016 PFOS HESD {U.S. EPA, 2016 3603365} investigated cardiac mediated apoptosis in weaned rats exposed to PFOS (0, 0.1, 0.6, or 2 mg/kg/day) on GD 2-21 {Zeng, 2014, 2851284}. The pups were sacrificed at the end of the lactation period, and trunk blood and the heart were recovered. Apoptotic cells in the heart tissue from six animals per dose group were measured using a Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining assay. PFOS exposure was associated with a dose dependent increase in the percentage of TUNEL positive nuclei. The 0.6 mg/kg/day dose was the LOAEL and the 0.1 mg/kg/day dose the NOAEL. The researchers found that biomarkers for apoptosis were supportive of the TUNEL results. The expression of BCL2-associated X protein and cytochrome c were upregulated and bcl-2 downregulated. The concentration of caspase 9 was significantly

increased above the control levels at all doses and caspase 3 levels were significantly increased for all but the lowest dose level.

3.4.4.3.7 Testicular Development

Two rat studies examined PFOS effects on testicular development. Zhang et al. (2013, 1598626) isolated primary Sertoli cells and gonocytes from 5-day-old rat pups and created a Sertoli cell/gonocyte coculture system to mimic *in vivo* interactions. PFOS exposure reduced cell viability and induced ROS production in a concentration-dependent manner, although PFOS did not appear to increase apoptosis. PFOS exposure altered and inhibited the cytoskeletal proteins vimentin and F-actin in Sertoli cells, indicating PFOS could adversely affect developing testes via ROS and cytoskeleton disruption. Li et al. (2018, 4241058) examined the effects of PFOS on pubertal Leydig cell development, both *in vitro* and *in vivo*. *In vitro*, PFOS inhibited androgen secretion via the downregulation of 17b-hydroxysteroid dehydrogenase 3 (HSD17B3, gene *Hsd18b3*), as measured by *Hsd18b3* mRNA expression. PFOS also promoted apoptosis of immature Leydig cells *in vitro* but did not affect cell proliferation. *In vivo*, PFOS exposure reduced serum testosterone levels, and reduced sperm production. LHCGR, CYP11A1, and CYP17A1 levels in Leydig cells were reduced, suggesting that PFOS exposure downregulates critical Leydig cell gene expression, indicating delayed maturation of these cells.

3.4.4.3.8 Neurological Development

PFOS effects on neurodevelopment and behavior in zebrafish were examined in two studies. In the zebrafish embryo assay by Jantzen et al. (2016, 3860114), embryonic exposure to PFOS resulted in hyperactive locomotor activity in larvae, possibly mediated through altered expression of development-associated genes (*calm3a, cdkn1a, cyp1a, flk1, tfc3a*, and *ap1s*). Stengel et al. (2018, 4238489) developed a neurodevelopmental toxicity test battery in zebrafish embryos and evaluated the effect of PFOS exposure. Although PFOS exposure had significant adverse effects on neuromast cells, including degeneration, no changes were observed in the olfactory or retinal toxicity assays.

Rat embryonic neural stem cells (NSCs) were used to examine the effects of PFOS on neuronal and oligodendrocytic differentiation. PFOS exposure at 25 or 50 nM reduced cell proliferation but showed increased protein levels in markers associated with differentiation (TuJ1, CNPase). Exposure also reduced the number of cells with spontaneous calcium activity. These effects appeared to be mediated through PPAR pathways, as indicated by increases in *PPARγ* and the downstream target *UCP2*. Results were confirmed using a PPARγ agonist that showed similar effects in the cells. This study also evaluated effects of PFOS exposure on the PPAR system *in vivo*. In PFOS-treated neonatal mice, *PPARγ* and *UCP3* were up-regulated in brain cortical tissue {Wan Ibrahim, 2013, 2919149}.

Lastly, Leung et al. (2018, 4633577) conducted a genome-wide methylation study on mothers and infants from the Faroese birth cohort study, which has been extensively studied for associations between neurodevelopmental deficits in children exposed to various chemicals, including PFAS. In cord blood samples from males, PFOS exposure was significantly associated with 10,598 methylation changes in CpG sites, 15% of which were enriched in cytobands of the X chromosome associated with neurological disorders. Other CpG sites were associated with genes in pathways of key physiological functions and diseases, including nervous system development, tissue morphology, digestive system development, embryonic development,

endocrine system development, cancer, eye disease, organ abnormalities, cardiovascular disease, and connective tissue disorders. The same effects were not observed in cord blood from females.

3.4.4.3.9 Conclusion

The available mechanistic studies suggest that the developing liver, developing heart, and placenta may be affected by PFOS at the molecular level (i.e., differential methylation of genes, gene expression changes, mitochondrial dysregulation), which may be related to developmental health effects described in Sections 3.4.4.1 and 3.4.4.2. Some effects tend to vary by sex or by developmental timepoint of outcome evaluation (e.g., early gastrulation, late gestation, lactation). Oxidative stress in parallel with epigenetic alterations in the placenta were consistently reported.

3.4.4.4 Evidence Integration

The evidence of an association between PFOS and developmental effects in humans is *moderate* based on the recent epidemiological literature. As noted in the epidemiological fetal growth restriction summary, there is evidence that PFOS may impact fetal growth restriction in humans. Several meta-analyses also support evidence of associations between maternal or cord blood serum PFOS and BWT or BWT-related measures {Verner, 2015, 3150627; Negri, 2017, 3981320; Dzierlenga, 2020, 7643488; Cao, 2021, 9959525; Yang, 2022, 10176603} (Table A-41, PFOS Appendix A). Comparing the postnatal growth results in infants with birth-related measures is challenging due to complex growth dynamics including rapid growth catch-up periods for those with fetal restriction. Nonetheless, the evidence for postnatal weight deficits was comparable to that seen for BWT. Overall, there was inconsistent evidence of PFOS impacts on rapid growth measures, postnatal height and postnatal adiposity measures up to age 2. There was less evidence available in recent studies of PFOS exposure for other endpoints such as fetal loss and birth defects. The evidence for an association between PFOS exposure and cryptorchidism or hypospadias were primarily negative but overall inconsistent. In contrast, there was fairly consistent evidence of an impact of PFOS exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) as the majority of studies showed some adverse associations. Several meta-analyses also show associations between PFOS and preterm birth {Deji, 2021, 7564388; Gao, 2021, 9959601l; Yang, 2022, 10176603} (Table A-41, PFOS Appendix A).

As noted previously there is some uncertainty as to what degree the available evidence may be impacted by pregnancy hemodynamic and sample timing differences across studies, as this may result in either confounding or reverse causality {Steenland, 2018, 5079861}. Additional uncertainty exists due to the potential for confounding by other PFAS. Very few of the existing studies performed multipollutant modeling in comparison with single pollutant estimates of PFOS associations. The results were often mixed from those that did this with some estimates increasing and some decreasing although PFOS was rarely chosen amongst dimension-reducing statistical approaches from models with various PFAS and or other environmental contaminants. There is some concern that controlling for other highly correlated co-exposures in the same model may amplify the potential confounding bias of another co-exposure rather than removing it {Weisskopf, 2018, 7325521}. Given these interpretation difficulties and potential for this co-exposure amplification bias, it remains unclear whether certain mutually adjusted models give a more accurate representation of the independent effect of specific pollutants for complex PFAS mixture scenarios.

The animal evidence for an association between PFOS exposure and developmental toxicity is *moderate* based on 16 *medium* confidence animal toxicological studies. Dose-dependent maternal and offspring effects were reported in mice, rats, and rabbits; however, a few studies did not observe effects. The studies evaluated demonstrate that PFOS exposure is associated with various developmental toxicity endpoints including increased mortality (pup mortality, fetal death, stillbirth, abortion), decreased body weight or body weight change (fetal, pup, and maternal), skeletal and soft tissue effects, and delayed eye opening. The most consistent effects observed across studies were decreased maternal body weight (encompassing decreases in maternal body weight and maternal body weight change), decreased offspring weight during the perinatal developmental period (encompassing fetal weight and pup weight prior to weaning), and increased mortality (encompassing abortion, stillbirth, fetal death, and pup mortality).

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

Similarly, Luebker et al. (2005, 757857; 2005, 1276160) observed decreased pup weights as an average per litter at lower dose levels than effects on viability endpoints including decreases in implantations, increased number of dams with all pups dying, and decreased number of live pups per litter. These results are supported by Lau et al. (2003, 757854) who found significant decreases in rat pup body weight at birth and increases in pup mortality in the first 24–48 hours after birth. Significant reductions in both endpoints occurred at the same dose of 2 mg/kg/day. A final study {Lee, 2015, 2851075} also observed increased fetal death and decreased fetal weight. However, in this study, increased incidence of fetal death was statistically significant at all dose levels whereas fetal weight was not affected at the lowest dose of 0.5 mg/kg/day.

The mechanistic data are primarily focused on gene expression changes and epigenetic alterations related to exposure to PFOS during developmental stages. PFOS-induced alterations to the expression of genes related to growth and development supports the observations in animals and humans (e.g., fetal growth restriction). Molecular alterations (primarily epigenetic alterations) were also measured in human cord blood and were related to PFOS levels in the same biological samples. Global hypomethylation, a marker of genomic instability, was associated with PFOS exposure, as was hypermethylation of genes related to xenobiotic metabolism. Another study in human cord blood reported changes in DNA methylation at genomic sites associated with genes related to normal development of several tissue and organ systems (e.g., nervous system development and endocrine system development, among others). However, the authors of these studies did not measure gene expression changes to confirm the epigenetic alterations affected the transcriptome, nor did the authors report any adverse post-natal effects to which to anchor the epigenetic alterations. In addition to human data, mechanistic data related to developmental effects and PFOS have been collected *in vivo* in zebrafish and rodent studies, as well as in human and rodent *in vitro* models. In zebrafish embryos exposed to PFOS, changes in genes that are related to growth and development (e.g., growth factors, among

others) were observed along with growth inhibition, decreased hatch rate, embryonic malformations, and other metrics of development, indicating that PFOS-induced effects on growth and development are related to alterations to the transcriptome of developing zebrafish. Alterations to individual genes or pathways that are also seen in tissues from adult animals in laboratory studies (e.g., PPAR and markers of apoptosis in the liver, or cardiac-specific pathways) were observed in developing animals and/or embryonic cell lines. Alterations to the epigenome were observed in several animal toxicological studies, including in the placenta of pregnant rodents exposed to PFOS. Such alterations occurred at the global and gene-specific levels, indicating that epigenetic regulation of normal development can be altered by PFOS exposure. Overall, there is *robust* evidence of an impact of PFOS exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) as most of the studies showed some adverse associations. This was strengthened by consistency in the reported magnitude of gestational age deficits despite different exposure levels and metrics examined. Although they were not as consistent in magnitude (60% of the PTB studies showed some adverse associations), some of the effect estimates were large for preterm birth in relation to PFOS exposures with limited evidence of exposure-response relationships. Few patterns were evident as explanatory factors for heterogeneous results based on our qualitative analysis.

3.4.4.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the available human and animal ***evidence indicates*** that PFOS exposure is likely to cause developmental toxicity in humans under relevant exposure circumstances (Table 3-12). This conclusion is based primarily on evidence of decreased birth weight from epidemiologic studies in which PFOS was measured during pregnancy, primarily with median PFOS ranging from 5.0 to 30.1 ng/mL. The conclusion is supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth and birth length) and consistent findings of dose-dependent decreases in fetal and maternal weight, with the effects observed in animal models gestationally exposed to PFOS at doses as low as 0.4 mg/kg/day. The available mechanistic information provides support for the biological plausibility of the phenotypic effects observed in exposed animals and humans.

Table 3-12. Evidence Profile Table for PFOS Developmental Effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Evidence from Studies of Exposed Humans (Section 3.4.4.1)					⊕⊕⊙ ***Evidence Indicates (likely)***
Fetal growth restriction 20 *High* confidence studies 13 *Medium* confidence studies 10 *Low* confidence studies	Some deficits in mean birth weight were observed in most studies (19/30) in the overall population, but evidence for the exposure-response relationship was limited. Most evidence for deficits in mean birth weight were reported from *high* or *medium* confidence studies (16/27). Studies on changes in standardized birth weight measures reported some inverse associations (9/15) in the overall population or either/both sexes. Seven of 11 studies observed increased risk of low birth weight or SGA. Deficits in birth weight-related measures were supported by in related FGR outcomes such as birth length (11/23) and head circumference (10/19).	*High* and *medium* confidence studies *Coherence* between different measures of FGR *Good* or *adequate sensitivity* in most studies	Limited evidence of exposure-response relationships based on categorical data *Potential bias* due to hemodynamic differences noted in studies using samples from later pregnancy	⊕⊕⊙ *Moderate* Evidence for developmental effects is based on consistent adverse effects for FGR including birthweight measures which are the most accurate endpoint. Some deficits were consistently reported for birth weight and standardized birth weight in many *high* and *medium* confidence cohort studies. Effects on birth weight were supported by findings for other measures of FGR, including birth length and head circumference, and impacts on gestational duration. Some uncertainty due to the potential impact of hemodynamics in later pregnancy due to use of biomonitoring samples from the second and	*Primary basis and cross-stream coherence:* Evidence consisted of decreased birth weight from epidemiologic studies in which PFOS was measured during pregnancy. This is supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth and birth length) and consistent findings of dose-dependent decreases in fetal weight, with the effects observed in animal models gestationally exposed to PFOS. *Human relevance and other inferences:* The available mechanistic information provides support for the biological plausibility of the phenotypic effects observed in exposed animals in support of the human relevance of the animal findings.

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
				third trimester or post-partum.	
Gestational duration 10 *High* confidence studies 5 *Medium* confidence studies 5 *Low* confidence studies	Some associations with gestational age measures in the overall population were observed (9/15), with most (7/9) considered *high* or *medium* confidence. Increased risk of preterm birth was also observed in most studies (7/11).	*High* and *medium* confidence studies *Consistency* in the magnitude of gestational age deficits	*Limited number* of studies examining preterm birth		
Fetal loss 2 *High* confidence studies 2 *Medium* confidence studies 1 *Low* confidence study	Increased risk of fetal loss was observed (3/5). One *medium* confidence study reported an inverse association.	*High* and *medium* confidence studies *Good sensitivity* across all studies *Consistent* magnitude of effect *Dose-dependent response*	No factors noted		
Post-natal growth 4 *High* confidence studies 4 *Medium* confidence studies 3 *Low* confidence studies	Most studies (7/8) reported an adverse association for infant weight changes. There was some evidence of a dose-response relationship in two studies (2/4) reporting categorical exposures. Decreases in infant height were only observed in two studies (2/4). Results for BMI and adiposity were mixed with studies reporting both increased (2/6) and decreased (3/6)	*High* and *medium* confidence studies *Dose-dependent response* *Good* or *adequate sensitivity* for most studies	*Inconsistent* timing of follow-up evaluation		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
	risk for adverse adiposity outcomes.				
Birth defects 3 *Medium* confidence studies 2 *Low* confidence studies	One *low* confidence study observed a small increased risk for total or combined birth defects. One *medium* confidence study reported increased risk for septal defects, conotruncal defects, and total congenital heart defects, but results were imprecise. Cryptorchidism was examined in three studies. Of the two *medium* confidence studies, one reported a non-significant inverse association and the other reported a null association.	*Medium* confidence studies	*Low* confidence studies *Imprecision* of some positive associations may suggest statistical power was limited *Limited number* of studies examining individual defects		
Evidence from *In Vivo* Animal Studies (Section 3.4.4.2)					
Maternal body weight 12 *Medium* confidence studies	Maternal body weight and/or body weight gain during gestation and lactation were dose-dependently reduced in several studies in rats, mice, and rabbits (8/12). Remaining studies (4/12) in mice found no effects on maternal body weight	*Medium* confidence studies *Dose-response* relationship	*Inconsistent direction* of effects	⊕⊕⊙ *Moderate* Evidence based on 16 *high* or *medium* confidence animal studies indicates that the developing fetus is a target of PFOS toxicity. Dose-dependent maternal and offspring effects were reported in mice, rats, and rabbits; however, a few studies	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
				did not observe effects. The studies evaluated demonstrate that PFOS exposure is associated with various developmental toxicity endpoints including increased mortality (pup mortality, fetal death, stillbirth, abortion), decreased body weight or body weight change (fetal, pup, and maternal), skeletal and soft tissue effects, and delayed eye opening.	
Offspring body weight 15 *Medium* confidence studies	Fetal body weights were dose-dependently reduced (4/8) in studies in rats, mice, and rabbits. Pup birth weights and/or body weights during lactation were dose-dependently reduced (4/9), with significant effects observed in rats but not mice.	*Medium* confidence studies *Dose-dependent* response	*Inconsistent direction* of effects across species for postnatal body weight		
Offspring mortality 11 *Medium* confidence studies	Increased fetal mortality (2/7) was reported in rats, mice, and rabbits that evaluated endpoints such as abortion, implantation, resorption, and dead/live fetus counts prior to parturition. Two studies exposed female rats prior	*Medium* confidence studies *Consistent direction* of effects *Dose-dependent* response	No factors noted		

Evidence Stream Summary and Interpretation				Evidence Integration Summary Judgment
	to mating through lactation, and the study with higher doses observed decreased number of implantation sites per delivered litter and liveborn litter size, and increased number of stillborn pups per litter (1/2). Four studies began exposure during gestation and allowed natural delivery of litters, and only one (1/4) observed decreased liveborn litter size. No studies reported an effect on sex ratio (percentage of male pups delivered per litter) (0/6). Postnatal survival was dose-dependently decreased in several studies in mice and rats (5/8). For the two studies with exposure prior to mating through lactation, both reported decreased pup viability index and increased numbers of dams with all pups dying in the first 4-5 days postpartum.			
Placental effects 6 *Medium* confidence studies	Decreased placental weight (2/3), decreased placental diameter (1/1), and decreased placental	*Medium* confidence studies *Dose-response* relationship	*Inconsistent direction* of effects *Limited number* of studies examining outcomes	

	Evidence Stream Summary and Interpretation			Evidence Integration Summary Judgment
	capacity (1/1) were observed in rat and mouse studies, but two other studies in rats and rabbits reported normal placental size and appearance. Histopathology was evaluated in two mouse studies; one study observed no changes in the placenta while the other study observed necrotic changes and dose-dependent decreases in trophoblasts.	*Coherence* of findings		
Structural abnormalities 2 *Medium* confidence studies	No external or visceral abnormalities were detected in mouse or rabbit fetuses (2/2). Lower incidence of diminished calcaneus ossification was observed in mice (1/1) and delayed skeletal ossification was observed in rabbits (1/1).	*Medium* confidence studies	*Limited number* of studies examining outcomes	
Developmental timing and organ maturation 4 *Medium* confidence studies	Delayed eye-opening (2/3) was reported in rats and mice following gestational PFOS exposure. In a two-generation study in rats, delayed pinna unfolding, air righting, and surface	*Medium* confidence studies *Coherence* of effects with other developmental delays	*Limited number* of studies examining outcomes	

Evidence Stream Summary and Interpretation		Evidence Integration Summary Judgment
	righting was also observed (1/1). In contrast, eye opening in mice exposed from PND 1-14 was unaffected (pup body weight was also unaffected in that study). In general, the studies that observed developmental delays also reported growth deficits and decreased viability during the lactation period. PFOS exposure from GD 12-18 affected lung development and maturation in rats when observed on PND 1-14 (1/1).	

Mechanistic Evidence and Supplemental Information (Section 3.4.4.3)

Summary of Key Findings, Interpretation, and Limitations	Evidence Stream Judgement
Key findings and interpretation: Evidence from zebrafish embryo assays demonstrate that PFOS exposure can lead to embryo and/or larva malformation and delays/reduction in hatching. Alterations to the expression of genes related to growth and development *in vivo* in zebrafish and rodents, and in human embryonic cell lines. Alterations to DNA methylation (global hypomethylation and gene-specific hypermethylation) in human cord blood and in placenta from rodent studies. **Limitations:** The role of epigenetic mechanisms in changes at the mRNA level is not clear, nor is the relationship between molecular changes and apical developmental outcomes.	The evidence demonstrates that PFOS exposure during development can alter the epigenome and the expression of genes that control regular growth and development; it is possible that such changes are related, although the relationship has not been directly measured.

Notes: SGA = small-for-gestational age; FGR = fetal growth restriction; PND = postnatal day; GD = gestational day; BMI = body mass index; DNA = deoxynucleic acid; mRNA = messenger ribonucleic acid.

3.4.5 Evidence Synthesis and Integration for Other Non-Cancer Health Outcomes

Consistent with the SAB's recommendation, EPA concluded that the non-cancer health outcomes with the strongest evidence are hepatic, immune, cardiovascular and developmental. For all other health outcomes (e.g., reproductive and endocrine), EPA concluded that the epidemiological and animal toxicological evidence available at this time from the preliminary scoping considered in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonate (PFOS) (CASRN 1763-23-1) in Drinking Water* is either *suggestive* of associations or *inadequate* to determine associations between PFOS and the health effects described. Based on this analysis, these outcomes were not prioritized for the MCLG assessment and the evidence synthesis and integration for these other outcomes are presented in the PFOS Appendix. In addition, Section 6.5 further describes rationale for evidence integration judgments for health outcomes which EPA determined had *evidence suggestive* of associations between PFOS and related adverse health effects, though the databases for those health outcomes shared some characteristics with the *evidence indicates* judgment.

3.5 Cancer Evidence Study Quality Evaluation, Synthesis, Mode of Action Analysis and Weight of Evidence

EPA identified 15 epidemiological and 1 animal toxicological study that investigated the association between PFOS and cancer. Of the epidemiological studies, 8 were classified as *medium* confidence, 6 as *low* confidence, and 1 was considered *uninformative* (Section 3.5.1). The single animal toxicological study was considered a *high* confidence study (Section 3.5.2). Studies have *mixed* confidence ratings if different endpoints evaluated within the study were assigned different confidence ratings. Though *low* confidence studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.5.1 Human Evidence Study Quality Evaluation and Synthesis

3.5.1.1 Introduction

There are 7 studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and cancer effects. Study quality evaluations for these 7 studies are shown in Figure 3-72.

The 2016 Health Effects Support Document for PFOS {U.S. EPA, 2016, 3603365} concluded that there was no evidence of carcinogenic effects for PFOS, but that the small number, breadth, and scope of the studies were not adequate to make definitive conclusions. Although an elevated risk of bladder cancer mortality was observed in an occupational study of workers at the 3M Decatur, Alabama plant {Alexander, 2003, 1291101}, a subsequent study to ascertain cancer incidence in the cohort observed elevated but non-significant incidence ratios that were 1.7- to 2-fold higher among exposed workers {Alexander, 2007, 4727072}. Mean PFOS serum levels were 94.1 ng/mL. In the same 3M cohort, {Grice, 2007, 4930271} observed that prostate, melanoma, and colon cancer were the most frequently reported malignancies. When cumulative exposure measures were analyzed, elevated odds ratios were reported for melanoma, colon, and

prostate cancer, however, they did not reach statistical significance. Length of follow-up may not have been adequate to detect cancer incidence in this cohort as approximately one-third of the participants had worked < 5 years in their jobs, and only 41.7% were employed ≥ 20 years.

No elevated bladder cancer risk was observed in a nested case-control study in a Danish cohort with plasma PFOS concentrations at enrollment ranging 1–130.5 ng/mL {Eriksen, 2009, 2919344}. Elevated non-significant ORs for prostate cancer were reported for the occupational cohort examined by Alexander and Olsen (2007, 4727072) and the Danish population-based cohort examined by Eriksen et al. (2009, 2919344), and no association was reported by another case-control study in Denmark {Hardell, 2014, 2968084}. A case-control study of breast cancer among Inuit females in Greenland with similar serum PFOS levels to those of the Danish population (1.5–172 ng/mL) reported an association of low magnitude that could not be separated from other perfluorosulfonated acids, and the association was not confirmed in a Danish population {Bonefeld-Jørgensen, 2011, 2150988; Bonefeld-Jørgensen, 2014, 2851186}. Some studies evaluated associations with serum PFOS concentration at the time of cancer diagnosis and the impact of this potential exposure misclassification on the estimated risks is unknown {Bonefeld-Jørgensen, 2011, 2150988; Hardell, 2014, 2968084}. No associations were adjusted for other perfluorinated chemicals in serum in any of the occupational and population-based studies.

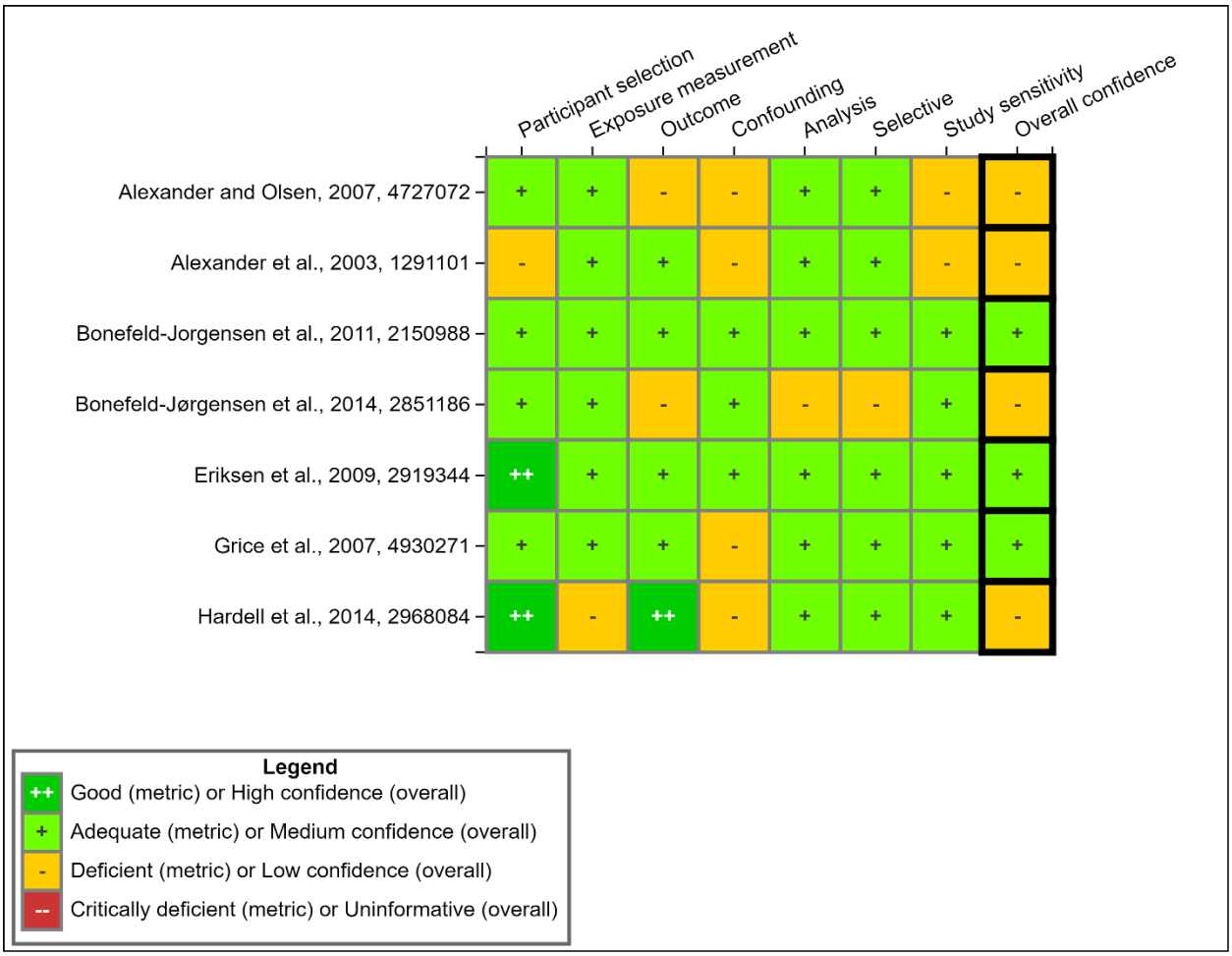


Figure 3-72. Summary of Study Evaluation for Pre-2016 Epidemiology Studies of PFOS and Cancer Effects

Interactive figure and additional study details available on HAWC.

Since publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}, 15 studies have been published that investigated the association between PFOS and cancer (see PFOS Appendix). All studies were conducted on the general population with one in a high-exposure community (i.e., C8 population). Different study designs were also used including two cohort studies {Fry, 2017, 4181820; Li, 2022, 9961926}, five case-control studies {Wielsoe, 2017, 3858479; Tsai, 2020, 6833693; Lin, 2020, 6835434; Itoh, 2021, 9959632; Liu, 2021, 10176563}, five nested case-control studies {Ghisari, 2017, 3860243; Hurley, 2018, 5080646; Cohn, 2020, 5412451; Mancini, 2020, 5381529; Shearer, 2021, 7161466}, and three cross-sectional studies {Christensen, 2016, 3858533; Ducatman, 2015, 3859843; Omoike, 2021, 7021502}. The studies were conducted in different study populations including populations from China {Lin, 2020, 6835434; Liu, 2021, 10176563}, Denmark {Ghisari, 2017, 3860243}, France {Mancini, 2020, 5381529}, Greenland {Wielsoe, 2017, 3858479}, Japan {Itoh, 2021, 9959632}, Sweden {Li, 2022, 9961926}, Taiwan {Tsai, 2020, 6833693}, and the United States {Fry, 2017, 4181820; Christensen, 2016, 3858533; Ducatman, 2015, 3859843; Shearer, 2021, 7161466; Hurley, 2018, 5080646; Cohn, 2020, 5412451; Omoike, 2021, 7021502}. All the studies measured PFOS in

study subject's blood components (i.e., serum or plasma) with one study measuring the levels in the maternal serum {Cohn, 2020, 5412451}. Cancers evaluated included breast {Cohn, 2020, 5412451; Ghisari, 2017, 3860243; Hurley, 2018, 5080646; Itoh, 2021, 9959632; Li, 2022, 9961926; Mancini, 2020, 5381529; Omoike, 2021, 7021502; Tsai, 2020, 6833693; Wielsoe, 2017, 3858479}, germ cell tumors {Lin, 2020, 6835434}, kidney {Shearer, 2021, 7161466}, melanoma {Li, 2022, 9961926}, ovarian {Omoike, 2021, 7021502}, prostate {Ducatman, 2015, 3859843; Omoike, 2021, 7021502}, thyroid {Liu, 2021, 10176563} uterine {Omoike, 2021, 7021502}, and any cancer {Christensen, 2016, 3858533; Fry, 2017, 4181820; Li, 2022, 9961926}.

3.5.1.2 Study Quality

There are 15 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and cancer effects. Study quality evaluations for these 15 studies are shown in Figure 3-73.

Of the 15 studies identified since the 2016 assessment (Figure 3-73), seven were considered *medium* confidence and six were *low* confidence {Christensen, 2016, 3858533; Itoh, 2021, 9959632; Lin, 2020, 6835434; Liu, 2021, 10176563; Omoike, 2021, 7021502; Tsai, 2020, 6833693}. One study conducted in the high exposure to PFAS Ronneby Register Cohort in Sweden was *uninformative* {Li, 2022, 9961926} because of concerns about exposure assessment and lack of data on important covariates. One study conducted in Greenland was considered *uninformative* { Wielsoe, 2017, 3858479} because of concerns about exposure assessment and participant selection, As a result, these two studies will not be further considered in this review. Concerns with the *low* confidence studies included the possibility of outcome misclassification, confounding or potential selection bias. Residual confounding was also a concern, including lack of considering co-exposures by other PFAS, and lack of appropriately addressing SES (SES) and other life-style factors, which could be associated with both exposure and cancer outcome. Although PFOS has a long half-life in the blood, concurrent measurements may not be appropriate for cancers with long latencies. Temporality of exposure measure in terms of cancer development wase noted to be an issue in several *low* confidence studies {Tsai, 2020, 6833693; Itoh, 2021, 9959632; Liu, 2021, 10176563; Omoike, 2021, 7021502}. Many of the *low* confidence studies also had sensitivity issues due to limited sample size.

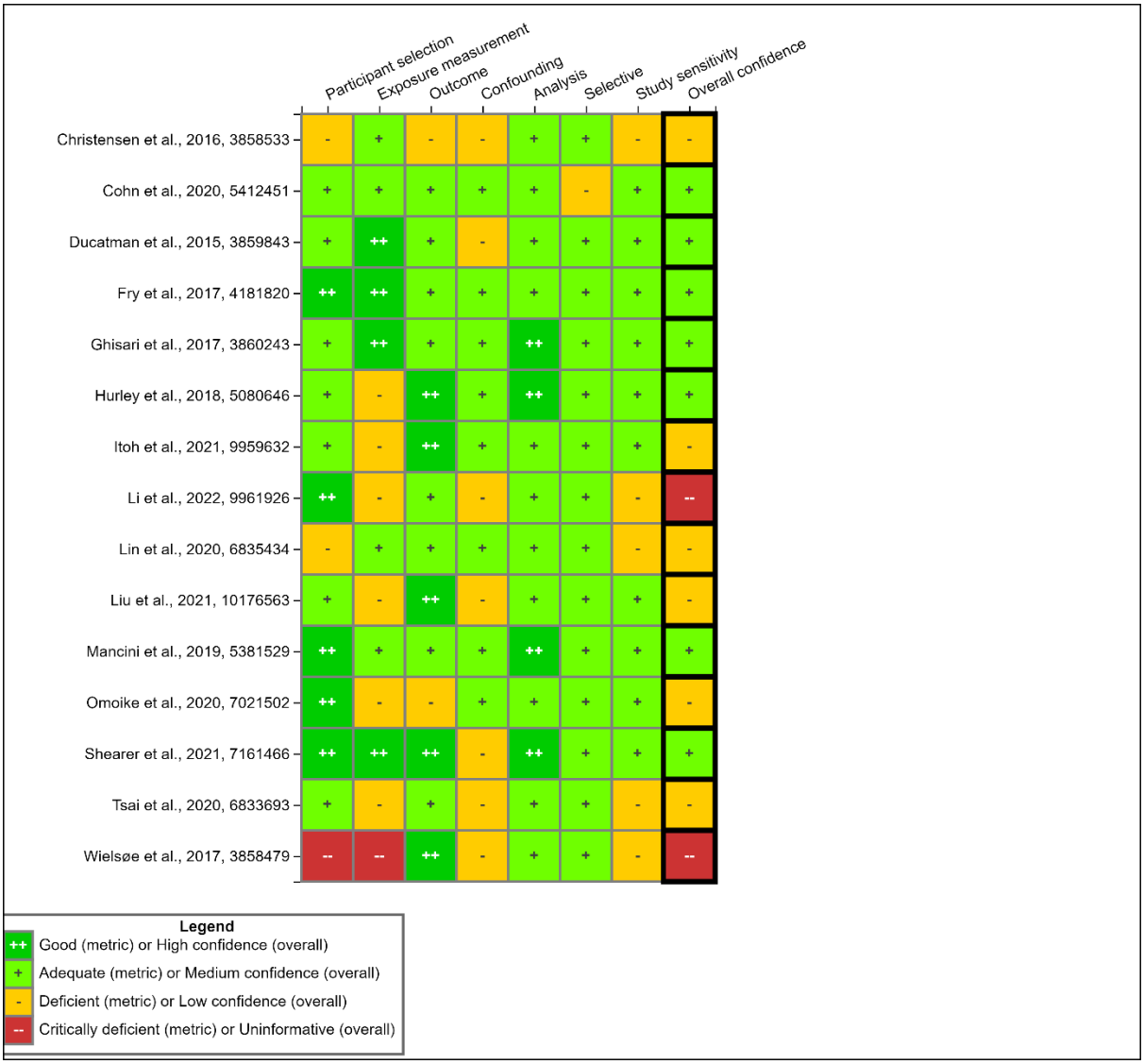


Figure 3-73. Summary of Study Evaluation for Epidemiology Studies of PFOS and Cancer Effects

Interactive figure and additional study details available on HAWC.

3.5.1.3 Findings from Children

One *low* confidence study examined cancers in children {Lin, 2020, 6835434} and reported a statistically significant higher median PFOS concentration in 42 pediatric germ cell tumor cases compared with 42 controls in blood samples collected from the children one week after diagnosis. However, the study did not observe an increased risk of germ cell tumors when evaluated on a per ng/mL increase in blood PFOS.

3.5.1.4 Findings from the General Adult Population

PFOS was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma) in a *medium* confidence study {Shearer, 2021, 7161466}. A case-control study nested within the

National Cancer Institute's (NCI) Prostate, Lung, Colorectal, and Ovarian Screening Trial, reported a statistically significant positive trend in risk of renal cell carcinoma with pre-diagnostic serum levels of PFOS (OR = 2.51; 95% CI: 1.28, 4.92 for the highest *vs.* lowest quartiles; p-trend = 0.009, or per doubling of PFOS: OR: 1.39; 95% CI: 1.04, 1.86) {Shearer, 2021, 7161466}. Although the trend was significant across quartiles, the effect in the third quartile was null (OR = 0.92; 95% CI: 0.45, 1.88). Additionally, the association with PFOS was attenuated after adjusting for other PFAS (OR = 1.14; 95% CI: 0.45, 2.88 for the highest *vs.* lowest quartiles; p-trend = 0.64), and it was lower in the third quartile than in the second quartile, indicating potential confounding by correlated PFAS exposures. There was no association when evaluated on a per doubling of PFOS after adjusting for other PFAS.

Seven general population studies published since the 2016 assessment, evaluated PFOS and risk for breast cancer {Cohn, 2020, 5412451; Ghisari, 2017, 3860243; Hurley, 2018, 5080646; Itoh, 2021, 9959632; Mancini, 2020, 5381529; Omoike, 2021, 7021502; Tsai, 2020, 6833693} with mixed results. All studies were case-control studies (with some nested case-controls), except for one cross-sectional NHANES-based study {Omoike, 2021, 7021502}. Three studies were considered *low* confidence {Itoh, 2021, 9959632; Omoike, 2021, 7021502; Tsai, 2020, 6833693} because of concerns about temporality of exposure measurements and breast cancer development, the control status was not confirmed via examination or medical records {Tsai, 2020, 6833693}, and potential for residual confounding due to SES, life-style factors and other PFAS. The remaining studies were all *medium* confidence. A nested case-control study did not observe an association between breast cancer identified through California cancer registry and PFOS concentrations in serum after case diagnosis, max PFOS concentration of 99.8 ng/mL {Hurley, 2018, 5080646}. A nested case-control study in a prospective (pregnancy) cohort study, the CHDS, suggested that maternal PFOS was associated with a decreased daughters' breast cancers risk in the first or fourth quartile of TC {Cohn, 2020, 5412451}, but the study did not examine breast cancer subtypes or genetic variants. Two nested case-control studies and one *low* confidence case-control study found associations between PFOS and breast cancer, but only in specific groups of participants {Ghisari, 2017, 3860243; Mancini, 2020, 5381529; Tsai, 2020, 6833693}. Ghisari et al. (2017, 3860243) reported an increased risk for breast cancer identified from the cancer registry with increasing PFOS concentrations only in participants with a CC genotype (n = 36 cases and 47 controls) in the CYP19 gene (cytochrome P450 aromatase). A nested case-control study (194 pairs of breast cancer cases and controls) within the French E3N cohort found an 86% higher risk of breast cancer in the 2nd and 3rd quartiles of PFOS (13.6–17.3 ng/mL, and 17.3–22.5 ng/mL) compared to the 1st quartile (5.8–13.6 ng/mL) (OR = 1.94; 95% CI: 1.00, 3.78, and OR = 2.03; 95% CI: 1.02, 4.04) in the full adjusted model{Mancini, 2020, 5381529}. Mancini et al. (2020, 5381529) reported that the risk for breast cancer (93% verified pathologically confirmed from medical records after self-reported cancer diagnosis) varied by type of cancer with a statistically significant increasing trend in estrogen receptor positive (ER+) and progesterone receptor positive (PR+) breast cancers. The study also observed a significant increase in estrogen receptor- (ER-) and progesterone receptor- (PR-) breast cancers in the second quartile with elevated risks also observed in the other quartiles, but with no trend. The sample size was small with 26 participants having ER- breast cancers and 57 having PR- breast cancers.

One *low* confidence study {Tsai, 2020, 6833693} conducted in Taiwan observed a statistically significant increase in risk of breast cancer with increasing log transformed PFOS, but only in

participants aged 50 years or younger and in ER+ breast cancer in participants aged 50 years or younger. Statistically significant increased odds of breast cancer were also observed in a *low* confidence NHANES study (2005–2012) {Omoike, 2021, 7021502} both per ng/mL increase in PFOS (OR = 1.011; 95% CI: 1.011, 1.011) and in the two highest quartiles of exposure. The association was significantly inverse in the second quartile compared to the lowest (OR = 0.87; 95% CI: 0.86, 0.89). One *low* confidence case-control study conducted in Japanese women {Itoh, 2021, 9959632} observed a significant inverse association across serum PFOS quartiles with a significant dose-response trend (p-value < 0.0001) (see PFOS Appendix). Median PFOS levels ranged from 7.6 ng/mL in the lowest quartile to 24.67 ng/mL in the highest quartile. The association remained significantly inverse in both pre- and postmenopausal women in the highest tertile of exposure, with a significant dose-response trend (p-values for trend = 0.007 and 0.001, respectively).

One *medium* confidence study based on the C8 Health Project {Ducatman, 2015, 3859843} examined prostate-specific antigen (PSA) as a biomarker for prostate cancer in adult males over age 20 years who lived, worked, or went to school in one of the six water districts contaminated by the DuPont Washington Works facility. No association was observed between PSA levels in either younger (i.e., aged 20–49 years) or older (i.e., aged 50–69 years) men and concurrent mean serum PFOS concentrations up to 25 ng/mL. In an NHANES population, Omoike et al. {2021, 7021502} observed a significantly inverse association with prostate cancer (OR = 0.994; 95% CI: 0.994, 0.994).

Omoike et al. (2021, 7021502) also observed statistically significant increased odds of ovarian cancer both per ng/mL increase in PFOS (OR = 1.012; 95% CI: 1.012, 1.013) and in the two highest quartiles of exposure, although the association was significantly inverse for the second quartile of PFOS exposure (see PFOS Appendix). A significant inverse association also was observed for uterine cancer (OR = 0.945; 95% CI: 0.944, 0.945 per ng/mL increase in PFOS) {Omoike, 2021, 7021502}.

One *low* confidence study conducted in Shandong Province, in eastern China {Liu, 2021, 10176563} observed a statistically significant inverse association with thyroid cancer across quartiles of serum PFOS (p-value for trend = 0.001). The median serum PFOS levels were higher in controls than in cases (7.5 *vs.* 5.5 ng/mL, p-value < 0.001). However, there is some concern about possible reverse causality. The ability to metabolize PFAS could change when the thyroid becomes cancerous, thereby changing the PFAS concentrations. The abnormality of thyroid hormones may also disturb the PFAS levels.

Two studies examined all cancers together, but collected different information on cancer (i.e., incidence verses mortality) and obtained the information using different methods. Cancer mortality based on Public-use Linked Mortality Files was not associated with PFOS exposure in a *medium* confidence study of participants over 60 years of age from NHANES, with median PFOS concentration 4.3 ng/g lipid {Fry, 2017, 4181820}; PFOS also was not found to be associated with self-reported cancer incidence in a *low* confidence study among male anglers over 50 years, median PFOS concentration 19 µg/L ({Christensen, 2016, 3858533}. Christensen, 2016, 3858533 was considered *low* confidence due to the potential of self-selection because participants were recruited from flyers and other methods and filled out an online survey including self-reported outcomes.

3.5.2 Animal Evidence Study Quality Evaluation and Synthesis

There is one study from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} that investigated the association between PFOS and cancer effects. Study quality evaluation for this one study is shown in Figure 3-74.

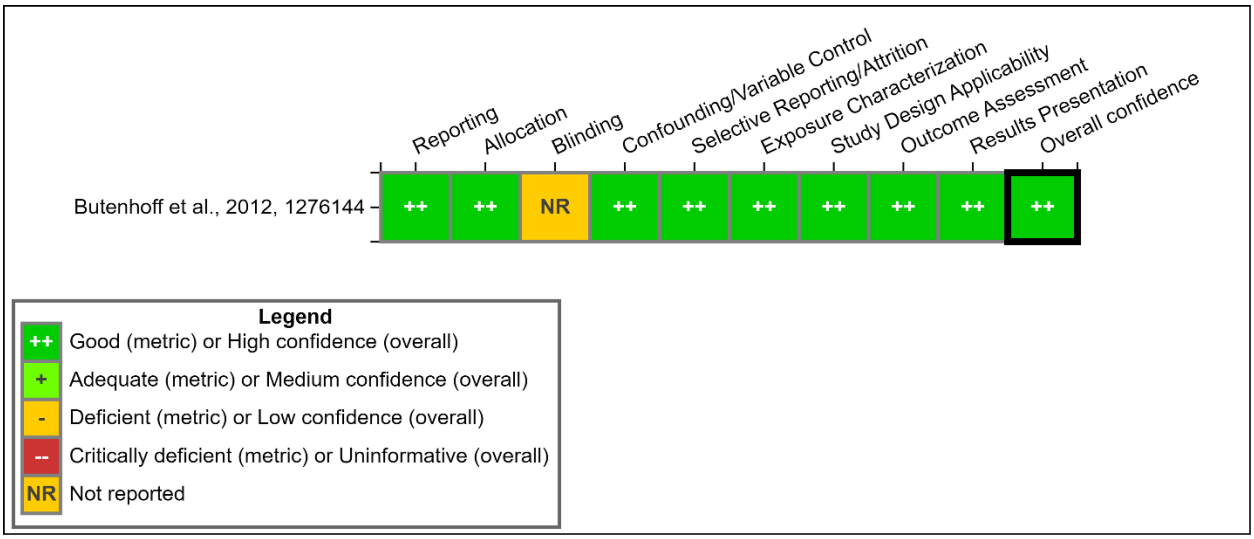


Figure 3-74. Summary of Study Evaluation for Toxicology Studies of PFOS and Cancer Effects

Interactive figure and additional study details available on HAWC.

Table 3-13. Incidences[a] of Hepatocellular and Pancreatic Tumors in Male and Female Sprague-Dawley Rats as Reported by Thomford (2002, 5029075)

Sex	Tumor Type	Treatment group				
		0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
Male	Hepatocellular Adenomas	0/41 (0%)**	3/42 (7%)	3/47 (6%)	1/44 (2%)	7/43 (16%)**
Female	Hepatocellular Adenomas	0/28 (0%)**	1/26 (4%)	1/15 (7%)	1/28 (4%)	5/31 (16%)*
Female	Hepatocellular Carcinomas	0/28 (0%)	0/29 (0%)	0/16 (0%)	0/31 (0%)	1/32 (3%)
Female	Combined Hepatocellular Adenomas and Carcinomas	0/28 (0%)**	1/29 (3%)	1/16 (6%)	1/31 (3%)	6/32 (19%)*
Male	Pancreatic Islet Cell Adenomas	4/44 (9%)	3/45 (7%)	4/48 (8%)	4/46 (9%)	4/44 (9%)
Male	Pancreatic Islet Cell Carcinomas	1/38 (3%)*	2/41 (5%)	2/44 (5%)	5/44 (11%)	5/40 (13%)
Male	Combined Pancreatic Islet Cell Adenomas and Carcinomas	5/44 (11%)	5/45 (11%)	6/48 (13%)	8/46[a] (17%)	9/44 (20%)

Notes:
*Statistically significant compared to the control group at $p \leq 0.05$. **Statistically significant compared to the control group at $p \leq 0.01$. Denoted significance for the control groups indicate statistically significant trends.
[a] Tumor incidence is expressed as the number of animals with tumors over the number of animals alive at the time of first occurrence of the tumor.

In addition to hepatocellular tumors, Thomford et al. (2002, 5029075) reported increased incidences of pancreatic islet cell carcinomas in males (Table 3-13). Though the slight increases in the number of animals with carcinomas in the 5 and 20 ppm dose groups were not statistically different from the control group, there was a statistically significant trend of increased incidence with increased dose.

Thyroid and mammary gland tumors were also observed but did not exhibit linear dose-response {Thomford, 2002, 5029075; Butenhoff, 2012, 1276144}. The most frequent thyroid tumor type in females was C-cell adenomas, but the highest incidence was that for the controls and there was a lack of dose-response among the exposed groups. There was also a high background incidence in mammary gland tumors in the female rats, primarily combined fibroma adenoma and adenoma, but the incidence lacked dose-response for all tumor classifications.

3.5.3 Mechanistic Evidence

Mechanistic evidence linking PFOS exposure to adverse cancer outcomes is discussed in Section 3.4.3 of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. There are 26 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the mechanisms of action of PFOS that lead to cancer effects. A summary of these studies is shown in Figure 3-75.

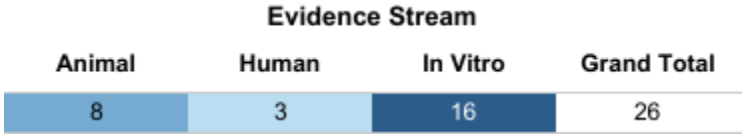


Figure 3-75. Summary of Mechanistic Studies of PFOS and Cancer Effects

Interactive figure and additional study details available on Tableau.

In 2016, ten key characteristics of carcinogens were selected by a multi-disciplinary working group of the International Agency for Research on Cancer (IARC), based upon common empirical observations of chemical and biological properties associated with human carcinogens (i.e., Group 1 carcinogens as determined by IARC) {Smith, 2016, 3160486}. In contrast to the "Hallmarks of cancer" as presented by Hanahan and Weinberg {Hanahan, 2022, 10164687; Hanahan, 2011, 758924; Hanahan, 2000, 188413}, the key characteristics focus on the properties of human carcinogens that induce cancer, not the phenotypic or genotypic traits of cancers. The ten key characteristics provide a framework to systematically identify, organize, and summarize mechanistic information for cancer hazard evaluations {Smith, 2016, 3160486}.

To aid in the evaluation of the carcinogenic potential of PFOS, the studies containing mechanistic data were organized by the proposed key characteristics of carcinogens for the following section. Evidence related to seven of the ten key characteristics of carcinogens was identified in the literature included in this assessment: 'Is Genotoxic', 'Induces Epigenetic Effects', 'Induces Oxidative Stress', 'Modulates Receptor Mediated Effects', 'Alters Cell

Proliferation, Cell Death, and Nutrient Supply', 'Is Immunosuppressive', and 'Induced Chronic Inflammation'. No studies from the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and recent systematic literature search and review efforts were identified for the following key characteristics: 'Is Electrophilic or Can Be Metabolically Activated to Electrophiles', 'Alters DNA Repair and Causes Genomic Instability', and 'Causes Immortalization'.

3.5.3.1 Key Characteristic #2: Is Genotoxic

Genotoxicity is a well-studied mode of action for carcinogens, defined as alterations to DNA through single or double strand breaks, alterations to DNA synthesis, and DNA adducts, all of which can result in chromosomal aberrations, formation of micronuclei, and mutagenesis if not effectively repaired.

3.5.3.1.1 Mutagenicity

3.5.3.1.1.1 *In Vivo* Evidence

Male *gpt* delta transgenic mice, a strain that was designed to facilitate the quantification of point mutations and deletions, were exposed to PFOS (4 and 10 mg/kg/day) for 28 days {Wang, 2015, 2850220}. The mutation frequencies at the targeted *redBA* and *gam* loci in the liver of exposed male mice were increased at concentrations of 4 and 10 mg/kg/day relative to controls, but the increase was not significant, and the variance of the high dose group was relatively large. The evidence for mutagenicity of PFOS *in vivo* is negative based on this single study (Table 3-14).

3.5.3.1.1.2 *In Vitro* Evidence

Several studies have demonstrated that PFOS is not mutagenic *in vitro* (Table 3-15). Of the four publications that tested PFOS for mutagenicity in *Salmonella typhimurium, Saccharomyces cerevisiae*, and *Escherichia coli* {Litton Bionetics, Inc., 1979, 10228135; Mecchi, 1999, 10228133; Simmon, 1978, 10228131; NTP, 2019, 5400978}, no evidence of DNA mutagenesis has been described in the presence or absence of metabolic activation. In contrast, Wang et al. (2015, 2850220) exposed *gpt* delta transgenic mouse embryonic fibroblast cells to PFOS and found concentration-dependent increases in mutation frequencies at the *redBA/gam* loci, a region often used to determine point mutations and deletions.

3.5.3.1.2 DNA Damage

3.5.3.1.2.1 *In Vivo* Evidence

Evaluations of PFOS exposure in rat, mouse, and zebrafish models were identified, which predominantly demonstrated evidence of genotoxicity (Table 3-16). The majority of studies presented data on potential micronuclei formation in bone marrow, peripheral blood, and/or the liver, though some also reported different metrics of DNA damage. It is important to note that rat models could be ineffective for determining micronucleus formation if study authors do not use appropriate methodologies because the spleen will remove micronucleated cells {Schlegel, 1984, 10368697}. However, this would generally bias studies towards the null, not result in false positives.

Particular methods such as flow cytometry can be used to effectively identify micronucleated cells prior to splenic removal {Dertinger, 2004, 10328871}. For instance, NTP (2019, 5400978) reported using flow cytometry to analyze micronuclei formation in immature polychromatic erythrocytes from the peripheral blood of male and female Sprague Dawley rats treated with

0.312–5 mg/kg/day PFOS by gavage for 28 days. No effects on the number of micronucleated polychromatic erythrocytes (PCEs) were observed in males, though there was a significant increase in the number of PCEs in the 5 mg/kg/day females. Importantly, NTP (2019, 5400978) noted that while there was a statistically significant trend for increasing micronucleated PCEs, and that the response in the 5 mg/kg/day group was statistically significant compared to controls indicating a positive test, the response was nonetheless within the range of historical control levels. NTP (2019, 5400978) also reported that there were significant dose-dependent decreases in the percentage of PCEs in the peripheral blood of both males and females, suggesting that PFOS exposure may induce bone marrow toxicity.

Three other studies published by the same primary authors also reported the induction of micronuclei formation in male or female Swiss Albino rats {Çelik, 2013, 2919161; Eke, 2016, 2850124; Eke, 2017, 3981318}. These studies used a valid fluorescence microscopy-based technique, though they did not use the OECD recommended number of cells according to OECD Guideline Test No. 474: Mammalian Erythrocyte Micronucleus Test (at least 500 erythrocytes for bone marrow and at least 2,000 erythrocytes for peripheral blood) {Dertinger, 2004, 10328871}. Çelik et al. (2013, 2919161) found that oral treatment with PFOS (≤2.5 mg/kg/day) administered every other day for 30 days induced genetic damage as measured with the comet assay, as well as the formation of micronuclei in female rat bone marrow samples. However, the study also demonstrated that PFOS exposure decreased the ratio of PCEs to normochromic erythrocytes (NCEs), similar to the results from NTP (2019, 5400978). These findings indicate that the genetic damage may be a result of bone marrow toxicity rather than direct genotoxicity of PFOS. Two subsequent studies in male rats using the same exposure paradigm (30-day exposure administered every other day) found similar results. Eke and Çelik (2016, 2850124) reported increased micronuclei formation and genetic damage indices (calculated using results of a comet assay) in peripheral blood while Eke et al. (2017, 3981318) reported increased micronuclei formation and genetic damage indices in liver tissue. Notably, these two studies did not report the ratio of PCEs to NCEs which limits the ability to interpret these data further. Based on the results from Çelik et al. (2013, 2919161) and considering the similarities in study design, it is reasonable to assume that the genetic damage observed may be due to bone marrow or hepatic toxicity.

Micronuclei frequency was higher in the bone marrow male *gpt* delta transgenic mice exposed to PFOS (4 and 10 mg/kg/day) for 28 days than in controls; however, these results were not statistically significant {Wang, 2015, 2850220}. This is a potential contradiction to previous findings reported in the EPA's 2016 HESD {U.S. EPA, 2016, 3603365} that found mouse bone marrow micronuclei assays to be negative after high dose acute exposures (approximately 24, 48, and 72 hours) to PFOS {Murli, 1996, 10228098}.

In another study, male and female zebrafish embryos were exposed to PFOS concentrations of 0.4, 0.8, or 1.6 mg/L for 30 days {Du, 2014, 2851143}. Following exposure, Du et al. (2014, 2851143) found significant dose-dependent increases in micronucleus formation. Du et al. (2014, 2851143) also reported increases in the number of DNA single-strand breaks, though none of the PFOS doses tested resulted in significant effects. Notably, the high dose exposure resulted in increased rates of developmental malformations, which could potentially confound these results.

Subchronic 28-day exposure of Sprague Dawley rats to PFOS did not alter micronuclei formation in reticulocytes in exposed males, while data derived from exposed female rats was equivocal {NTP, 2019, 5400978).

3.5.3.1.2.2 *In Vitro* Evidence

3.5.3.1.2.2.1 Chromosomal aberrations

EPA's 2016 HESD {U.S. EPA, 2016, 3603365} reports that PFOS exposure did not induce chromosomal aberrations in human lymphocytes (Table 3-17) {Murli, 1999, 10228132}. No new studies were identified that measure chromosomal aberrations after PFOS exposure in the updated literature search.

3.5.3.1.2.2.2 DNA Synthesis

A study by Cifone (1999, 10228136) evaluated the effects of 15 different PFOS concentrations ranging from 0.25 µg/mL to 4,000 µg/mL in Fisher 344 male rat hepatocytes. No evidence of increased DNA synthesis was observed, denoted by the lack of elevated mean net nuclear grains. Cytotoxicity significantly increased at approximately 50 µg/mL.

An additional study, detailed elsewhere, noted increased DNA synthesis (increased cells in S phase) following exposure in rodent hepatocytes. For additional information, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.1.2.2.3 DNA Damage

Several assays of DNA damage have been performed on a variety of *in vitro* models (Table 3-17). Wang et al. (2015, 2850220) exposed gpt delta transgenic mouse embryonic fibroblasts to PFOS and found evidence of concentration-dependent increase in phosphorylated histone H2AX (γ-H2AX), a biomarker of DNA double strand breaks (DSBs), after exposure to 1 or 20 µM PFOS (no statistical analysis was reported). Direct exposure of suspended calf thymus DNA to 10 µM PFOS for 30 minutes modified DNA structure, attenuated DNA charge transport, and led to PFOS-DNA adduct formation {Lu, 2012, 2919198}.

In contrast, several studies found no evidence of DNA damage after exposure. Jacquet et al. (2012, 2919219) exposed Syrian hamster embryos to PFOS (≤50 µg/mL) and found no evidence of DNA damage by a comet assay. Similarly, there was no evidence of DNA damage via a comet assay in the protist species *Paramecium caudatum* exposed to 10–100 µM for 24 hours {Kawamoto, 2010, 1274162}.

Florentin et al. (2011, 2919235) exposed HepG2 cells to PFOS (5–300 µM) for 1 or 24 hours. There was no evidence of DNA damage in a comet assay nor change in micronucleus frequency at any concentration or time point. However, within the 24-hour exposure assay, significant cytotoxic effects were noted at 300 µM. In contrast, a study conducted by Wielsoe et al. (2015, 2533367) exposed HepG2 cells to PFOS (2×10^{-7} to 2×10^{-5} M) for 24 hours and used a comet assay to measure DNA damage. Following exposure, the cells demonstrated a dose-dependent increase in DNA damage at all tested concentrations.

Table 3-14. Mutagenicity Data from *In Vivo* Studies

Reference	Species, Strain (Sex)	Tissue	Results	PFOS Concentration (Dosing Regimen)
Wang et al. (2015, 2850220)	Mouse, *Gpt* delta transgenic (Male)	Liver	Negative	1–10 mg/kg/day (daily via gavage for 28 days)

Table 3-15. Mutagenicity Data from *In Vitro* Studies

Reference	Cell Line or Bacterial Strain	Results		Concentration (Duration of exposure)
		S9-Activated	**Non-Activated**	
Litton Bionetics, Inc. (1979, 10228135)	*Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100)	Negative	Negative	0.1 – 1,000 µg/plate
Litton Bionetics, Inc. (1979, 10228135)	*Saccharomyces cerevisiae* (D4)	Not Reported	Negative	0.1 - 1,000 µg/plate
Mecchi (1999, 10228133)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537)	Negative	Negative	0.333 – 5,000 µg/plate
Mecchi (1999, 10228133)	*Escherichia coli* (WP2*uvr*A)	Negative	Negative	33.3 – 5,000 µg/plate
NTP (2019, 5400978)	*Salmonella typhimurium* (TA98, TA100)	Negative	Negative	100 – 5,000 µg/plate
NTP (2019, 5400978)	*Escherichia coli* (WP2*uvr*A/pkM101)	Negative	Negative	100 – 10,000 µg/plate
Simmon (1978, 10228131)	*Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100)	Negative	Negative	10 – 5,000 µg/plate
Simmon (1978, 10228131)	*Salmonella cerevisiae* (D3)	Negative	Negative	0.1 – 5 µg/plate
Wang et al. (2015, 2850220)	*gpt* Delta transgenic mouse embryonic fibroblasts	Not reported	Positive[a]	1 – 20 µM (24 hours)

[a] Mutagens were present in cells exposed ≥10 µM.

Table 3-16. DNA Damage Data from *In Vivo* Studies

Reference	Species, Strain (Sex)	Tissue	Results	PFOS Concentration (Dosing Regimen)
DNA Damage via Comet Assay				
Çelik et al. (2013, 2919161)	Rat, Swiss Albino (Female)	Bone marrow	Positive	0.6 – 2.5 mg/kg/day (every other day via gavage for 30 days)
Du et al. (2014, 2851143)	Zebrafish, AB (Male and female)	Peripheral blood cells	Negative	0.4 – 1.6 mg/L (single dose to rearing water)
Eke and Çelik (2016, 2850124)	Rat, Swiss Albino (Male)	Peripheral blood cells	Positive	0.6 – 2.5 mg/kg/day (every other day via gavage for 30 days)
Eke et al. (2017, 3981318)	Rat, Swiss Albino (Male)	Liver	Positive	0.6 – 2.5 mg/kg/day (every other day via gavage for 30 days)
Micronuclei Formation				
Çelik et al. (2013, 2919161)	Rat, Swiss Albino (Female)	Bone marrow	Positive	0.6 – 2.5 mg/kg/day (every other day via gavage for 30 days)
Du et al. (2014, 2851143)	Zebrafish, AB (Male and female)	Peripheral blood cells	Positive	0.4 – 1.6 mg/L (single dose to rearing water for 30 days)
Eke and Çelik (2016, 2850124)	Rat, Swiss Albino (Male)	Peripheral blood cells	Positive	0.6 – 2.5 mg/kg/day (every other day via gavage for 30 days)
Eke et al. (2017, 3981318)	Rat, Swiss Albino (Male)	Liver	Positive	0.6 – 2.5 mg/kg/day (every other day via gavage for 30 days)
Murli (1996, 10228098)	Mouse, Crl:CD-1 (Male and female)	Bone marrow	Negative	__[a]
NTP (2019, 5400978)	Rat, Sprague Dawley (Male)	Peripheral blood cells	Negative	0.312 – 5 mg/kg/day (daily via gavage for 28 days)
NTP (2019, 5400978)	Rat, Sprague Dawley (Female)	Peripheral blood cells	Equivocal	0.312 – 5 mg/kg/day (daily via gavage for 28 days)
Wang et al. (2015, 2850220)	Mouse, *Gpt* delta transgenic (Male)	Bone marrow	Negative	1 – 10 mg/kg/day (daily via gavage for 28 days)

[a] Findings based on the 2016 EPA's Health Effects Support Document for Perfluorooctane Sulfonic Acid {U.S. EPA, 2016, 3603365}, concentration(s) unknown.

Table 3-17. DNA Damage Data from *In Vitro* Studies

Reference	*In Vitro* Model (Assay)	Results	Concentration (Duration of exposure)
	Chromosomal Aberrations		
Murli (1999, 10228132)	Human lymphocytes	Negative	10 – 470 µg/mL (3 hours)
	Unscheduled DNA Synthesis		
Cifone (1999, 10228136)	Fisher 344 male rat hepatocytes	Negative	0.25 – 4,000 µg/mL
	DNA Damage		
Wang et al. (2015, 2850220)	*gpt* Delta transgenic mouse embryonic fibroblasts (γ-H2AX foci)	Positive	0 – 30 µM (24 hours)
Jacquet et al. (2012, 2919219)	Syrian hamster embryo cells (comet assay)	Negative	2×10^{-4} – 50 µg/mL (7 days)
Kawamoto et al. (2010, 1274162)	*Paramecium caudatum* (comet assay)	Negative	10 – 100 µM (1 – 24 hours)
Lu et al. (2012, 2919198)	Calf thymus DNA (X-ray photoelectron spectroscopic and electrochemical impedance spectroscopy)	Positive	10 µmol/L (30 minutes)
Wielsoe et al. (2015, 2533367)	HepG2 (comet assay)	Positive	2×10^{-7} – 2×10^{-5} M (24 hours)
Florentin et al. (2011, 2919235)	HepG2 (comet assay)	Negative	5 – 300 µM (1 or 24 hours)

3.5.3.2 Key Characteristic #4: Induces Epigenetic Alterations

Epigenetic alterations are modifications to the genome that do not change genetic sequence. Epigenetic alterations include DNA methylation, histone modifications, changes in chromatin structure, and dysregulated microRNA expression, all of which can affect the transcription of individual genes and/or genomic stability {Smith, 2016, 3160486}.

3.5.3.2.1 *In Vivo* Evidence

3.5.3.2.1.1 1.2.1.1. Humans

A cohort of singleton term births were recruited from Faroese hospitals over an eighteen-month period from 1986 to 1987 {Leung, 2018, 4633577}. At delivery, samples of umbilical cord whole blood and scalp hair from the mothers were collected and used to measure toxicant levels as well as evaluation of DNA methylation. PFOS levels were significantly correlated with the number of methylated CpG sites (10,598 sites) in male newborn umbilical cord whole blood samples. Data from the male samples were then used to evaluated potential gene networks or pathways enriched based on the genes related to the methylated CpG sites; specifically, to evaluate potential relationships between physiological functions/diseases and the PFOS-induced aberrant methylation patterns. The top physiological function related to the methylation changes was "nervous system development and function." Additionally, CpG sites for which PFOS exposure altered the methylation status were associated with individual genes related to cancer.

A subset of adults enrolled in the C8 Health Project between August 1, 2005 and August 31, 2006 were evaluated for exposure to perfluoroalkyl acids (PFAAs) via drinking water {Watkins, 2014, 2850906}. The cross-sectional survey consisted only of residents within the mid-Ohio River Valley. A second, short-term follow-up study including another sample collection was conducted in 2010 to evaluate epigenetic alterations in relation to serum PFOS concentrations. Serum concentrations of PFOS decreased slightly between enrollment (2005-2006) and follow-up (2010). Methylation of long interspersed nuclear elements (LINE-1) transposable DNA elements in peripheral blood leukocytes at the follow-up timepoint in 2010 was significantly associated with PFOS exposure, with an unadjusted 0.265% increase in LINE-1 methylation (per 12 ng/mL increase in mean serum PFOS). This association between LINE-1 methylation and PFOS exposure remained significant after adjusting for covariates; a 0.20% increase was observed when the data were adjusted for age, gender, BMI, smoking status, and drinking status.

Additional epidemiological studies of prenatal or birth cohorts have identified epigenetic alterations associated with PFOS, indicating exposure can induce global DNA methylation changes and alterations to methylation of CpG sites that are associated with genes involved in several physiological functions and diseases related to development. For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.2.1.2 1.2.1.2. Animals

Dysregulation of long non-coding RNAs in rodent *in vivo* studies following PFOS exposure has been demonstrated, leading to reduced placental size. For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive Tableau for additional supporting information and study details). It should be noted that such effects were not seen in other tissues or in relation to other effects that may be more relevant to cancer outcomes.

Additional rodent evidence examined liver microRNA (miRNA) expression and found an increase in the expression of *miR-34a-5p,* which is involved in p53-mediated apoptosis, following exposure to PFOS. For additional information, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.2.2 *In Vitro* Evidence

Pierozan et al. (2020, 6833637) evaluated PFOS (10 µM) in the MCF-10A breast cell line. After 72 hours of exposure, PFOS-treated cells exhibited decreased acetylation of histone H3K9 (H3K9ac). In contrast, no alterations were found in the levels of H3K9 methylation and H3K26 acetylation.

Several additional studies have evaluated the potential of PFOS to alter the epigenome within various *in vitro* systems designed to test developmental effects. The available mechanistic studies suggest that the developing liver, developing heart, and placenta may be affected by PFOS at the molecular level (i.e., differential methylation of genes, gene expression changes, mitochondrial dysregulation). For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.3 Key Characteristic #5: Induce Oxidative Stress

Reactive oxygen and nitrogen species (ROS and RNS, respectively) are byproducts of energy production that occur under normal physiological conditions. An imbalance in the detoxification of reactive such species can result in oxidative (or nitrosative) stress, which can play a role in a variety of diseases and pathological conditions, including cancer. The primary mechanism by which oxidative stress leads to the carcinogenic transformation of normal cells is by inducing oxidative DNA damage that leads to genomic instability and/or mutations {Smith et al., 2016, 3160486}.

3.5.3.3.1 *In Vivo* Evidence

3.5.3.3.1.1 Humans

Several human epidemiological studies have reported that PFOS exposure induces oxidative stress, leading to cardiological dysregulation (e.g., endothelial dysfunction, impaired vasodilation, increased 8-OHdG and 8-NO2Gua). For additional information, please see the cardiovascular mechanistic section (Section 3.4.3.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.3.1.2 Animals

Male Sprague Dawley rats were administered 1 or 10 mg/kg/day PFOS orally for 28 days {Han, 2018, 4238554}. Following exposure, significant increases in ROS production and nitric oxide synthase mRNA expression were noted in the liver. Elevation of oxidative stress was associated with decreased intracellular antioxidant defense by aberrant catalase and superoxide dismutase activities.

Liu et al. (2009, 757877) studied markers of oxidative stress in the liver and brain in KM mice exposed to PFOS and found that there was no treatment effect. The authors found that levels of

malondialdehyde (MDA) did not differ between controls and exposed animals, and that superoxide dismutase activity was lower in treated vs. control mice. indicating that oxidative stress was not induced.

Evidence of increased oxidative stress in the liver, including increased ROS levels, changes in GSH and GSSG levels, and decreases in antioxidant enzymes, was observed in rodents *in vivo* following oral exposure to PFOS. For additional information, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.3.2 *In Vitro* Evidence

Several studies have evaluated ROS production in HepG2 cells exposed to PFOS, reporting varied results. A study by Hu and Hu (2009, 2919334) demonstrated PFOS exposure (50-200 µmol/L; 24-72 hours) induced a significant increase in ROS. This effect correlated with decreased mitochondrial membrane potential and apoptosis. Furthermore, PFOS exposure caused increased superoxide dismutase, catalase, and glutathione reductase levels but decreased glutathione-*S*-transferase and glutathione peroxidase levels in cells. In contrast, Florentin et al. (2011, 2919235) exposed HepG2 cells to PFOS (5-300 µM) for 24 hours and found a decrease in ROS generation by approximately 23%.

A study by Wang et al. (2015, 2850220) used mouse embryonic fibroblast (MEF) cells to identify intercellular ROS induced by PFOS exposure (1 or 20 µM). Using a fluorescent free radical probe CM-H_2DCFDA kit to evaluate ROS levels, cells exposed to 20 µM PFOS had a significantly higher level of florescence than controls, indicating PFOS induced intercellular oxidative stress. To better understand the role of H_2O_2 in this PFOS-induced cytotoxicity (Section 3.5.3.7) and genotoxicity (Section 3.5.3.1), Wang et al. treated cells concurrently with a cell membrane-permeating catalase to initiate the breakdown of H_2O_2 and protect cells from oxidative damage. In the presence of catalase, cytotoxicity and DNA double strand break frequency were decreased in PFOS-exposed cells. Mutation frequencies were also significantly suppressed in cells exposed to both PFOS and catalase when compared to cells exposed to PFOS alone. These results in Wang et al. (2015, 2850220) suggest that PFOS-induced genotoxicity is mediated by the induction of ROS.

Wielsoe et al. (2014, 2533367) exposed HepG2 cells to PFOS (2×10^{-7} to 2×10^{-5} M) for 24 hours. Following exposure, the cells demonstrated significant increase in intercellular ROS at all tested PFOS concentrations.

Several studies have identified the potential of PFOS to induce oxidative stress within various *in vitro* testing systems that are designed to understand effects during developmental stages. The available mechanistic studies demonstrated that oxidative stress mediates alterations in development and gross morphology following PFOS exposure. PFOS. For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive Tableau for additional supporting information and study details).

Further evidence of the ability of PFOS to induce oxidative stress is described elsewhere. PFOS exposure has been shown to be associated with increased markers of oxidative damage and decreased activity of protective antioxidants that play a role in the reduction of oxidative

damage. PFOS. For additional information, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.4 Key Characteristic #6: Induces Chronic Inflammation

The induction of chronic inflammation includes increased white blood cells, altered chemokine and/or cytokine production, and myeloperoxidase activity {Smith, 2016, 3160486}. Chronic inflammation has been associated with several forms of cancer, and a role of chronic inflammation in the development of cancer has been hypothesized. However, there are biological links between inflammation and oxidative stress and genomic instability, such that the contribution of each in carcinogenic progression is not always clear.

Several studies have identified the potential of PFOS to increase inflammation within various *in vivo* and *in vitro* models. It is important to note that *in vitro* models may be used for the evaluation of changes in inflammatory markers and response, they are generally not effective in modeling the events that are associated with chronic inflammation. For additional information, please see the immune (Section 3.4.2.3), hepatic (Section 3.4.1.3), developmental (Section 3.4.4.3), and cardiovascular (Section 3.4.3.3) mechanistic sections (refer to the interactive Tableau for additional supporting information and study details).

3.5.3.5 Key Characteristic #7: Is Immunosuppressive

Immunosuppression refers to the reduction in the response of the immune system to antigen, which is important in cases of tumor antigens {Smith, 2016, 3160486}. It is important to note that immunosuppressive agents do not directly transform cells, but rather can facilitate immune surveillance escape of cells transformed through other mechanisms (e.g., genotoxicity).

Studies have identified the immunosuppressive potential of PFOS in *in vivo* and *in vitro* testing systems. Specifically, PFOS has been associated with depression of natural killer cell activity, reduced macrophage function, and changes in the cellularity and immunophenotypes of lymphocytes. For additional information, please see the immune mechanistic section (Section 3.4.2.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.6 Key Characteristic #8: Modulates Receptor-Mediated Effects

Modulation of receptor-mediated effects involves the activation or inactivation of receptors (e.g., PPAR, AhR) or the modification of endogenous ligands (including hormones) {Smith, 2016, 3160486}.

3.5.3.6.1 *In Vivo* Evidence

Several studies have reported the potential of PFOS to modulate nuclear receptor- and hormone-mediated effects within various *in vivo* and *in vitro* testing systems, specifically models relevant to the hepatic system.

PFOS has been shown to activate several nuclear receptors, including PPARα, PPARγ, PPARβ/δ, CAR/PXR, and LXR/RXR. Many of these nuclear receptors, including PPARα and CAR, are known to play an important role in liver homeostasis and have been implicated in liver dysfunction. PFOS exposure may lead to liver toxicity through the activation of multiple nuclear receptors in both rodents and humans. For additional information, please see the hepatic

mechanistic section (Section 3.4.1.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.6.2 *In Vitro* Evidence

3.5.3.6.2.1 PPAR Mediated Effects

Liver-expressed peroxisome PPARα regulates transcription of genes involved in peroxisome proliferation, cell cycle control, apoptosis, and lipid metabolism. Data for PFOS illustrates the ability of PFOS to activate PPARα {Shipley, 2004, 2990378; Martin, 2007, 758419; Wolf, 2008, 716635; Wolf, 2014, 2850908}.

Jacquet et al. (2012, 2919219) exposed Syrian hamster embryo (SHE) cells to PFOS (≤50 µg/mL) for 5 and 24 hours. Evaluation of PPAR gene expression by qPCR indicated a 3.0-fold increase of *ppar-b/d* mRNA level at a PFOS concentration of 0.2 µg/mL after 24 hours. Subsequent exposure of SHE cells to PFOS (0.02-20 µg/mL) for 1 week found overexpression of PPAR-target genes and a significant increase of *ppar-b/d* mRNA at 0.2 µg/mL (2-fold increase) and 2 µg/mL (2.5-fold increase). mRNA levels of *ppar-y* were significant increased after 7 days at all PFOS exposure concentrations. Interestingly, upregulation of the *ppar-a* gene was found at the lowest concentration tested (0.2 µg/mL). A study using MCF-7 human breast cancer cells demonstrated that PFOS increased proliferation in a dose-dependent manner at concentrations of 0.01 and 30 µg/mL, a response that was observed in tandem with the maximal estrogen (E_2) response, suggesting that PFOS may be an estrogen receptor agonist at these concentrations {Henry, 2013, 1805116}.

3.5.3.7 Key Characteristic #10: Alters Cell Proliferation, Cell Death, or Nutrient Supply

Aberrant cellular proliferation, cell death, and/or nutrient supply is a common mechanism among carcinogens. This mechanism includes aberrant proliferation, decreased apoptosis or other evasion of terminal programming, changes in growth factors, angiogenesis, and modulation of energetics and signaling pathways related to cellular replication or cell cycle control {Smith, 2016, 3160486}.

3.5.3.7.1 *In Vivo* Evidence

3.5.3.7.1.1 Humans

Epidemiological studies found an association between PFOS exposure and increased markers of endothelial and platelet apoptosis. For additional information, please see the cardiovascular mechanistic section (Section 3.4.3.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.3.7.1.2 Animals

Proliferation of peroxisomes has been suggested as a mechanism of action for several non-genotoxic carcinogens that induce liver tumors upon chronic administration to rats and mice {Ashby, 1994, 630327; Rao and Reddy, 1996, 1334694}, and PFOS has been shown to activate PPARs. In a study of male and female Sprague Dawley rats administered PFOS in the diet at 0, 0.5, 2, 5, or 20 ppm for 4 or 14 weeks, there was no evidence of increased hepatic cell proliferation {Seacat, 2003, 1290852}. However, the same authors continued this same dietary

PFOS exposure in Sprague Dawley rats for up to two years and found liver effects consistent with PPAR activation {Thomford 2002, 5029075; Butenhoff, 2012, 1276144}. This two-year cancer bioassay found that the only neoplastic response that was attributable to PFOS exposure was an increased incidence of hepatocellular adenoma in both male and female rats in the 20 ppm PFOS group.

3.5.3.7.2 *In Vitro* Evidence

Two human giant cell tumor (GCT)-derived cell lines (COV434 and KGN) were exposed to PFOS (0.08-8,000 ng/mL) for 72 hours {Gogola, 2018, 5016947}. PFOS significantly increased proliferation in both cell lines in a dose-dependent manner. Specifically, PFOS treatment at 0.08 ng/mL increased COV434 and KGN proliferation by 1.4-fold and 1.9-fold, respectively. Follow up studies by the same authors did not observe any change in caspase 3 or 7 activities in cells exposed to concentrations of PFOS (0.8, 8, or 80 ng/ml; 72 hours), both of which play a role in apoptosis {Gogola. 2020, 6316203; Gogola, 2020, 6316206}.

The potential of PFOS to induce tumorigenic activity (proliferation, cell-cycle progression, and malignant phenotype) was evaluated in MCF-10A breast epithelial cells {Pierozan, 2018, 4238459}. Exposure to 10 µM promoted proliferation by accelerating G0/G1-to-S phase transition of the cell cycle after 24, 48, and 72 hours of exposure. PFOS exposure increased CDK4 while simultaneously decreased p27, p21, and p53 levels in MCF-10A cells. Furthermore, 10 µM PFOS exposure for 72 hours stimulated MCF-10A cell migration and invasion. A follow up study evaluating PFOS (10 µM; 72 hours) in MCF-10A cells induced proliferation and alteration of regulatory cell-cycle proteins (cyclin D1, CDK6, p21, p53, p27, ERK1, ERK2, and p38) {Pierozan, 2020, 6833637}. Additionally, PFOS exposure increased cell migration and invasion in unexposed daughter cells of exposed cells, as evidenced by a reduction in the levels of E-cadherin, occludin, and β-integrin. A study in MCF-7 human breast cancer cells demonstrated that PFOS increased proliferation in a dose-dependent manner at concentrations of 0.01 and 30 µg/mL, a response that may be the result of estrogen receptor activation {Henry, 2013, 1805116}. These results elucidate PFOS's potential carcinogenic effects through alteration of cell proliferation.

In contrast to these results, no changes in cellular proliferation were observed in MCF-7 breast adenocarcinoma cells exposed to PFOS (0.1–100 µM) for 24 hours {Maras, 2006, 2952988}. However, a small but significant downregulation of estrogen-responsive genes (*TFFI* and *ESR1*) was noted following PFOS exposure.

In a study designed to determine the effect of PFOS effect on the tumor suppressor protein SHP-2, HepG2 cells were exposed to sub-cytotoxic concentrations of PFOS for 24 hours before SHP-2 was immunoprecipitated from the cell lysates {Yang, 2017, 3981427}. While PFOS exposure increased SHP-2 gene expression in a concentration-dependent manner, it was also found to have an inverse proportional decrease in SHP-2 enzyme activity. Interestingly, a 1.4-fold increase in SHP-2 protein levels was observed in exposed cells, indicating that PFOS inhibits SHP-2 by blocking enzymatic activity post-translationally.

For additional information, please see the developmental mechanistic section (Section 3.4.4.3; refer to the interactive Tableau for additional supporting information and study details).

3.5.4 Weight Of Evidence for Carcinogenicity

3.5.4.1 Summary of Evidence

Several epidemiological studies and a single chronic cancer bioassay comprise the evidence database for the carcinogenicity of PFOS. The available epidemiology studies report elevated risk of bladder, prostate, kidney, and breast cancers after chronic PFOS exposure. However, the study designs, analyses, and mixed results do not allow for a definitive conclusion on the relationship between PFOS exposure and cancer outcomes in humans. The sole animal chronic cancer bioassay study provide support for multi-site tumorigenesis in male and female rats.

3.5.4.1.1 Evidence from Epidemiological Studies

Studies of the association between PFOS serum concentrations and bladder cancer have mixed findings. An elevated risk of bladder cancer mortality was associated with PFOS exposure in an occupational study {Alexander, 2003, 1291101} but a subsequent study to ascertain cancer incidence in the cohort observed elevated but not statistically significant incidence ratios that were 1.7- to 2-fold higher among workers with higher cumulative exposure {Alexander, 2007, 4727072}. The risk estimates lacked precision because the number of cases was small, and the study did not control for the potential confounding of smoking. A nested case-control study in a general population Danish cohort did not observe elevated bladder cancer risk with increasing PFOS serum levels {Eriksen, 2009, 2919344}.

Elevated non-significant ORs for prostate cancer were reported for the occupationally exposed cohort examined by Alexander and Olsen (2007, 4727072) and the Danish population-based cohort examined by Eriksen et al. (2009, 2919344). In the same occupational cohort studied by Alexander and Olsen (2007, 4727072), Grice et al. (2007, 4930271) observed that prostate cancers were among the most frequently reported malignancies. When cumulative exposure measures were analyzed, elevated ORs were reported for prostate cancer, however, they did not reach statistical significance. Length of follow-up may not have been adequate to detect cancer incidence in this cohort as approximately one-third of the participants had worked < 5 years in their jobs, and only 41.7% were employed ≥ 20 years. No association between PFOS exposure and prostate cancer was reported in either a second case-control study in Denmark {Hardell, 2014, 2968084} or in a study of the association between PFOS serum concentrations and prostate specific antigen (a biomarker of prostate cancer) from the C8 Health Project {Ducatman, 2015, 3859843}. In an NHANES population, Omoike et al. (2021, 7021502) observed a significantly inverse association with prostate cancer.

One study in the general population reported a statistically significant increase in risk of renal cell carcinoma in the highest PFOS exposure quartile and per doubling of PFOS concentration {Shearer, 2021, 7161466}. Although the trend was significant across quartiles, the effect in the third quartile was null. Additionally, the association with PFOS was attenuated after adjusting for other PFAS, and it was lower in the third quartile than in the second quartile, indicating potential confounding by correlated PFAS exposures. There was no association when evaluated on a per doubling of PFOS after adjusting for other PFAS.

The majority of studies examining associations between PFOS and cancer outcomes were on breast cancer. No association was identified between PFOS and breast cancer in either a case-control or a nested case-control studies of Danish and California cancer registry populations,

respectively {Bonefeld-Jørgensen, 2014, 2851186; Hurley, 2018, 5080646}. One study of Inuit females in Greenland observed positive associations between PFOS levels and risk for breast cancer {Bonefeld-Jørgensen, 2011, 2150988}, although the association was of a low magnitude and could not be separated from the effects of other perfluorosulfonated compound exposures. Three studies indicated potential associations between PFOS exposure and increased breast cancer risk in specific subgroups or increased risk for specific breast cancer subtypes. Ghisari et al. (2017, 3860243) found that increased breast cancer risk was associated with increased PFOS serum concentrations in Danish individuals with a specific polymorphism in the CYP19 gene (aromatase; associated with estrogen biosynthesis and metabolism). Mancini et al. (2019, 5381529) reported that increased PFOS serum concentrations were associated specifically with increased risk of ER+ and PR+ tumors, whereas risk of ER- and PR- tumors did not follow a dose-dependent response. In a Taiwanese population Tsai et al. (2020, 6833693) observed a statistically significant increase in risk of breast cancer, but only in participants aged 50 years or younger, and in ER+ breast cancer in participants aged 50 years or younger. Another general population study in the U.S. suggested that maternal PFOS exposure combined with high maternal cholesterol may decrease the daughters' risk of breast cancer but did not examine breast cancer subtypes or genetic variants {Cohn, 2020, 5412451}. Significantly increased breast cancer risk was also observed in an NHANES population in the two highest quartiles of exposure, but the association was inverse in the second quartile {Omoike, 2021, 7021502}. A recent study in a Japanese population observed inverse association across serum PFOS quartiles with a significant dose-response trend {Itoh, 2021, 9959632}. The association remained significantly inverse in both pre- and postmenopausal women in the highest tertile of exposure, with a significant dose-response trend. However, in some of the studies PFOS levels were measured after or near the time of cancer diagnosis {Tsai, 2020, 6833693; Omoike, 2021, 7021502}. Given the long half-life of PFOS in human blood, the exposure levels measured in these studies could represent exposures that occurred prior to cancer development. However, this is currently difficult to evaluate since data on the latency of PFOS-related cancer is not available.

Overall, study design issues, lack of replication of the results, and a lack of mechanistic understanding of PFOS on specific breast cancer subtypes or in subpopulations limit firm conclusions regarding PFOS and breast cancer. These findings are supported by other recent assessments and reviews {ATSDR 2021, 9642134; Steenland, 2021, 7491705; CalEPA, 2021, 9416932}.

3.5.4.1.2 Evidence from Animal Bioassays

The single available chronic toxicity/carcinogenicity bioassay for PFOS in animals is a 104-week dietary study in rats {Thomford, 2002, 5029075; Butenhoff, 2012, 1276144}. Statistically significant increases in the incidence of hepatocellular adenomas in the high dose (20 ppm) male (7/43; 16%) and female rat groups (5/31; 16%) and combined adenomas/carcinomas in the females (6/32; 19%; 5 adenomas, 1 carcinoma) were observed. The observation of a carcinoma in the female rats is a relatively rare occurrence according to NTP's historical controls for female Sprague-Dawley rats (1/639 historical control incidence) {NTP, 2020, 10368689}. Historical control incidence rates for these tumor types were not provided by Thomford (2002, 5029075). Additionally, there were statistically significant trends in the hepatic tumor responses of both males and females. A statistically significant trend of increased incidence of pancreatic islet cell carcinomas with increased PFOS dose was also observed in the male rats, though the individual dose groups were not statistically different from the control group. The percentages of animals

with islet cell carcinomas in the highest dose group (12.5%) exceeds NTP's historical controls for male Sprague-Dawley rats by over an order of magnitude (12/638; 1.9%) {NTP, 2020, 10368689}.

Thyroid tumors (follicular cell adenomas and carcinomas) were observed in males and females, though these responses were not statistically significant in any dose group, nor was there a linear dose-response trend. In males, the incidence of thyroid tumors was significantly elevated only in the high-dose, recovery group males exposed for 52 weeks (10/39) but not in the animals receiving the same dose for 105 weeks. However, Thomford (2002, 5029075) indicated that the number of thyroid tumors observed in the recovery group males were outside the range of historical control values at that time, similar to what NTP (2020, 10368689) has reported for its laboratories (3/637 combined follicular cell adenoma or carcinoma). There were very few follicular cell adenomas/carcinomas in the females (4 total, excluding the recovery group) with a non-linear dose-response. There was also a high background incidence of mammary gland tumors in the female rats, primarily combined fibroma adenoma and adenoma, but the incidence lacked dose-response for all tumor classifications.

3.5.4.2 Mode of Action for Hepatic Tumors

The strongest evidence of the carcinogenicity of PFOS comes from a *high* confidence chronic rodent study identifying hepatocellular tumors in both male and female rats {Butenhoff, 2012, 1276144; Thomford, 2002, 5029075}. As described in the subsections below, the available mechanistic data suggest that multiple MOAs may underlie the hepatocellular tumors observed after PFOS exposure. Specifically, the available studies provide varying levels of support for the role of several plausible MOAs: PPARα activation, CAR activation, HNF4α suppression, cytotoxicity, genotoxicity, oxidative stress, and immunosuppression.

3.5.4.2.1 PPARα activation

There is considerable debate over the relevance of PFAS-induced hepatic tumors to human health. Exposure to some PFAS have been shown to activate PPARα, which is characterized by downstream cellular or tissue alterations in peroxisome proliferation, cell cycle control (e.g., apoptosis and cell proliferation), and lipid metabolism {U.S. EPA, 2016, 3603365}. Notably, human expression of PPARα mRNA and protein is only a fraction of what is expressed in rodent models, though there are functional variant forms of PPARα that are expressed in human liver to a greater extent than rodent models {Klaunig, 2003, 5772415; Corton, 2018; 4862049}. Therefore, for PPARα activators that act solely or primarily through PPARα-dependent mechanisms (e.g., Wyeth-14,643, di-2-ethyl hexyl phthalate), the hepatic tumorigenesis observed in rodents may be expected to be reduced in frequency or severity or not observed in humans {Klaunig, 2003, 5772415; Corton, 2014, 2215399; Corton, 2018, 4862049}.

The adverse outcome pathway (AOP) for the PPARα MOA for hepatic tumors has been characterized to include the following set of key events: 1) PPARα activation in hepatic cells; 2) alterations in cell growth signaling pathways (e.g., increases in Kupffer cell activation leading to increases in TNFα); 3) perturbations of hepatocyte growth and survival (i.e., increased cell proliferation and inhibition of apoptosis); and 4) selective clonal expansion of preneoplastic foci cells leading to 5) increases in hepatocellular adenomas and carcinomas {Klaunig, 2003, 5772415; Corton, 2014, 2215399; Corton, 2018, 4862049}. This AOP is associated with but not necessarily causally related to non-neoplastic effects including peroxisome proliferation,

hepatocellular hypertrophy, Kupffer cell-mediated events, and increased liver weight. There is also some overlap between signaling pathways and adverse outcomes, including tumorigenesis, associated with PPARα activation and the activation or degradation of other nuclear receptors, such as CAR, PXR, HNF4α, and PPARγ {Rosen, 2017, 3859803; Huck, 2018, 5079648; Beggs, 2016, 3981474; Corton, 2018, 4862049}.

Table 3-18. Evidence of Key Events Associated with the PPARα Mode of Action in Male Sprague-Dawley Rats Exposed to PFOS

Dose (mg/kg/day)	Key Event 1 (PPARα activation)	Key Event 2 (altered cell growth signaling)	Key Event 3a (altered hepatocyte growth)	Key Event 3b (altered hepatocyte survival)	Key Event 4 (preneoplastic clonal expansion)	Key Event 5 (hepatic tumors)
0.024	–	–	- (4 & 14w)	- (14 & 103w)	–	- (103w)
0.098	- (4w)	–	- (4 & 14w)	- (14 & 103w)	–	- (103w)
0.242	–	–	- (4 & 14w)	- (14 & 103w)	–	- (103w)
0.312	↑ (4w)	–	–	- (4w)	–	–
0.625	↑ (4w)	–	–	- (4w)	–	–
0.984–1	↑ (4w, GD 1–PND 20 F_1 PND 21)	↑ (4w)	↑/- (4w) - (14 & 53w)	- (14 & 53w) ↓ (103w)	–	↑ (103w)
1.5 – 1.93	- (1d) ↑ (7d & 4w)	–	- (1d) ↑ (7d) ↑/- (4w)	- (1d) ↓ (7d) -/↑ (4w)	–	–

Notes: ↑ = statistically significant increase in response compared to controls; - = no significant response; ↓ = statistically significant decrease in response compared to controls; a "/" separating symbols for direction of effect indicates that multiple studies assessed the key event at the same dose and time point but reported conflicting results; d = day(s); w = week(s).
Data represented in table extracted from NTP (2019, 5400978); Chang et al. (2009, 757876); Elcombe et al. (2012, 1332473); Elcombe et al. (2012, 1401466); Curran et al. (2008, 757871); Han et al. (2018, 4355066); Butenhoff et al. (2012, 1276144)/ Thomford (2002, 5029075).

The published *in vivo* and *in vitro* literature suggests that PFOS is a relatively weak PPARα agonist compared to other known PPARα agonists such as PFOA {Martin, 2007, 758419; Wolf, 2012, 1289836; Behr, 2020, 6305866; Rosen, 2013, 2919147}. While *in vitro* PPARα activation assay results indicate overall effective activation of PPARα by PFOS, the magnitude of that activation has been found to be relatively lower than chemicals that induce toxicity primarily through PPARα activation (e.g., di-2-ethyl hexyl phthalate). There is *in vivo* rodent assay evidence of PFOS-induced PPARα-associated transcriptional and enzymatic responses (e.g., upregulation of *Acox1* and acyl-CoA activity) as well. However, consistent with the *in vitro* activation assays, these *in vivo* responses were relatively weaker than PFOA and/or other PPARα activators and were often reported to be accompanied by transcriptional responses associated with other nuclear receptor signaling pathways (e.g., CAR and PPARγ), consistent with multiple modes of action {Martin, 2007, 758419; Dong, 2016, 3981515; NTP, 2019, 5400978; Chang, 2009, 757876; Elcombe, 2012, 1332473; Elcombe, 2012, 1401466}. For further details, see Section 3.4.1.3. Consistent with these findings, studies of WT and PPARα-null mice reported that 808 differentially expressed genes responsive to a 7-day 10 mg/kg/day PFOS exposure were expressed in PPARα-null mouse livers while 906 genes were differentially expressed in WT mice, corroborating the likelihood of an active PPARα-independent MOA(s) {Rosen, 2010, 1274165}. Robust PPARα-independent effects in null mice were observed even at the lowest dose of PFOS (3 mg/kg/day; 630 differentially expressed genes in PPARα-null mice vs. 81

differentially expressed genes in WT mice) compared to responses in mice treated with 3 mg/kg/day Wyeth-14,643 or PFOA (902 genes WT, 10 genes PPARα-null and 879 genes WT, 176 genes PPARα-null, respectively) {Rosen, 2010, 1274165}, consistent with multiple MOAs for PFOS hepatic effects.

Table 3-19. Evidence of Key Events Associated with the PPARα Mode of Action in Female Sprague-Dawley Rats Exposed to PFOS

Dose (mg/kg/day)	Key Event 1 (PPARα activation)	Key Event 2 (altered cell growth signaling)	Key Event 3a (altered hepatocyte growth)	Key Event 3b (altered hepatocyte survival)	Key Event 4 (preneoplastic clonal expansion)	Key Event 5 (hepatic tumors)
0.029	–	–	- (4 & 14w)	- (14 & 103w)	–	- (103w)
0.12	↓ (4w)	–	- (4 & 14w)	- (14 & 103w)	–	- (103w)
0.299	–	–	- (4 & 14w)	- (14 & 103w)	–	- (103w)
0.312	↑ (4w)	–	–	- (4w)	–	–
0.625	↑ (4w)	–	–	- (4w)	–	–
1.251	- (4w & GD 1-GD 20 dam)	–	- (4, 14 & 53w)	- (14 & 53w) ↓ (103w)	–	↑ (103w)

Notes: ↑ = statistically significant increase in response compared to controls; - = no significant response; ↓ = statistically significant decrease in response compared to controls; d = day(s); w = week(s).
Data represented in table extracted from NTP (2019, 5400978); Chang et al. (2009, 757876); Curran et al. (2008, 757871); Butenhoff et al. (2012, 1276144)/ Thomford (2002, 5029075).

There is evidence from *in vivo* animal bioassays and *in vitro* studies of Kupffer cell activation, an indicator of alterations in cell growth, in response to PFOS treatment. Though this mechanism is itself PPARα-independent, factors secreted upon Kupffer cell activation may be required for increased cell proliferation by PPARα activators {Corton, 2018, 4862049}. Two short-term exposure *in vivo* rodent studies reported increased serum TNFα levels after 3–4 weeks of PFOS administration {Han, 2018, 4355066; Su, 2019, 5080481}; TNFα is a pro-inflammatory cytokine that can be released upon activation of Kupffer cells {Corton, 2018, 4862049}. In addition to serum TNFα levels, Han et al. (2018, 4355066) reported increased TNFα mRNA in hepatic tissues of PFOS-exposed rats. The authors also extracted primary Kupffer cells from untreated rats and cultured them with PFOS *in vitro* for 48 hours and reported increased supernatant TNFα levels and cellular TNFα mRNA levels. These results indicate that rodent hepatic tissues may be primed for perturbations of PPARα-dependent cell growth upon PFOS exposure. However, further study is needed to understand the potential role of other mediators of Kupffer cell activation since unlike PPARα, PPARγ is expressed in Kupffer cells and can also be activated by PFOS.

While there is some evidence of alterations in cell growth signaling pathways due to PFOS exposure, there is conflicting evidence related to the ability of PFOS to induce hepatic cell proliferation and inhibit apoptosis. The available rodent *in vivo* study results indicate that increases in proliferation may be dose- and exposure duration-dependent whereas changes in apoptosis may be species- or dose-dependent. In the only available chronic rodent bioassay for PFOS {Thomford, 2002, 5029075; Butenhoff, 2012, 1276144}, significant increases in the number of hepatic tumors were observed at the highest dose levels in each sex (20 ppm in diet or approximately 1 mg/kg/day) without corresponding increases in the incidence or severity of cell proliferation at 52 weeks in the livers of male or female rats. Additionally, there were transient

effects on hepatic peroxisomal proliferation in males or females at weeks 4 and 14 as indicated by the palmitoyl-CoA assay {Thomford, 2002, 5029075; Seacat, 2003, 1290852}. In contrast, there is evidence of hepatic cell and/or peroxisome proliferation from short-term studies that administered higher PFOS dose levels than the Thomford report (2002, 5029075) (i.e., 2-10 mg/kg/day) {Elcombe, 2012, 1401466; Elcombe, 2012, 1332473; NTP, 2019, 5400978; Han, 2018, 4355066}. Results were not always consistent across time points or sexes and were accompanied by evidence of increased activation of other nuclear receptors (i.e., CAR and PXR), which could also influence cell proliferation. The characteristics of typical PPARα-induced cell proliferation includes an early burst that recovers to a level that is slightly higher than background, the latter of which is difficult to detect for compounds that are weak PPARα activators {Corton, 2014, 4862049}. This likely explains, at least in part, the inconsistencies in cell proliferation patterns across timepoints and lends support to the evidence of relatively weak PPARα activation by PFOS. Additionally, Elcombe et al. (2012, 1401466) reported substantially greater palmitoyl-CoA oxidation after 50 ppm Wyeth-14,643 administration in male Sprague-Dawley rats compared to 20 or 100 ppm (approximately 1.7 and 7.9 mg/kg/day, respectively) PFOS administration for up to 28 days, lending further support for PFOS as a relatively weak PPARα activator.

In addition to the observation of increased hepatic cell proliferation on day 1 of recovery in male rats administered 20 or 100 ppm PFOS (approximately 1.93 and 9.65 mg/kg/day, respectively) for 7 days, Elcombe et al. (2012, 1332473) also reported decreased hepatic apoptotic indices (i.e., the percent of apoptotic nuclei out of the total number cell nuclei in a unit of area) in both dose groups, which is an indication of PPARα-dependent hepatotoxicity. However, these results were inconsistent with the results of the second Elcombe et al. (2012, 1401466) study, which reported an increased apoptotic index after 7 days of 20 ppm dietary PFOS administration. The authors observed no other statistically significant changes in the apoptotic indices of rats from the 20 ppm group in the two additional timepoints tested (1 day and 28 days), though they did report decreases in the apoptotic indices of rats in the 100 ppm group at all three time points, similar to the results of Elcombe et al. (2012, 1332473; 2012, 1401466). The underlying reason for the inconsistent apoptosis findings in the 20 ppm dose groups between the two studies is unclear. Increased hepatic apoptosis was observed in mice administered 2.5–10 mg/kg/day PFOS for 30 days {Xing, 2016, 3981506}, and short-term PFOS studies in both rats and mice reported increases in apoptosis-related hepatic gene expression and/or protein activity/expression {Eke, 2017, 3981318; Wan, 2016, 3981504; Han, 2018, 4238554; Lv, 2018, 5080395}. Further descriptions of these *in vivo* studies, as well as *in vitro* studies examining hepatic cell proliferation and apoptosis can be found in Section 3.4.1.3.

There are several studies of the hepatic effects resulting from PFOS exposure observed in PPARα-null mice with either short-term or gestational exposure durations but therefore, lack an ability to assess tumor incidence or chronic histopathological effects. The studies of Qazi et al. (2009, 1937260), Abbott et al. (2009, 2919376), and Rosen et al. (2010, 1274165) all observed increased absolute and/or relative liver weight in PPARα-null adults orally administered PFOS or pups exposed to PFOS *in utero.* Along with the PPARα-independent cell signaling effects in PPARα-null mice reported by Rosen et al. (2010, 1274165; 2017, 3859803), these studies corroborate that the hepatomegaly observed in WT rodents administered PFOS is not entirely PPARα-dependent. Several other signaling pathways may contribute to the observed hepatomegaly due to PFOS exposure, though the relationship of these liver effects with tumor

formation is unclear. Further descriptions of studies utilizing PPARα-null mice can be found in Section 3.4.1.3.

In general, PPARα activators are not expected to induce cell proliferation or suppress apoptosis of hepatocytes in humans {Corton, 2018, 4862049}. Specifically, there is strong consensus that the MOA for liver tumor induction by PPARα activators in rodents has limited-to-no relevance to humans, due to differences in cellular expression patterns of PPARα and related proteins (e.g., cofactors and chromatin remodelers), as well as differences in binding site affinity and availability {Corton, 2018, 4862049; Klaunig, 2003, 5772415}. Nonetheless, several studies have reported increased cell proliferation or markers of cell proliferation *in vitro* in human liver cell lines exposed to PFOS {Cui, 2015, 3981568; Song, 2016, 9959776; Louisse, 2020, 6833626} (see Section 3.4.1.3). For example, Cui et al. (2015, 3981568) found increased proliferation using the MTT assay in the non-tumor fetal human liver cell line HL-7702. These increases in cell proliferation were accompanied by corresponding proteomic changes indicative of increased proliferation. Using flow cytometry, Cui et al. (2015, 3981568) also found that increased percentages of cells were in cell phases associated with DNA synthesis and/or interphase growth and mitosis (S and G2/M phases), depending on the length of exposure and dose of PFOS. Corroborative transcriptional results were observed in two additional human cell lines (HepG2 and HepaRG) {Song, 2016, 9959776; Louisse, 2020, 6833626}. There was no mention of changes in apoptosis accompanying increased cell proliferation in two of the studies of human hepatocytes {Cui, 2015, 3981568; Louisse, 2020, 6833626}, while Song et al. (2016, 9959776) reported that genes related to "regulation of apoptosis" were significantly altered, although the direction of the change is not specified. Beggs et al. (2016, 3981474) reported that a human primary cell line exposed to PFOS predominantly showed changes in the expression of genes involved in carcinogenesis and cell death signaling, among other biological pathways/functions related to hepatotoxicity and hepatic diseases. The authors linked these transcriptional changes to the loss of HNF4α functionality which is known to promote the development of hepatocellular carcinoma, providing evidence of a PPARα-independent mechanism of hepatotoxicity and carcinogenicity. In addition to HNF4α-mediated hepatocarcinogenicity, Benninghoff et al. (2012, 1274145) proposed that promotion of hepatocarcinogenesis by PFOS in an initiation-promotion model in rainbow trout, which are similarly insensitive to PPARα as humans, is potentially the result of activation of the trout liver estrogen receptor. Specifically, dietary PFOS treatment promoted hepatocarcinogenesis (i.e., increased the incidence of hepatocellular carcinomas and adenomas) and increased tumor promotion and cell proliferation in rainbow trout exposed to aflatoxin B_1 as a cancer initiator {Benninghoff, 2012, 1274145}.

3.5.4.2.2 Other Nuclear Receptors

In addition to PPARα, there is some evidence that other nuclear receptors may play a role in the MOA for hepatic tumors resulting from PFOS exposure. For example, CAR, which has an established adverse outcome pathway of key events similar to PPARα, has been implicated in hepatic tumorigenesis in rodents. The key events of CAR-mediated hepatic tumors are: 1) activation of CAR; 2) altered gene expression specific to CAR activation; 3) increased cell proliferation; 4) clonal expansion leading to altered hepatic foci; and 5) liver tumors {Felter, 2018, 9642149}. Associative events include hypertrophy, induction of CAR-specific CYP enzymes (e.g., CYP2B) and inhibition of apoptosis. As described in Section 3.4.1.3, there is both *in vivo* and *in vitro* evidence that PFOS can activate CAR and initiate altered gene expression

and associative events {Dong, 2016, 3981515; NTP, 2019, 5400978; Martin, 2007, 758419; Elcombe, 2012, 1401466; Chang, 2009, 757876; Elcombe, 2012, 1332473; Rosen, 2010, 1274165; Rosen, 2013, 2919147; Rosen, 2017, 3859803}. Some studies, such as NTP (2019, 5400978), report greater activation of CAR with PFOS treatment compared to PPARα, depending on the sex and/or model of interest. As with PPARα-mediated tumorigenesis, there are claims that CAR-mediated tumorigenesis is not relevant to humans because CAR activators such as phenobarbital have been shown to induce cell proliferation and subsequent tumorigenesis in rodents but do not induce cell proliferation in human cell lines {Elcombe, 2014, 2343661}. However, as outlined above, several studies have reported increased cell proliferation or markers of cell proliferation due to PFOS treatment in human cell lines {Cui, 2015, 3981568; Song, 2016, 9959776; Louisse, 2020, 6833626}. Further study is needed to understand the mechanistic underpinnings of PFOS-induced hepatic cell proliferation and whether it is related to CAR activation.

Table 3-20. Evidence of Key Events Associated with the CAR Mode of Action in Male Sprague-Dawley Rats Exposed to PFOS

Dose (mg/kg/day)	Key Event 1 (CAR activation)	Key Event 2 (Altered Gene Expression)	Key Event 3 (Cell Proliferation)	Key Event 4 (clonal expansion)	Key Event 5 (Hepatic Tumors)
0.024	–	–	- (4 & 14 w)	–	- (103 w)
0.098	–	–	- (4 & 14 w)	–	- (103 w)
0.242	–	–	- (4 & 14 w)	–	- (103 w)
0.312	–	↑ (4 w)	–	–	–
0.625	–	↑ (4 w)	–	–	–
0.984–1	–	↑ (GD 1–PND 20, PND 21 F_1)	↑/- (4 w) - (14 & 53 w)	–	↑ (103 w)

Notes: ↑ = statistically significant increase in response compared to controls; - = no significant response; ↓ = statistically significant decrease in response compared to controls; a "/" separating symbols for direction of effect indicates that multiple studies assessed the key event at the same dose and time point but reported conflicting results; d = day(s); w = week(s).
Data represented in table extracted from NTP (2019, 5400978); Chang et al. (2009, 757876); Elcombe et al. (2012, 1332473); Elcombe et al. (2012, 1401466); Han et al. (2018, 4355066); Butenhoff et al. (2012, 1276144)/ Thomford (2002, 5029075).

Table 3-21. Evidence of Key Events Associated with the CAR Mode of Action in Female Sprague-Dawley Rats Exposed to PFOS

Dose (ppm)	Key Event 1 (CAR activation)	Key Event 2 (Altered Gene Expression)	Key Event 3 (Cell Proliferation)	Key Event 4 (clonal expansion)	Key Event 5 (Hepatic Tumors)
0.024/ 0.029	–	–	- (4 & 14 w)	–	- (103 w)
0.098/ 0.12	–	–	- (4 & 14 w)	–	- (103 w)
0.242/ 0.299	–	–	- (4 & 14 w)	–	- (103 w)
0.312	–	↑ (4 w)	–	–	–
0.625	–	↑ (4 w)	–	–	–
0.984/ 1.251	–	↑ (GD 1–GD 20, dam)	- (4, 14 & 53 w)	–	↑ (103 w)

Notes: ↑ = statistically significant increase in response compared to controls; - = no significant response; ↓ = statistically significant decrease in response compared to controls; d = day(s); w = week(s).

Data represented in table extracted from NTP (2019, 5400978); Chang et al. (2009, 757876); Butenhoff et al. (2012, 1276144)/ Thomford (2002, 5029075).

HNF4α is known as a master regulator of hepatic differentiation and plays a role in tumor suppression as well as general liver maintenance and function {Beggs, 2016, 3981474}. Interestingly, PFOS exposure appears to downregulate HNF4α and its target genes. Studies utilizing primary human hepatocytes, HepG2 cells, and *in vivo* mouse models have reported decreased HNF4α protein expression as well as corresponding changes in downstream HNF4α target genes with PFOS treatment {Beggs, 2016, 3981474; Behr, 2020, 6505973}. Beggs et al. (2016, 3981474) reported that PFOS induced changes in genes involved in carcinogenesis and cell death signaling and linked the loss of HNF4α functionality to potential hepatocellular tumor promotion. The authors also suggested that loss of HNF4α functionality may play a role in non-cancer hepatic effects including hepatomegaly, steatosis, altered lipid metabolism, and fatty liver disease.

There is additional evidence from *in vivo* and *in vitro* studies that PFOS has the ability to activate and modulate the targets of other nuclear receptors. As described in Section 3.4.1.3, PFOS has been reported to modulate the activity of PPARs other than PPARα (i.e., PPARβ/δ, and PPARγ), PXR, LXR, RXR, RAR, and Erβ, though the evidence of activation is sometimes conflicting across different cell lines, assays, and species. Several of these nuclear receptors, such as PPARγ, are known to play a role in liver homeostasis and disease and may be driving factors in the hepatotoxicity observed after PFOS exposure, though their role in tumorigenesis is less clear. As described in Section 3.5.3, there is also evidence that PFOS modulates endogenous ligands for nuclear receptors, most notably thyroid and reproductive hormones. However, it is also unclear what role, if any, these receptors and ligands may be playing in PFOS-induced hepatic tumorigenesis.

3.5.4.2.3 Cytotoxicity

There is suggestive evidence that PFOS may act through a cytotoxic MOA. Felter et al. (2018, 9642149) identified the following key events for establishing a cytotoxicity MOA: 1) the chemical is not DNA reactive; 2) clear evidence of cytotoxicity by histopathology such as the presence of necrosis and/or increased apoptosis; 3) evidence of toxicity by increased serum enzymes indicative of cellular damage that are relevant to humans; 4) presence of increased cell proliferation as evidenced by increased labeling index and/or increased number of hepatocytes; 5) demonstration of a parallel dose response for cytotoxicity and formation of tumors; and 6) reversibility upon cessation of exposure. As discussed above in the genotoxicity section, there is some evidence that PFOS can induce DNA damage and/or micronuclei formation in liver tissue {Eke, 2017, 3981318; Wang, 2015, 2850220}. These data indicate that PFOS may be DNA reactive (either directly or indirectly), but it is unclear if this DNA reactivity is the source of the tumor findings {Holsapple, 2006, 194740}. Quantitative liver histopathology is limited to three studies, however the one available chronic study {Butenhoff, 2012, 1276144} reported significant trends in increased individual hepatocyte necrosis in male and female Sprague-Dawley rats which was also statistically significant in the highest dose groups. Liver histopathology in humans is also limited, however, Jin et al. (2020, 6315720) reported higher odds (not necessarily statistically significant) of nonalcoholic steatohepatitis ($p < 0.05$), ballooning, fibrosis, and portal inflammation.

Table 3-22. Evidence of Key Events Associated with the Cytotoxicity Mode of Action in Male Sprague-Dawley Rats

Dose (mg/kg/day)	Key Event 1 (Cytotoxicity)	Key Event 2 (Serum Enzymes)	Key Event 3 (Regenerative Proliferation)	Key Event 4 (Hyperplasia and/or Preneoplastic Lesions)	Key Event 5 (Hepatic Tumors)
0.024	- (14 & 103 w)	- (4, 14, 27 & 53 w)	- (4 & 14 w)	- (14 & 103 w)	- (103 w)
0.098	- (14 & 103 w)	- (4, 14, 27 & 53 w)	- (4 & 14 w)	- (14 & 103 w)	- (103 w)
0.242	- (14 & 103 w)	- (4, 14, 27 & 53 w)	- (4 & 14 w)	- (14 & 103 w)	- (103 w)
0.312	- (4 w)	- (4 w)	–	- (4 w)	–
0.625	- (4 w)	↑ (4 w)	–	- (4 w)	–
0.984	- (4, 14 & 53 w) ↑ (103 w)	↑ (4, 14 & 53 w) - (27 w)	↑/- (4 w) - (14 & 53 w)	- (14 & 53 w) ↑ (103 w)	↑ (103 w)

Notes: ↑ = statistically significant increase in response compared to controls; - = no significant response; ↓ = statistically significant decrease in response compared to controls; a "/" separating symbols for direction of effect indicates that multiple studies assessed the key event at the same dose and time point but reported conflicting results; d = day(s); w = week(s).
NTP (2019, 5400978); Elcombe et al. (2012, 1332473); Elcombe et al. (2012, 1401466); Han et al. (2018, 4355066); Butenhoff et al. (2012, 1276144)/ Thomford (2002, 5029075).

There is evidence in both humans and animals that exposure to PFOS increases serum liver enzymes. Specifically, statistically significant positive associations between ALT and PFOS (i.e., increased ALT as a continuous measure with higher PFOS exposure levels) were observed in several studies {Salihovic, 2018, 5083555; Nian, 2019, 5080307; Jain, 2019, 5381541; Costa, 2009, 1429922; Gallo, 2012, 1276142; Olsen, 2003, 1290020}. These individual findings are supported by a meta-analysis of epidemiological studies reporting biomarkers of liver injury reporting a statistically significant ($p < 0.001$) weighted z-score suggesting a positive association between PFOS and increased ALT in adults and children {Costello, 2022, 10285082}. Statistically significant increases in serum enzymes (i.e., ALT, AST, ALP, and GGT) were also observed in several animal toxicological studies, though these increases were generally less than two-fold (100% change relative to control) compared to control {Seacat, 2003, 1290852; Curran, 2008, 757871; Butenhoff, 2012, 1276144; Xing, 2016, 3981506; Yan, 2014, 2850901; NTP, 2019, 5400978; Han, 2018, 4355066}. However, these changes in serum enzyme levels were accompanied by histopathological evidence of damage, as outlined above, and coherence is observed in humans.

Table 3-23. Evidence of Key Events Associated with the Cytotoxicity Mode of Action in Female Sprague-Dawley Rats

Dose (mg/kg/day)	Key Event 1 (Cytotoxicity)	Key Event 2 (Serum Enzymes)	Key Event 3 (Regenerative Proliferation)	Key Event 4 (Hyperplasia and/or Preneoplastic Lesions)	Key Event 5 (Hepatic Tumors)
0.029	- (14 & 103 w)	- (4, 14, 27 & 53 w)	- (4 & 14 w)	- (14 & 103 w)	- (103 w)

Dose (mg/kg/day)	Key Event 1 (Cytotoxicity)	Key Event 2 (Serum Enzymes)	Key Event 3 (Regenerative Proliferation)	Key Event 4 (Hyperplasia and/or Preneoplastic Lesions)	Key Event 5 (Hepatic Tumors)
0.12	- (14 & 103 w)	- (4, 14, 27 & 53 w)	- (4 & 14 w)	- (14 & 103 w)	- (103 w)
0.299	- (14 & 103 w)	- (4, 14, 27 & 53 w)	- (4 & 14 w)	- (14 & 103 w)	- (103 w)
0.312	- (4 w)	- (4 w)	–	- (4 w)	–
0.625	- (4 w)	- (4 w)	–	- (4 w)	–
1.251	- (4, 14 & 53 w) ↑ (103 w)	- (4, 14, 27 & 53 w)	- (4, 14 & 53 w)	- (14 & 53 w) ↑ (103 w)	↑ (103 w)

Notes: ↑ = statistically significant increase in response compared to controls; - = no significant response; ↓ = statistically significant decrease in response compared to controls; d = day(s); w = week(s).
NTP (2019, 5400978); Butenhoff et al. (2012, 1276144)/ Thomford (2002, 5029075).

As highlighted in the PPARα activation section, several studies have reported increased cell proliferation or markers of cell proliferation in human cell lines {Cui, 2015, 3981568; Song, 2016, 9959776; Louisse, 2020, 6833626}, though there is limited quantitative histopathological data to determine the ability of PFOS to induce hepatic hyperplasia. Finally, the available data indicate a parallel dose response for cytotoxicity and the formation of liver tumors as evidence in Table 3-24 and Table 3-25, though dose spacing (i.e., the gap in dosing between the mid-high and high doses administered) may limit the precision of a dose response curve.

Table 3-24. Incidences of Liver Tumor and Nonneoplastic Lesions in Male Sprague-Dawley Rats at 103 weeks, as Reported by Thomford (2002, 5029075)

	0 mg/kg/day	0.024 mg/kg/day	0.098 mg/kg/day	0.242 mg/kg/day	0.984 mg/kg/day
Hepatocellular Adenomas	0/41**	3/42	3/47	1/44	7/43**
Necrosis, Individual Hepatocyte	3/50	2/50	6/50	4/50	10/50
Altered Hepatocellular, Clear/Eosinophilic Cell	13/50	21/50	23/50	24/50	24/50
Cystic Degeneration	5/50	15/50	19/50	17/50	22/50
Hyperplasia, Bile Duct	19/50	20/50	25/50	24/50	25/50

Notes:
Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

Table 3-25. Incidences of Liver Tumor and Nonneoplastic Lesions in Female Sprague-Dawley Rats at 103 weeks, as Reported by Thomford (2002, 5029075)

	0 mg/kg/day	0.029 mg/kg/day	0.120 mg/kg/day	0.299 mg/kg/day	1.251 mg/kg/day
Combined Hepatocellular	0/28**	1/29	1/16	1/31	6/32*

	0 mg/kg/day	0.029 mg/kg/day	0.120 mg/kg/day	0.299 mg/kg/day	1.251 mg/kg/day
Adenomas & Carcinomas					
Necrosis, Individual Hepatocyte	3/50	4/50	4/50	5/50	9/50
Infiltrate, Macrophage, Pigmented	2/50	3/50	5/50	6/50	20/50
Infiltrate, Lymphohistiocytic	33/50	37/50	33/50	36/50	42/50
Hyperplasia, Bile Duct	21/50	25/50	19/50	17/50	27/50

Notes:
Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

3.5.4.2.4 Genotoxicity

Several relatively recent studies, primarily published by the same laboratory, have shown the potential for PFOS to act as a genotoxicant (see Section 3.5.3); previously, EPA had not identified evidence supporting genotoxicity as a potential MOA for PFOS {U.S. EPA, 2016, 3603365}. Two *in vivo* studies, the first a 30-day study in male Swiss Albino rats and the second a 28-day study in male *gpt* delta transgenic mice, provided evidence of DNA damage and/or micronuclei formation in liver tissue of animals administered up to 2.5 or 10 mg/kg/day PFOS, respectively {Eke, 2017, 3981318; Wang, 2015, 2850220}. However, there are concerns about the Interpretation of these studies regarding the genotoxicity and mutagenicity of PFOS because results reported as not statistically significant, concerns about the study design, or unclear relationship of the observed effects to genotoxicity of PFOS vs. Secondary effects from hepatoxicity (e.g., oxidative stress).

Several other 28–30-day studies in male and female rats and mice also observed DNA damage and/or micronuclei formation in bone marrow or peripheral blood cells {Çelik, 2013, 2919161; Eke, 2016, 2850124; NTP, 2019, 5400978}, though there are similar concerns about whether these responses are attributable to direct genotoxicity of PFOS. For example, NTP (2019, 5400978) reported increased numbers of micronucleated polychromatic erythrocytes in the blood of female rats administered 5 mg/kg/day PFOS (highest dose group) for 28 days, but also reported concomitant decreases in the percentage of polychromatic erythrocytes in the peripheral blood, indicative of bone marrow toxicity. This potential bone marrow toxicity may be driving micronuclei formation rather than the direct mutagenicity of PFOS. NTP (2019, 5400978) also noted that the observed responses of the high dose females were within historical control ranges and considered these results to be equivocal. From this very limited database, it does not appear that genotoxicity in male and female Sprague-Dawley rats occurs at doses at or below those that result in tumorigenesis.

In addition to rodent studies, Du et al. (2014, 2851143) reported increased DNA strand breaks and micronuclei formation in peripheral blood cells of male and female zebrafish exposed to PFOS for 30 days and several other studies reported increased DNA damage *in vitro* {Wang, 2015, 2850220; Lu, 2012, 2919198; Wielsoe, 2014, 2533367}. However, the majority of *in vitro*

studies (described in Section 3.5.3) report negative results for genotoxic endpoints including chromosomal aberrations, unscheduled DNA synthesis, mutagenicity, and various types of DNA damage.

The available *in vivo* evidence suggests that exposure to PFOS may result in genotoxicity and is particularly compelling for the potential indirect genotoxicity that could stem from hepatotoxicity and/or bone marrow toxicity. At this time, there are no generally accepted mechanistic explanations for PFOS directly interacting with genetic material. Additionally, while there is some *in vivo* evidence of PFOS-induced mutagenicity as primarily evidenced by micronuclei formation in rats, mice, and zebrafish, there are several uncertainties that limit the interpretation of these results. There is currently no robust evidence to support a mutagenic MOA for PFOS, though overall, genotoxicity cannot be ruled out as a potential MOA for PFOS.

3.5.4.2.5 Consideration of Other Plausible MOAs

In addition to the evidence supporting modulation of receptor-mediated effects, and potential genotoxicity, PFOS also exhibits several other key characteristics (KCs) of carcinogens (see Section 3.5.3), some of which are similarly directly evident in hepatic tissues.

For example, PFOS appears to induce oxidative stress, another KC of carcinogens, particularly in hepatic tissues (see Section 3.4.1.3). Several studies in rats and mice showed evidence of increased oxidative stress and reduced capacity for defense against oxidants and oxidative damage in hepatic tissues. Interestingly, two studies, one 28-day study in rats and one 30-day study in mice, reported reduced Nrf2 protein levels or expression in hepatic tissues after PFOS exposure {Wan, 2016, 3981504; Lv, 2018, 5080395}. Nrf2 is an important regulator of antioxidant response elements and is generally activated in response to pro-oxidant exposure and oxidative stress. Accordingly, these studies and others noted a reduction in the hepatic expression of genes that are implicated in antioxidant, anti-inflammatory, and/or stress response functions (e.g., *hmox1, nqo1*) as well as reduced antioxidant enzyme levels and activities (e.g., CAT, SOD) {Wan, 2016, 3981504; Lv, 2018, 5080395; Han, 2018, 4238554; Liu, 2009, 757877; Xing, 2016, 3981506}. Several *in vivo* exposure studies also noted increases in hepatic ROS and markers of oxidative damage (e.g., MDA) {Han, 2018, 4238554; Liu, 2009, 757877; Xing, 2016, 3981506; Wan, 2016, 3981504; Lv, 2018, 5080395}. Notably, Han et al. (2018, 4238554) reported several indicators of oxidative stress in male Sprague-Dawley rats gavaged for 28 days with 1 mg/kg/day PFOS (lowest dose tested in the study), a comparable dose to that which caused tumorigenesis in the chronic study in male rats. Taken together, these results provide some support for disruption of the oxidative stress response in hepatic tissues leading to accumulation of ROS and subsequent oxidative damage.

Immunosuppression is the reduction of an individual's immune system to respond to foreign cells or antigens, including tumor cells {Smith, 2020, 6956443}. The immune system plays an important role in the identification and eventual destruction of cancer cells; immunosuppression may allow for the evasion of this process by cancer cells and subsequently lead to tumorigenesis. As discussed in Section 3.4.2.1.1, PFOS serum levels are associated with markers of immunosuppression, particularly in children. Several studies reported inverse associations between PFOS serum concentrations and antibody production following vaccinations in children {Grandjean, 2017, 3858518; Grandjean, 2017, 4239492; Mogensen, 2015, 3981889; Timmermann, 2020, 6833710}. Additionally, one *medium* confidence study reported higher odds

of total infectious diseases with increasing PFOS serum concentrations {Goudarzi, 2017, 3859808}, though it should be noted that studies reporting odds ratios for individual infectious diseases had mixed results. Animal toxicological studies also report markers of immunosuppression, including reductions in natural killer cell activity. As described in Section 3.4.2.2, there are several reports of decreased natural killer cell activity in male and female, adult and F_1 generation mice from short-term, subchronic, and gestational studies {Dong, 2009, 1424951; Peden-Adams, 2008, 1424797; Keil, 2008, 1332422; Zhong, 2016, 3748828; Zheng, 2009, 1429960}. While one short-term study in male mice reported increases in splenic T-helper ($CD3^+CD4^+$) and T-cytotoxic ($CD3^+CD8^+$) lymphocytes {Lv, 2015, 3981558}, two gestational studies reported reductions in thymic $CD4^+$ cells in male offspring {Zhong, 2016, 3748828; Keil, 2008, 1332422}. There is also limited evidence of immunosuppression in the form of reduced white blood cell counts (primarily lymphocytes) from two short-term rodent studies in male mice and rats, respectively {Qazi, 2009, 1937259; NTP, 2019, 5400978}. This short-term report is the only available study in Sprague-Dawley rats and does not indicate that immunosuppressive effects are occurring at or below doses that result in tumorigenesis {NTP, 2019, 5400978}. However, it is difficult to discount immunosuppression as a potential MOA for PFOS, given the limited database for rats and stronger databases indicating immunosuppression in mice and humans.

3.5.4.2.6 Conclusions

Based on the weight of evidence evaluation of the available peer-reviewed scientific evidence, PFOS has the potential to induce hepatic tumors via multiple MOAs in rodents, most notably via the modulation of nuclear receptors (i.e., PPARα and CAR) and cytotoxicity. There is also limited evidence supporting potential MOAs of genotoxicity, immunosuppression, and oxidative stress. The conclusions from the weight of evidence analysis of the available data for PFOS are consistent with literature reviews recently published by two state health agencies which concluded that the hepatotoxic effects of PFOS are not entirely dependent on PPARα activation {CalEPA, 2021, 9416932; NJDWQI, 2018, 5026035}.

As described in the *Guidelines for Carcinogen Risk Assessment* {U.S. EPA, 2005, 6324329}, "[i]n the absence of sufficiently, scientifically justifiable mode of action information, EPA generally takes public health-protective, default positions regarding the interpretation of toxicologic and epidemiologic data; animal tumor findings are judged to be relevant to humans, and cancer risks are assumed to conform with low dose linearity." For the available data regarding the MOA of PFOS-induced hepatic carcinogenesis, there is an absence of definitive information supporting a single, scientifically justified MOA; in fact, there is evidence supporting the potential for multiple plausible MOAs. Therefore, EPA concludes that the hepatic tumors observed by Thomford (2002, 5029075) and Butenhoff et al. (2012, 1276144) can be relevant to human health and support the positive, albeit, limited, tumor findings from epidemiological studies.

3.5.5 Cancer Classification

Under the *Guidelines for Carcinogen Risk Assessment* {U.S. EPA, 2005, 6324329}, EPA reviewed the weight of the evidence and determined that PFOS is *Likely to Be Carcinogenic to Humans*, as "the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor *Carcinogenic to Humans.*" The *Guidelines*

provide descriptions of data that may support the *Likely to Be Carcinogenic to Humans* descriptor; the available PFOS data are consistent with the following factors:

- "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;
- a rare animal tumor response in a single experiment that is assumed to be relevant to humans; or
- a positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case" {U.S. EPA, 2005, 6324329}.

The epidemiological evidence of associations between PFOS and cancer found mixed results across tumor types. However, the available study findings support a plausible correlation between PFOS exposure and carcinogenicity in humans. The single chronic cancer bioassay performed in rats is positive for multi-site and -sex tumorigenesis {Thomford, 2002, 5029075; Butenhoff, 2012, 1276144}. In this study, statistically significant increases in the incidences of hepatocellular adenomas or combined adenomas and carcinomas were observed in both male and female rats. There was also a statistically significant trend of this response in both sexes indicating a relationship between the magnitude/direction of response and PFOS dose. As described in Section 3.5.4.2, the available mechanistic evidence is consistent with multiple potential MOAs for this tumor type; therefore, the hepatocellular tumors observed by Thomford/Butenhoff et al. (2002, 5029075; 2012; 1276144) may be relevant to humans. In addition to hepatocellular tumors, Thomford/Butenhoff et al. (2002, 5029075; 2012; 1276144) reported increased incidences of pancreatic islet cell tumors with a statistically significant dose-dependent positive trend, as well as modest increases in the incidence of thyroid follicular cell tumors. The findings of multiple tumor types provide additional support for potential multi-site tumorigenesis resulting from PFOS exposure.

The PFOA carcinogenicity database includes both epidemiological studies and animal bioassays that support its designation as *Likely to be Carcinogenic to Humans*. Structural similarities between PFOS and PFOA add to the weight of evidence for carcinogenicity of PFOS. Notably, a similar set of non-cancer effects have been observed after exposure to either PFOA or PFOS in humans and animal toxicological studies including similarities in hepatic, developmental, immunological, cardiovascular, and endocrine effects, among others.

Table 3-26. Comparison of the PFOS Carcinogenicity Database with the *Likely* Cancer Descriptor as Described in the Guidelines for Carcinogen Risk Assessment {U.S. EPA, 2005, 6324329}

Likely to be Carcinogenic to Humans	
An agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments	Epidemiological studies evaluating the association between human exposure to PFOS and cancer are mixed. Supporting carcinogenicity data are available from animal experiments.
An agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans	**PFOS data are consistent with this description**. PFOS has tested positive in animal experiments in more than one sex and site. Hepatic tumors were observed in male and female rats (statistically significant at high dose and statistically significant trend tests for each) and islet cell carcinomas show a statistically significant positive trend in male rats.
A positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset	**This description is not applicable to PFOS.**
A rare animal tumor response in a single experiment that is assumed to be relevant to humans	**PFOS data are consistent with this description**. The hepatocellular carcinoma observed in the high-dose female rats is a rare tumor type in this strain {NTP, 2020, 7330145}.
A positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case	**PFOS data are consistent with this description.** The positive multi-site, multi-sex chronic cancer bioassay is supported by mechanistic data indicating that PFOS is associated with events generally known to be associated with tumor formation such as inducing nuclear receptor activation, cytotoxicity, genotoxicity, oxidative stress, and immunosuppression.

While reviewing the weight of evidence for PFOS, EPA evaluated consistencies of the carcinogenicity database with other cancer descriptors according to the Guidelines for Carcinogen Risk Assessment {U.S. EPA, 2005, 6324329}. A discussion on these findings is presented in Section 6.4.

4 Dose-Response Assessment

4.1 Non-Cancer

4.1.1 Study and Endpoint Selection

There is evidence from both epidemiological and animal toxicological studies that oral PFOS exposure may result in adverse health effects across many health outcomes (Section 3.4). Per recommendations made by the SAB and the conclusions presented in EPA's preliminary analysis, *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water,* EPA has focused its toxicity value derivation efforts "on those health outcomes that have been concluded to have the strongest evidence" {U.S. EPA, 2022, 10476098}. EPA prioritized health outcomes and endpoints with the strongest overall weight of evidence (evidence ***demonstrates*** or evidence ***indicates***) based on human and animal evidence (Section 3.4 and 3.5) for POD derivation using the systematic review methods described in Section 2 and the Appendix (see PFOS Appendix). For PFOS these health outcomes are immunological, developmental, cardiovascular (serum lipids), and hepatic effects. EPA considered both epidemiological and animal toxicological studies for POD derivation.

In the previous section, for hazard judgment decisions (Section 3.4 and 3.5), EPA qualitatively considered *high*, *medium*, and, at times, *low* confidence studies to characterize the weight of evidence for each health outcome. However, given the robust database for PFOS, only well-conducted *high* or *medium* confidence human and animal toxicological studies were considered for POD derivation, as recommended in the IRIS Handbook {U.S. EPA, 2022, 10367891}. Such human epidemiological studies were available for immunotoxicity, developmental, serum lipid, and hepatic effects. Preferred animal toxicological studies consisted of *medium* and *high* confidence studies of longer exposure duration (e.g., chronic or subchronic studies vs. 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 toxicological studies evaluating exposure periods relevant to developmental outcomes. These types of animal toxicological studies increase the confidence in the RfD relative to other animal toxicological studies because they are based on data with relatively low risk of bias and are associated with less uncertainty related to low-dose and exposure duration extrapolations. See Section 6.3 for a discussion of animal toxicological studies and endpoints selected for POD derivation for this updated assessment compared to those selected for the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}.

For all other health outcomes (e.g., reproductive, endocrine, nervous, hematological, musculoskeletal), the evidence integration summary judgment for the human and animal evidence was ***suggestive*** or ***inadequate*** and these outcomes were not assessed quantitatively. Uncertainties related to health outcomes for which the results were *suggestive* are discussed in the evidence profile tables provided in the Appendix (See PFOS Appendix), as well as Section 6.5.

4.1.1.1 Hepatic effects

As reviewed in Section 3.4.1.4, ***evidence indicates*** that elevated exposures to PFOS are associated with hepatic effects in humans. As described in Table 3-5, the majority of

epidemiological studies assessed endpoints related to serum biomarkers of hepatic injury (8 *medium* confidence studies), while several studies also reported on liver disease or injury (3 *medium* confidence studies) and other serum markers of liver function (2 *medium* confidence studies). EPA prioritized endpoints related to serum biomarkers of injury for quantitative analyses as the reported effects on these endpoints, particularly ALT, were well-represented within the database and were generally consistent across the available *medium* confidence studies. Specifically, three *medium* confidence studies (out of five total) reported statistically significant positive associations between PFOS serum concentrations and ALT in adults. Findings for AST and GGT in adults were generally positive and are supportive of the selection of ALT as an endpoint for dose-response modeling, though results from stratified analyses of these two endpoints were less consistent.

Serum ALT measures are considered a reliable indicator of impaired liver function because increased serum ALT is indicative of leakage of ALT from damaged hepatocytes {Boone, 2005, 782862; Liu, 2014, 10473988; U.S. EPA, 2002, 625713}. Additionally, evidence from both human epidemiological and animal toxicological studies indicates that increased serum ALT is associated with liver disease {Ioannou, 2006, 10473853; Ioannou, 2006, 10473854; Kwo, 2017, 10328876; Roth, 2021, 9960592}. Human epidemiological studies have demonstrated that even low magnitude increases in serum ALT can be clinically significant. For example, a Scandinavian study in people with no symptoms of liver disease observed that relatively small increases in serum ALT were associated with liver diseases such as steatosis and chronic hepatitis C {Mathiesen, 1999, 10293242}. Additionally, a study in Korea found that the use of lowered thresholds for "normal" serum ALT values showed good prediction power for liver-related adverse outcomes such as mortality and hepatocellular carcinoma {Park, 2019, 10293238}. Others have questioned the biological significance of relatively small increases in serum ALT (i.e., less than 2-fold) reported in animal toxicological studies {Hall, 2012, 2718645}, though measures of ALT in these studies can be supported by histopathological evidence of liver damage.

Additionally, numerous studies have demonstrated an association between elevated ALT and liver-related mortality (reviewed by Kwo et al. (2017, 10328876)). Furthermore, the American Association for the Study of Liver Diseases (AASLD) recognizes serum ALT as an indicator of overall human health and mortality {Kim, 2008, 7757318}. For example, as reported by Kwo et al. (2017, 10328876), Kim et al. (2004, 10473876) observed that higher serum ALT concentrations corresponded to an increased risk of liver-related death in Korean men and women; similarly, Ruhl and Everhart (2009, 3405056; 2013, 2331047) analyzed NHANES data and observed an association between elevated serum ALT and increased mortality, liver-related mortality, coronary heart disease in Americans, and Lee et al. (2008, 10293233) found that higher serum ALT was associated with higher mortality in men and women in Olmstead County, Minnesota. Furthermore, the American College of Gastroenterology (ACG) recommends that people with ALT levels greater than 33 (men) or 25 IU/L (women) undergo screenings and assessments for liver diseases, alcohol use, and hepatotoxic medication use {Kwo, 2017, 10328876}. Results of human and animal toxicological studies as well as the positions of the AASLD and the ACG demonstrate the clinical significance of increased serum ALT. It is also important to note that while evaluation of direct liver damage is possible in animal studies, it is difficult to obtain biopsy-confirmed histological data in humans. Therefore, liver injury is typically assessed using serum biomarkers of hepatotoxicity {Costello et al, 2022, 10285082}.

Results reported in animal toxicological studies are consistent with the observed elevated ALT indicative of hepatic damage in epidemiological studies. Specifically, studies in rodents found that oral PFOS treatment resulted in increased liver weight (11/14 *high* and *medium* confidence studies in adult rodents), alterations in levels of serum biomarkers of liver injury, particularly in male rodents (i.e., ALT (7/7 studies), AST (4/7 studies), ALP (3/4 studies), and GGT (1/1 study)), and evidence of histopathological alterations including hepatocellular damage (5/7 *high* and *medium* confidence studies). These hepatic effects, particularly the increases in serum enzymes and histopathological evidence of liver damage are supportive of elevated ALT observed in human populations. Mechanistic studies in rodents and limited evidence from *in vitro* studies and animal models provide additional support for the biological plausibility and human relevance of the apical effects observed in animals and suggest possible PPARα-dependent and -independent MOA for PFOS induced liver toxicity. EPA prioritized studies that quantitatively reported histopathological evidence of hepatic damage for dose-response modeling because these endpoints are more direct measures of liver injury than serum biomarkers. However, the observed increases in liver enzymes in rodents are supportive of the hepatic damage confirmed during histopathological examinations in several studies.

Three *medium* confidence epidemiological studies {Gallo, 2012, 1276142; Lin, 2010, 1291111; Nian, 2019, 5080307} and one *high* confidence animal toxicological study {Butenhoff, 2012, 1276144} were considered for POD derivation (Table 4-1). The largest study of PFOS and ALT in adults is by Gallo et al. (2012, 1276142), which was conducted in over 30,000 adults from the C8 Study. Two additional studies {Lin, 2010, 1291111; Nian, 2019, 5080307} were considered by EPA for POD derivation because they reported significant associations in general populations in the U.S and a highly exposed population in China, respectively. Nian et al. (2019, 5080307) examined a large population of adults in Shenyang (one of the largest fluoropolymer manufacturing centers in China) as part of the Isomers of C8 Health Project. In an NHANES adult population, Lin et al. (2010, 1291111) observed elevated ALT levels per log-unit increase in PFOS in the models adjusted for age, gender, and race/ethnicity, but not in the fully adjusted models, or in the models additionally adjusted for PFOA, PFHxS and PFNA. While this is a large nationally representative population, several methodological limitations, including lack of clarity about base of logarithmic transformation applied to PFOS concentrations in regression models and the choice to model ALT as an untransformed variable ultimately preclude its use for POD derivation.

EPA identified one study in male rats by Butenhoff et al. (2012, 1276144), a chronic dietary study, for POD derivation. Butenhoff et al. (2012, 1276144) conducted histopathological examinations of liver tissue in male and female rats and reported dose-dependent increases in the incidence of individual hepatocellular necrosis. As this is the only available chronic PFOS toxicity studies with a large sample size, numerous and relatively low dose levels, and a comprehensive suite of endpoints, individual cell necrosis in the liver in females was considered for derivation of PODs. This effect was also observed in males but was accompanied by inflammatory cell responses in the livers of female animals.

4.1.1.2 Immunological Effects

As reviewed in Section 3.4.2.4, ***evidence indicates*** that elevated exposures to PFOS are associated with immunological effects in humans. As described in Table 3-10, the majority of epidemiological studies assessed endpoints related to immunosuppression (1 *high* and 16

medium confidence studies) and immune hypersensitivity (1 *high* and 17 *medium* confidence studies), while one study (*medium* confidence) also reported on endpoints related to autoimmune disease. Endpoints related to autoimmune diseases were not further considered for quantitative assessments as there were a limited number of *medium* and *high* confidence studies and a limited number of total studies that assessed the same specific diseases (e.g., rheumatoid arthritis, celiac disease). Endpoints related to immune hypersensitivity were also not considered for dose-response analyses. Although the majority (6/9) of the available *medium* confidence studies reported consistent increases in the odds of asthma, there were inconsistencies in effects reported in subgroups across studies. These inconsistencies limited the confidence needed to select particular studies and populations for dose-response modeling. Other immune hypersensitivity endpoints, such as odds of allergies and rhinoconjunctivitis, had less consistent results reported across *medium* and *high* confidence studies and were therefore excluded from further consideration, though they are supportive of an association between PFOS and altered immune function.

Evidence of immunosuppression in children reported by epidemiological studies were consistent across studies and endpoints. Specifically, epidemiological studies reported reduced humoral immune response to routine childhood immunizations, including lower levels of tetanus and anti-diphtheria antibodies {Timmerman, 2021, 9416315; Grandjean, 2012, 1248827; Budtz-Jørgensen, 2018, 5083631} and rubella {Granum, 2013, 1937228; Pilkerton, 2018, 5080265; Stein, 2016, 3108691} antibody titers. Reductions in antibody response were observed at multiple timepoints throughout childhood, using both prenatal and childhood exposure levels, and were consistent across study populations from *medium* confidence studies.

Measurement of antigen-specific antibodies following vaccinations is an overall measure of the ability of the immune system to respond to a challenge. The antigen-specific antibody response is extremely useful for evaluating the entire cycle of adaptive immunity and is a sweeping approach to detect immunosuppression across a range of cells and signals {Myers, 2018, 10473136}. The SAB's PFAS review panel noted that reduction in the level of antibodies produced in response to a vaccine represents a failure of the immune system to respond to a challenge and is considered an adverse immunological health outcome {U.S. EPA, 2022, 10476098}. This is in line with a review by Selgrade (2007, 736210) who suggested that specific immunotoxic effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards—which has the potential to be a more adverse effect that just a single immunotoxic effect. Thus, decrements in the ability to maintain effective levels of antitoxins following immunization may be indicative of wider immunosuppression in these children exposed to PFOS.

As noted by Dewitt et al. (2017, 5926400; 2019, 5080663) as well as subject matter experts on the SAB's PFAS review panel {U.S. EPA, 2022, 10476098}, the clinical manifestation of a disease is not a prerequisite for a chemical to be classified as an immunotoxic agent and the ability to measure clinical outcomes as a result of mild to moderate immunosuppression from exposure to chemicals in traditional epidemiological studies can be challenging. Specifically, the SAB noted that "[d]ecreased antibody responses to vaccines is relevant to clinical health outcomes and likely to be predictive of risk of disease" {U.S. EPA, 2022, 10476098}. The WHO *Guidance for immunotoxicity risk assessment for chemicals* similarly recommends measures of

vaccine response as a measure of immune effects as "childhood vaccine failures represent a significant public health concern" {WHO, 2012, 10633091}. This response is also translatable across multiple species, including rodents and humans, and extensive historical data indicate that suppression of antigen-specific antibody responses by exogenous agents is predictive of immunotoxicity.

When immunosuppression occurs in the developing immune system, the risks of developing infectious diseases and other immunosuppression-linked diseases may increase {Dietert, 2010, 644213}. Immunosuppression linked with chemical stressors is not the same as an immunodeficiency associated with, for example, genetic-based diseases, but still is an endpoint associated with potential health risks. Studies of individuals exposed at the extremes of age, those with existing immunodeficiencies, and those exposed to chronic stress, show that what may be considered mild to moderate immunosuppression in the general population could result in increased risk of infections in these more susceptible populations {Selgrade, 2007, 736210}. Finally, the immune system continues developing after birth; because of this continued development, exposures to PFAS may have serious and long-lasting consequences {DeWitt, 2019, 5080663; MacGillivray, 2014, 6749084; Selgrade, 2007, 736210}. Hessel et al. (2015, 5750707) reviewed the effect of exposure to nine toxicants on the developing immune system and found that the developing immune system was at least as sensitive or more sensitive than the general (developmental) toxicity parameters. Immunotoxicity that occurs in the developing organism generally occurs at doses lower than required to affect the adult immune system, thus providing a more sensitive endpoint for assessing risk {vonderEmbse, 2018, 6741321}. Luster et al. (2005, 2174509) similarly noted that responses to childhood vaccines may be sensitive enough to detect changes in populations with moderate degrees of immunosuppression, such as those exposed to an immunotoxic agent.

Results reported in animal toxicological studies are consistent with the observed immunosuppression in epidemiological studies. Specifically, studies in rodents found that oral PFOS treatment resulted in reduced immune responses (e.g., reduced plaque-forming cell (PFC) responses, reduced natural killer (NK) cell activity) (4 *medium* confidence studies) and altered immune cell populations (e.g., bone marrow hypocellularity, altered splenic and thymic cellularity, white blood cell counts) (2 *high* and 3 *medium* confidence studies). EPA prioritized endpoints from both categories for quantitative analyses for several reasons. First, immunosuppression evidenced by functional assessments of the immune responses, such as analyses of PFC and NK responses, are coherent decreased antibody responses seen in human populations. EPA prioritized PFC responses over NK cell activity for POD derivation because several studies {Dong, 2009, 1424951; Peden-Adams, 2008, 1424797; Zhong, 2016, 3748828} reported non-monotonic dose-response curves for NK cell activity. Second, altered immune cell populations were reported in two *high* confidence studies and supported by several *medium* confidence studies, strengthening the weight of evidence for these endpoints. EPA prioritized results from NTP (2019, 5400978) as this was a *high* confidence study reporting consistent effects of PFOS treatment on multiple endpoints related to immune cellularity (i.e., increased bone marrow hypocellularity, increased splenic extramedullary hematopoiesis, and reduced leukocytes, neutrophils, and white blood cell counts) in male rats.

Two *medium* confidence epidemiologic studies {Budtz-Jørgensen, 2018, 5083631; Timmerman, 2021, 9416315} and one *high* and one *medium* confidence animal toxicological studies {Zhong,

2016, 3748828; NTP, 2019, 5400978} were considered for POD derivation (Table 4-1). The candidate epidemiological studies offer data characterizing antibody responses to vaccinations in children using a variety of PFOS exposure measures across various populations and vaccinations. Budtz-Jørgensen and Grandjean (2018, 5083631) investigated anti-tetanus and anti-diphtheria responses in Faroese children aged 5–7 and Timmerman et al. (2021, 9416315) investigated anti-tetanus and anti-diphtheria responses in Greenlandic children aged 7–12. In addition to the results from epidemiological studies, altered PFC response in male PNW 4 mice gestationally exposed to PFOS from GD 1–17 reported in Zhong et al. (2016, 3748828) and extramedullary hematopoiesis in male and female rats gavaged with PFOS for 28 days reported in NTP (2019, 5400978), supported the evidence of immunotoxicity in humans and were also considered for POD derivation.

4.1.1.3 Cardiovascular effects

As reviewed in Section 3.4.3.4, ***evidence indicates*** that elevated exposures to PFOS are associated with cardiovascular effects in humans. As described in Table 3-11, the majority of epidemiological studies assessed endpoints related to serum lipids (2 *high* and 20 *medium* confidence studies) and blood pressure and hypertension (2 *high* and 16 *medium* confidence studies), while several studies also reported on cardiovascular disease (1 *high* and 4 *medium* confidence studies) and atherosclerosis (1 *high* and 4 *medium* confidence studies). Endpoints related to cardiovascular disease and atherosclerosis were not prioritized for dose-response as they reported mixed or primarily null results. Endpoints related to blood pressure and hypertension were also not prioritized for quantitative analyses because studies reported no effects or generally mixed associations, though there was evidence of associations between PFOS exposure and at least one measure of continuous blood pressure in adults (5 *medium* or *high* confidence studies reported positive associations). There is some uncertainty associated with the blood pressure endpoints as there was not often concordance between SBP and DBP within study populations. However, these results are supportive of an association between PFOS and cardiovascular effects in humans.

Studies in adults from the general population, including high-exposure communities, reported positive associations between PFOS serum concentrations and serum lipids. Specifically, *medium* confidence epidemiological studies in the general population reported positive associations between PFOS exposure and total cholesterol (TC) (9/10 studies) and low-density lipoprotein (LDL) (6/6 studies). Associations between PFOS and high-density lipoprotein (HDL) or triglycerides in the general population were inconsistent. EPA prioritized TC for quantitative assessments because the association was consistently positive in adults, with some studies reporting statistically significant ORs, the response was more consistently positive with a greater magnitude of change in other populations (i.e., children and pregnant women) compared to LDL, and elevations in TC were reported in a marginally larger number of studies. Additionally, the positive associations with TC were supported by a recent meta-analysis restricted to 14 general population studies in adults {U.S. EPA, 2022, 10369698}.

Increased serum cholesterol is associated with changes in incidence of cardiovascular disease events such as myocardial infarction (MI, i.e., heart attack), ischemic stroke (IS), and cardiovascular mortality occurring in populations without prior CVD events {D'Agostino, 2008, 10694408; Goff, 2014, 3121148; Lloyd-Jones, 2017, 10694407}. Additionally, disturbances in cholesterol homeostasis contribute to the pathology of non-alcoholic fatty liver disease (NAFLD)

and to accumulation of lipids in hepatocytes {Malhotra, 2020, 10442471}. Cholesterol is made and metabolized in the liver, and thus the evidence indicating that PFOS exposure disrupts lipid metabolism, suggests that toxic disruptions of lipid metabolism by PFOS are indications of hepatoxicity. Associations between PFOS and other serum lipids (i.e., TG and HDL) were less certain, though there was some evidence of positive associations with blood pressure and hypertension in adults.

Though results reported in animal toxicological studies support the alterations in lipid metabolism observed in epidemiological studies, variations in the direction of effect with dose increases the uncertainty of the biological relevance of these responses in rodents to humans. Additionally, the available mechanistic data does not help to explain the non-monotonicity of serum lipid levels and decreased serum lipid levels at higher PFOS dose levels in rodents (Section 3.4.3.3). EPA did not derive PODs for animal toxicological studies reporting cardiovascular effects, such as altered serum lipid levels, due to uncertainties about the human relevance of these responses.

Three *medium* confidence epidemiologic studies were considered for POD derivation (Table 4-1) {Dong, 2019, 5080195; Lin, 2019, 5187597; Steenland, 2009, 1291109}. These candidate studies offer a variety of PFOS exposure measures across various populations. Dong et al. (2019, 5080195) investigated the NHANES population (2003–2014), while Steenland et al. (2009, 1291109) investigated effects in a high-exposure community (the C8 Health Project study population). Lin et al. (2019, 5187597) collected data from prediabetic adults from the Diabetes Prevention Program (DPP) and DPP Outcomes Study at baseline (1996–1999). Dong et al. (2019, 5080195) and Steenland et al. (2009, 1291109) excluded individuals prescribed cholesterol medication from their analyses, a potential confounder for the total cholesterol endpoint, whereas Lin et al. (2019, 5187597) did not.

4.1.1.4 Developmental effects

As reviewed in Section 3.4.4.4, ***evidence indicates*** that elevated exposures to PFOS are associated with developmental effects in humans. As described in Table 3-12, the majority of epidemiological studies assessed endpoints related to fetal growth restriction (20 *high* and 13 *medium* confidence studies) and gestational duration (10 *high* and 5 *medium* confidence studies), while several studies also reported on endpoints related to fetal loss (2 *high* and 2 *medium* confidence studies) and birth defects (3 *medium* confidence studies). Findings from the small number of studies reporting on birth defects were mixed and generally limited in terms of the number of studies reporting specific effects and therefore were not prioritized for quantitative assessments. Although half of the available *high* and *medium* confidence studies reported increased incidence of fetal loss (2/4), EPA did not prioritize this endpoint for dose-response analyses as there were a relatively limited number of studies compared to endpoints related to gestational duration and fetal growth restriction and the evidence from *high* confidence studies was mixed. The impacts observed on fetal loss are supportive of an association between PFOS exposure and adverse developmental effects.

Approximately half of the available studies reporting metrics of gestational duration observed increased risk associated with PFOS exposure. Seven of the thirteen *medium* or *high* confidence studies reported adverse effects on gestational age at birth and seven of the eleven *medium* or *high* confidence studies reported an association with preterm birth. There were generally

consistent associations with adverse effects on preterm birth, particularly from the *high* confidence studies, with several studies reporting statistically significant results. However, several studies did not report exposure-response relationships and no definitive patterns or explanations were seen based on study characteristics that would help to explain why some studies reported associations while others did not. Results for gestational age were also relatively consistent but there was similar uncertainty due to a lack of rationale explaining inconsistent responses between studies. While overall there appears to be associations between PFOS exposure and gestational duration, the inconsistencies in the database reduce the level of confidence in the responses preferred for endpoints prioritized for dose-response modeling.

The adverse effects on gestational duration were consistent with effects on fetal growth restriction. The majority of *high* and *medium* confidence epidemiological studies (16/27) reported associations between PFOS and decreased mean birth weight in infants. Studies on changes in standardized birth weight measures (i.e., z-scores) also generally reported inverse associations (8/12 studies; 6 *high* and 2 *medium* confidence). Low birth weight is clinically defined as birth weight less than 2,500 g (approximately 5.8 lbs.) and can include babies born small for gestational age (SGA; birth weight below the 10th percentile for gestational age, sex, and parity) {JAMA, 2002, 10473200; McIntire, 1999, 15310; U.S. EPA, 2013, 4158459}. Low birth weight is widely considered a useful measure of public health {Cutland, 2017, 10473225; Lira, 1996, 10473966; Vilanova, 2019, 10474271; WHO, 2004, 10473140} and is on the World Health Organization's (WHO's) global reference list of core health indicators {WHO, 2014, 10473141; WHO, 2018, 10473143}.

Substantial evidence links low birth weight to a variety of adverse health outcomes at various stages of life. It has been shown to predict prenatal mortality and morbidity {Cutland, 2017, 10473225; U.S. EPA, 2013, 4158459; WHO, 2014, 10473141} and is a leading cause of infant mortality in the United States {CDC, 2020, 10473144}. Low-birth-weight infants are also more likely to have underdeveloped and/or improperly functioning organ systems (e.g., respiratory, hepatic, cardiovascular), clinical manifestations of which can include breathing problems, red blood cell disorders (e.g., anemia), and heart failure {Guyatt, 2004, 10473298; JAMA, 2002, 10473200; U.S. EPA, 2013, 4158459; WHO, 2004, 10473140; Zeleke, 2012, 10474317}. Additionally, low-birth-weight infants evaluated at 18 to 22 months of age demonstrated impaired mental development {Laptook, 2005, 3116555}.

Low birth weight is also associated with increased risk for diseases in adulthood, including obesity, diabetes, and cardiovascular disease {Gluckman, 2008, 10473269; Osmond, 2000, 3421656; Risnes, 2011, 2738398; Smith, 2016, 10474151; Ong, 2002, 10474127, as reported in Yang et al. (2022, 10176603). Poor academic performance, cognitive difficulties {Hack, 2002, 3116212; Larroque, 2001, 10473940}, and depression {Loret de Mola, 2014, 10473992} in adulthood have also been linked to low birth weight. These associations between low birth weight and infant mortality, childhood disease, and adult disease establish low birth weight as an adverse effect. Given the known consequences of this effect, as well as the consistency of the database and large number of *high* confidence studies reporting statistically significant odds of this effect, the endpoint of low birth weight in humans was considered for dose-response modeling.

Results reported in animal toxicological studies are consistent with the observed developmental toxicity in epidemiological studies. Specifically, studies in rodents found that gestational PFOS

treatment resulted in reduced offspring weight (8/14 *medium* confidence studies), decreased offspring survival (5/9 *medium* confidence studies), and altered maternal weight (6/12 *medium* confidence studies). Though limited in number, several other studies also reported consistent effects on placental endpoints, reduced ossification, and developmental delays.

Given the large number of adverse effects identified in the animal toxicological database for the developmental health outcome, EPA considered only the most sensitive effects in pups supported by multiple studies for derivation of PODs. EPA focused on the animal studies with effects in the offspring, as opposed to maternal effects, because these effects provide concordance with the approximate timing of low birth weight observed in human infants. The one study reporting altered maternal weight without confounding effects on the offspring {Argus, 2000, 5080012} could not be considered for derivation of a POD because the study was in rabbits and the pharmacokinetic model EPA used to predict internal dose in the animal models is parameterized for mice, rats and monkeys but rabbits. EPA also focused on endpoints for which multiple studies corroborated the observed effect, thereby increasing the confidence in that effect. Multiple animal toxicological studies observed effects at low dose levels and demonstrated a dose-related response in pups for decreased fetal and pup body weight and decreased offspring survival. EPA also focused on studies with exposure durations lasting through the majority of gestation and/or lactation (i.e., from GD 1 until postnatal development) rather than those that targeted a specific period of gestation as they were more likely to be sensitive for detection of developmental effects. Overall, the developmental effects seen in the offspring of rodents treated with PFOS are supportive of low birth weight and potential consequences of low birth weight observed in human populations.

Six *high* confidence epidemiologic studies {Chu, 2020, 6315711; Darrow, 2013, 2850966; Sagiv, 2018, 4238410; Starling, 2017, 3858473; Wikström, 2020, 6311677; Yao, 2021, 9960202} and 2 *medium* confidence animal toxicological studies {Lee, 2015, 2851075; Luebker, 2005, 757857} were considered for POD derivation (Table 4-1). The candidate epidemiological studies offer a variety of PFOS exposure measures across the fetal and neonatal window. All studies reported their exposure metric in units of ng/mL and reported the β coefficients per ng/mL or ln(ng/mL), along with 95% confidence intervals, estimated from linear regression models. Given the consistency of effects on offspring weight and survival across studies and species, decreased pup body weight at LD 5 from a reproductive study (exposure to dams from 42 days prior to mating until LD 5) as reported by Luebker et al. (2005, 757857), and increased fetal death and decreased fetal weight in offspring exposed to PFOS from GD 11–16 reported by Lee et al. (2015, 2851075) were considered for the derivation of PODs.

Table 4-1 summarizes the studies and endpoints considered for POD derivation.

Table 4-1. Summary of Endpoints and Studies Considered for Dose-Response Modeling and Derivation of Points of Departure for All Effects in Humans and Rodents

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Derived?	Notes
Immune Effects				
Reduced Antibody Concentrations for Diphtheria and Tetanus	Budtz-Jørgensen and Grandjean (2018, 5083631)[a] Medium Timmerman et al. (2021, 9416315) Medium	Human, male and female children	Yes	. Decreases in childhood antibody responses to pathogens such as diphtheria and tetanus were observed at multiple timepoints in childhood, using both prenatal and childhood exposure levels. Effect was large in magnitude and generally coherent with epidemiological evidence for other antibody effects
Reduced Antibody Concentrations for Rubella	Granum et al. (2013, 1937228) Medium	Human (male and female children)	No	Effect was large in magnitude and generally coherent with epidemiological evidence for other antibody effects, however, the data were not suitable for application of a BMR of 1 SD and ½ SD to provide a reasonably good estimate of 10% and 5% extra risk. The Benchmark Dose Technical Guidance {U.S. EPA, 2012, 1239433} explains that in a control population where 1.4% are considered to be at risk of having an adverse effect, a downward shift in the control mean of one SD results in about 10% extra risk of being at risk of having an adverse effect. The cut off value of 0.1 IU/mL resulted in 0.003% of the control population at risk of having an adverse effect, a value much smaller than 1.4% which in turn did not result in 10% extra risk (see PFOS Appendix).
Decreased Plaque Forming Cell (PFC) Response to SRBC	Zhong et al. (2016, 3748828) Medium	C57BL/6 Mice, F_1 males	Yes	Indicative of immunosuppression. Effect was consistently observed across multiple studies: Peden-Adams et al. (2008, 1424797), Dong et al. (2009, 1424951), Zheng et al. (2009, 1429960), and Keil et al. (2008, 1332422). Zhong et al. (2016, 3748828) was selected because the study tested a relatively low dose range and the effect was measured in a sensitive lifestage and time point (pups at PNW 4).
Extramedullary Hematopoiesis in the Spleen	NTP (2019, 5400978) High	Sprague-Dawley Rats, male and female	Yes	Blood cell production outside of the bone marrow which occurs when normal cell production is impaired. Selected for POD derivation because the results were from a *high* confidence study, histopathologically confirmed, consistent across both sexes, accompanied by evidence of bone marrow hypocellularity, and consistent with other studies that reported alterations in circulating immune cells, splenic cellularity, and thymic cellularity.
Developmental Effects				

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Derived?	Notes
Decreased Birth Weight	Chu et al. (2020, 6315711) High Darrow et al. (2013, 2850966) High Sagiv et al. (2018, 4238410) High Starling et al. (2017, 3858473) High Wikström et al. (2020, 6311677) High Yao et al. (2021, 9960202) High	Human, male and female infants	Yes	Evidence for developmental effects is based on consistent adverse effects for FGR including birthweight measures which are the most accurate endpoint. Some deficits were consistently reported for birth weight and standardized birth weight in many high and medium confidence cohort studies. Effect was generally large in magnitude and coherent with epidemiological evidence for other biologically related effects.
Decreased Fetal Body Weight	Lee et al. (2015, 2851075) Medium	CD-1 Mice, F_1 males and females	Yes	Effect was consistently observed across multiple studies and species {Argus, 2000, 5080012; Li, 2016, 3981495} and is coherent with epidemiological evidence of low birth weight. Lee et al. (2015, 2851075) was selected because there is a pharmacokinetic model available to extrapolate from exposures in mice to exposures in humans, the study tested a relatively low dose range, and mice appear to be a more sensitive model for this endpoint than rats.
Decreased Pup Body Weight	Luebker et al. (2005, 757857) Medium	Sprague-Dawley Rats, F_1 male and female	Yes	Effect was consistently observed across multiple studies and species {Luebker, 2005, 1276160; Lau, 2003, 757854} and is coherent with epidemiological evidence of low birth weight. Luebker et al. (2005, 757857) was selected because the study tested a relatively large number of dose groups and a low dose range. This study was previously selected as the overall RfD for PFOS in the 2016 HESD {U.S. EPA, 2016, 3603365}.
Increased Number of Dead Fetuses	Lee et al. (2015, 2851075) Medium	CD-1 Mice, females	Yes	Decreased offspring survival was consistently observed across multiple studies and species and is also consistent with other developmental effects related to survival observed in rodents {Luebker, 2005, 1276160; Argus, 2000, 5080012; Lau, 2003, 757854; Luebker, 2005, 757857}. Lee et al. (2015, 2851075) was selected because there is a pharmacokinetic model available to extrapolate from exposures in mice to exposures in humans, the study tested a relatively low dose range, and mice appear to be a more sensitive model for this endpoint than rats.

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Derived?	Notes
		Serum Lipid Effects		
Increased Total Cholesterol	Dong et al. (2019, 5080195) Medium Lin et al. (2019, 5187597) Medium Steenland et al. (2009, 1291109)[b] Medium	Human, male and female	Yes	Effect supported by an association in PFOS and blood pressure from the epidemiological studies. Effect observed in studies designed to exclude individuals prescribed cholesterol medication, minimizing concerns of bias due to medical intervention {Dong, 2019, 5080195; Steenland, 2009, 1291109}.
		Hepatic Effects		
Increased ALT	Gallo et al. (2012, 1276142) Medium Nian et al. (2019, 5080307) Medium	Human (male and female adults)	Yes	Effect was consistent and observed across multiple populations including general population adults {Lin, 2010, 1291111} (NHANES) and high-exposure communities {Gallo, 2012, 1276142} (C8 Health Project); Nian, 2019, 5080307} (Isomers of C8 Health Project in China)
Increased ALT	Lin et al. (2010, 1291111) Medium	Human (male and female adults)	No	While this is a large nationally representative population, several methodological limitations preclude its use for POD derivation. Limitations include lack of clarity about base of logarithmic transformation applied to PFOS concentrations in regression models, and the choice to model ALT as an untransformed variable, a departure from the typically lognormality assumed in most of the ALT literature.
Individual Cell Necrosis in the Liver	Butenhoff et al. (2012, 1276144) High	Sprague-Dawley rats, females	Yes	Effect was supported by a similar response in males from the same study {Butenhoff, 2012, 1276144}. Effect was accompanied by a hepatic inflammatory cell response in females. Effect was qualitatively observed in Xing et al. (2016, 3981506) and Cui et al. (2009, 757868), and further supported by increases in serum enzyme levels associated with hepatic damage in both animals and humans.

Notes: PNW = postnatal week; ALT = alanine transaminase; F_1 =first generation.

[a] Supported by Grandjean et al. (2012, 1248827); Grandjean et al. (2017, 3858518); Grandjean et al. (2017, 4239492).

[b] See Section 6.6.3 for discussion on the approach to estimating BMDs from regression coefficients.

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 intended to represent a minimal, biologically significant level of change. The *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433} describes a hierarchy by which BMRs are selected, with the first and preferred approach being the use of 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 guidance document recommends BMRs that could be used in the absence of information about a minimal clinical or biological level of change considered to be adverse—specifically, a BMR of one standard deviation (SD) change 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 the *Benchmark Dose Technical Guidance* suggests that studies of developmental effects can support lower BMRs. BMDs for these effects may employ a BMR of 0.5 SD change from the control mean for continuous data or a BMR of 5% for dichotomous data {U.S. EPA, 2012, 1239433}. A lower BMR can also be used if it can be justified on a biological and/or statistical basis. The *Benchmark Dose Technical Guidance* (page 23; {U.S. EPA, 2012, 1239433}) shows that in a control population where 1.4% are considered to be at risk of having an adverse effect, a downward shift in the control mean of one SD results in a ~10% extra risk of being at risk of having an adverse effect. A BMR smaller than 0.5 SD change from the control mean is generally used for severe effects (e.g., 1% extra risk of cancer mortality).

Based on rationales described in EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}, the IRIS Handbook {U.S. EPA, 2022, 10367891} and past IRIS assessment precedent, BMRs were selected for dose-response modeling of PFOS-induced health effects for individual study endpoints as described below and summarized in Table 4-2 along with the rationales for their selection. For this assessment, EPA took statistical and biological considerations into account to select the BMR. For dichotomous responses, the general approach was to use 10% extra risk as the BMR for borderline or minimally adverse effects and either 5% or 1% extra risk for adverse effects, with 1% reserved for the most severe effects. For continuous responses, the preferred approach for defining the BMR was to use a preestablished cutoff for the minimal level of change in the endpoint at which the effect is generally considered to become biologically significant (e.g., greater than or equal to 42 IU/L serum ALT in human males {Valenti, 2021, 10369689}) In the absence of an established cutoff, a BMR of 1 SD change from the control mean, or 0.5 SD for effects considered to be severe, was generally selected. Specific considerations for BMR selection for endpoints under each of the priority non-cancer health outcomes are described in the subsections below. Considerations for BMR selection for cancer endpoints are described in Section 4.2.

4.1.2.1 Hepatic Effects

Modeling elevated human ALT used cutoff levels of 42 IU/L for males and 30 IU/L for females, based on the most recent sex-specific upper reference limits {Valenti, 2021, 10369689}. The baseline prevalence of elevated ALT is estimated as 14% and 13% in U.S. male and female

adults (aged 20 and older), respectively (see PFOS Appendix). Therefore, the BMR was defined as a 5% increase in the number of people with ALT values above the cutoffs. Although the *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433} recommends a BMR of 10% extra risk for dichotomous data when biological information is not sufficient to identify the BMR, in this situation, such a BMR would result in a doubling of risk.

For the adverse effect of individual cell necrosis observed in livers of rats following PFOS exposure, there is currently inadequate available biological or toxicological information to permit determination of a minimal biologically significant response level. Therefore, in accordance with EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}, a BMR of 10% extra risk was used (dichotomous data; see Table 4-2).

4.1.2.2 Immune Effects

For the developmental immune endpoint of decreased diphtheria and tetanus antibody response in children found to be associated with PFOS exposure, the BMD and the BMDL were estimated using a BMR of 0.5 SD change from the control mean (see Table 4-2). Consistent with EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}, EPA typically selects a 5% or 0.5 standard deviation (SD) benchmark response (BMR) when performing dose response modeling of data from an endpoint resulting from developmental exposure. Because Budtz-Jørgensen and Grandjean (2018, 5083631) and Timmerman et al. (2021, 9416315) assessed antibody response after PFAS exposure during gestation and childhood, these are considered developmental studies {U.S. EPA, 1991, 732120} based on EPA's *Guidelines for Developmental Toxicity Risk Assessment*, which includes the following definition:

> "Developmental toxicology - The study of adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the lifespan of the organism."

EPA guidance recommends the use of a 1 or 0.5 SD change in cases where there is no accepted definition of an adverse level of change or clinical cut-off for the health outcome {U.S. EPA, 2012, 1239433}. A 0.5 SD was selected since the health outcome is developmental and there is no accepted definition of an adverse level of change or clinical cut-off for reduced antibody concentrations in response to vaccination. Therefore, EPA performed the BMDL modeling using a BMR equivalent to a 0.5 SD change in log2-transformed antibody concentrations, as opposed to a fixed change in the antibody concentration distributions {U.S. EPA, 2012, 1239433}.

For the adverse effects of decreased plaque forming cell (PFC) response to SRBC observed in mice and splenic extramedullary hematopoiesis in rats following PFOS exposure, there is currently inadequate available biological or toxicological information to permit determination of minimal biologically significant response levels. Therefore, in accordance with EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}, a BMR of 1 SD change from the control mean was employed for the effect on PFC response (continuous data) and a BMR of 10% extra risk was used for the increased incidence of extramedullary hematopoiesis (dichotomous data) (see Table 4-2).

4.1.2.3 Cardiovascular Effects

Modeling human cholesterol used a cutoff level of 240 mg/dL for elevated serum total cholesterol, consistent with the American Heart Association's definition of hypercholesterolemia {NCHS, 2019, 10369680}. Recent data (for years 2015-2018) show that the percentage of U.S. adults aged 20 and older with total cholesterol ≥240 mg/dL is 11.5% {NCHS, 2019, 10369680}. Therefore, the BMR was defined as a 5% increase in the number of people with total cholesterol values above 240 mg/dL. Although the *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433} recommends a BMR of 10% extra risk for dichotomous data when biological information is not sufficient to identify the BMR, in this situation, such a BMR would result in a doubling of risk.

4.1.2.4 Developmental Effects

For the developmental endpoint of decreased birth weight in infants associated with PFOS exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk, given that this level of response is typically used when modeling developmental responses from animal toxicology studies, and that low birthweight confers increased risk for adverse health effects throughout life {Hack, 1995, 8632216; Reyes, 2005, 1065677; Tian, 2019, 8632212}. Low birth weight is clinically defined as birth weight less than 2,500 g (approximately 5.8 lbs) and can include babies born SGA (birth weight below the 10th percentile for gestational age, sex, and parity) {JAMA, 2002, 10473200; McIntire, 1999, 15310; U.S. EPA, 2013, 4158459}.

For decreased fetal and pup weights and decreased pup survival observed in animal studies, a BMR of 5% relative deviation and 0.5 SD from the control was employed, respectively (see Table 4-2). This is consistent with EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433} and the IRIS Handbook {U.S. EPA, 2022, 10367891}, which note that studies of adverse developmental effects represent a susceptible lifestage and can support BMRs that are lower than 10% extra risk (dichotomous data) and 1 SD change from the control mean (continuous data).

A 5% relative deviation in markers of growth in gestational exposure studies (e.g., fetal weight) that do not lead to death has generally been considered an appropriate biologically significant response level and has been used as the BMR in final IRIS assessments (e.g., U.S. EPA (2003, 1290574), U.S. EPA (2004, 198783), and U.S. EPA (2012, 3114808)). Additionally, the 5% BMR selection is statistically supported by data which compared a BMR of 5% relative deviation for decreased fetal weight to NOAELs and other BMR measurements, including 0.5 standard deviation, and found they were statistically similar {Kavlock, 1995, 75837}.

Table 4-2. Benchmark Response Levels Selected for BMD Modeling of Health Outcomes

Endpoint	BMR	Rationale
Immune Effects		
Reduced antibody concentrations for diphtheria and tetanus in children (developmental immune endpoint)	0.5 SD	**Consistent with EPA guidance.** EPA typically selects a 5% or 0.5 standard deviation (SD) benchmark response (BMR) when performing dose response modeling of data from an endpoint resulting from developmental exposure and selects a 1 or 0.5 SD change in cases where there is no accepted definition of an

Endpoint	BMR	Rationale
		adverse level of change or clinical cut-off for the health outcome {U.S. EPA, 2012, 1239433}
Decreased Plaque Forming Cell (PFC) Response to SRBC	1 SD	**Insufficient information available to determine minimal biologically significant response level.** The available biological or toxicological information does not allow for determination of a minimal biologically significant response level for this adverse effect, and so a BMR of one SD was used as per EPA guidance {U.S. EPA, 2012, 1239433}
Extramedullary Hematopoiesis in the Spleen	10%	**Insufficient information available to determine minimal biologically significant response level.** The available biological or toxicological information does not allow for determination of a minimal biologically significant response level for this adverse effect, and so a BMR of 10% was used as per EPA guidance {U.S. EPA, 2012, 1239433}
Developmental Effects		
Decreased Birth Weight in Infants or Decreased Pup Body Weight in Rodent Offspring	5%	**Consistent with EPA guidance.** EPA typically selects a 5% or 0.5 standard deviation (SD) benchmark response (BMR) when performing dose response modeling of data from an endpoint resulting from developmental exposure {U.S. EPA, 2012, 1239433}
Increased Number of Dead Fetuses	0.5 SD	**Consistent with EPA guidance.** EPA typically selects a 5% or 0.5 standard deviation (SD) benchmark response (BMR) when performing dose response modeling of data from an endpoint resulting from developmental exposure {U.S. EPA, 2012, 1239433}
Serum Lipids		
Increased Cholesterol	5%	**Response rate of 5% extra risk is reasonable, whereas a 10% BMR would result in a doubling of risk.** Although EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433} recommends a BMR based on a 10% extra risk for dichotomous endpoints when biological information is not sufficient to identify the BMR, in this situation such a BMR would result in a highly improbable doubling of risk.
Hepatic Effects		
Increased ALT	5%	**Response rate of 5% extra risk is reasonable, whereas a 10% BMR would result in a doubling of risk.** Although EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433} recommends a BMR based on a 10% extra risk for dichotomous endpoints when biological information is not sufficient to identify the BMR, in this situation such a BMR would result in a highly improbable doubling of risk
Individual Cell Necrosis	10%	**Insufficient information available to determine minimal biologically significant response level.** The available biological or toxicological information does not allow for determination of a minimal biologically significant response level for this adverse effect, and so a BMR of 10% was used as per EPA guidance {U.S. EPA, 2012, 1239433}

Notes: ALT = alanine transaminase; BMD = benchmark dose; BMR = benchmark response; CDC = Centers for Disease Control; SD = standard deviation.

4.1.3 Pharmacokinetic Modeling Approaches to Convert Administered Dose to Internal Dose in Animals and Humans

4.1.3.1 Pharmacokinetic 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 PFOS dosimetry in experimental animals based on the following criteria:

- availability of model parameters across the species of interest,
- agreement with out-of-sample datasets (see PFOS Appendix), and
- flexibility to implement life course modeling.

These criteria originated from the goal of accurately predicting internal dose metrics for toxicology studies that were selected for dose-response analysis. These studies involved rats, mice, and non-human primates, and these were the species of interest necessary to have available model parameters. Good agreement with out-of-sample datasets shows that the model performance is good compared to both the data used to identify model parameters and to external data. This increases confidence that the model can be used to make accurate predictions of internal dose metrics for the toxicology studies, which can also be seen as external. The ability to implement life-course modeling was necessary to properly predict internal dose metrics for developmental studies and endpoints as the animal transitioned through numerous life-stages.

In this case, an oral dosing version of the original model structure introduced by Andersen et al. (2006, 818501) and summarized in Section 3.3.2 was selected for having the fewest number of parameters that would need estimation. 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 toxicological studies of PFOS.

The Wambaugh et al. (2013, 2850932) model was selected for pharmacokinetic modeling for animal internal dosimetry for several important reasons: 1) it allowed for sex-dependent concentration-time predictions for PFOS across all three species of interest, 2) it adequately predicted dosimetry of newer datasets published after model development, and 3) it was amendable to addition of a life stage component for predicting developmental study designs. These analyses are further described below. Uncertainties and limitations of the selected modeling approach are described in Section 6.6.1.

4.1.3.1.1 Animal Model Parameters

Table 4-3. PK Parameters from Wambaugh et al. (2013, 2850932) Meta-Analysis of Literature Data for PFOS

Parameter	Units	CD1 Mouse (F)[a]	CD1 Mouse (M)[a]	Sprague- Dawley Rat (F)[a]	Sprague- Dawley Rat (M)[a]	CynomolgusMonkey (M/F)[a]
Body weight[b] (BW)	kg	0.02	0.02	0.203	0.222	3.42
Cardiac Output[c] (Q_{cc})	L/h/kg$^{0.74}$	8.68	8.68	12.39	12.39	19.8
Absorption rate (k_a)	1/h	1.16 (0.617–42,400)	433.4 (0.51–803.8)	4.65 (3.02–1,980)	0.836 (0.522–1.51)	132 (0.225–72,100)
Central Compartment Volume (V_{cc})	L/kg	0.264 (0.24–0.286)	0.292 (0.268–0.317)	0.535 (0.49–0.581)	0.637 (0.593–0.68)	0.303 (0.289–0.314)
Intercompartment transfer rate (k_{12})	1/h	0.0093 ($2.63 \times e^{-10}$–38,900)	2,976 ($2.8 \times e^{-10}$–$4.2 \times e^{4}$)	0.0124 ($3.1 \times e^{-10}$–46,800)	0.00524 ($2.86 \times e^{-10}$–43,200)	0.00292 ($2.59 \times e^{-10}$–34,500)
Intercompartment ratio ($R_{V2:V21}$)	Unitless	1.01 (0.251–4.06)	1.29 (0.24–4.09)	0.957 (0.238–3.62)	1.04 (0.256–4.01)	1.03 (0.256–4.05)
Maximum resorption rate (T_{maxc})	µmol/h	57.9 (0.671–32,000)	$1.1 \times e^{4}$ ($2.1–7.9 \times e^{4}$)	1,930 (4.11–83,400)	$1.34 \times e^{-6}$ ($1.65 \times e^{-10}$–44)	15.5 (0.764–4,680)
Renal resorption affinity (K_T)	µmol	0.0109 ($1.44 \times e^{-5}$–1.45)	381 ($2.6 \times e^{-5}$–$2.9 \times e^{3}$)	9.49 (0.00626–11,100)	2.45 ($4.88 \times e^{-10}$–60,300)	0.00594 ($2.34 \times e^{-5}$–0.0941)
Free fraction	Unitless	0.00963 (0.00238–0.0372)	0.012 (0.0024–0.038)	0.00807 (0.00203–0.0291)	0.00193 (0.000954–0.00249)	0.0101 (0.00265–0.04)
Filtrate flow rate (Q_{filc})	Unitless	0.439 (0.0125–307)	27.59 (0.012–283)	0.0666 (0.0107–8.95)	0.0122 (0.0101–0.025)	0.198 (0.012–50.5)
Filtrate volume (V_{filc})	L/kg	0.00142 ($4.4 \times e^{-10}$–6.2)	0.51 ($3.5 \times e^{-10}$–6.09)	0.0185 ($8.2 \times e^{-7}$–7.34)	0.000194 ($1.48 \times e^{-9}$–5.51)	0.0534 ($1.1 \times e^{-7}$–8.52)

Notes: F = female; M = male.
Means and 95% credible intervals (in parentheses) from Bayesian analysis are reported. For some parameters the distributions are quite wide, indicating uncertainty in that parameter (i.e., the predictions match the data equally well for a wide range of values).
[a] Data sets modeled for the mouse and rat were from Chang et al. (2012, 1289832) and for the monkey from Seacat et al. (2002, 757853) and Chang et al. (2012, 1289832).
[b] Average bodyweight for species:individual-specific bodyweights.
[c] Cardiac outputs obtained from Davies and Morris (1993, 192570).

4.1.3.1.2 Out-of-Sample Comparisons

To evaluate the model's ability to predict PFOS concentration-time data in the species of interest, EPA compared model fits to *in vivo* datasets published following the 2016 HESD (Table 4-4). For rats, the data of Kim et al. (2016, 3749289) and Huang et al. (2019, 7410147) were used. Model simulations demonstrated good agreement with available data for adult time-course PFOS PK predictions in the rat. However, there was no comparable PK dataset for PFOS in mice. Therefore, only the original study used for parameter determination {Chang, 2012, 1289832} was compared to model simulations. This comparison approach demonstrated agreement with the *in vivo* data.

Using the Wambaugh et al. (2013, 2850932) model, EPA predicted the half-life, V_d, and clearance and compared these species-specific predictions to values obtained from *in vivo* studies when data were available.

Following out-of-sample dataset evaluation of the female rat PK parameters (Table 4-4) and visual inspection of the resulting concentration-time fits, EPA determined that only male PK model parameters would be used for all rat-specific modeling. This assumption agrees with Kim et al. (2016, 3749289) where they report no PK differences between the sexes for PFOS ADME.

Table 4-4. Model Predicted and Literature PK Parameter Comparisons for PFOS

	Male			Female		
	t1/2,β (days)	**Vd,β (L/kg)**	**CL (L/d/kg)**	**t1/2,β (days)**	**Vd,β (L/kg)**	**CL (L/d/kg)**
			Rat			
Model	44.13	0.638	0.01	282.05	0.538	0.0013
Literature	28.7[a], 39.7[b]	0.382[a], 0.681[b]	0.0092[a], 0.013[b]	24.8[a], 32.8[b]	0.288[a], 0.421[b]	0.008[a], 0.009[b]
			Mouse			
Model	134.83	0.472	0.0024	38.4	1.41	0.0255
Literature	–	–	–	–	–	–

Notes: CL = clearance; PK = pharmacokinetic; $t_{1/2,\beta}$ = terminal-phase elimination half-life; V_d, β = volume of distribution during the terminal phase.
[a] Information obtained from Kim et al. (2016, 3749289).
[b] Information obtained from Huang et al. (2019, 5387170).

4.1.3.1.3 Life Course Modeling

The Wambaugh et al. (2013, 2850932) model was modified to allow for a gestation, lactation, and post-weaning phase (Figure 4-1). Using the original model structure and published parameters, simulations assumed that dams were dosed prior to conceptions and up to the date of parturition. Following parturition, a lactational phase involved PFOS transfer from the breastmilk to the suckling pup where the pup was modeled 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.

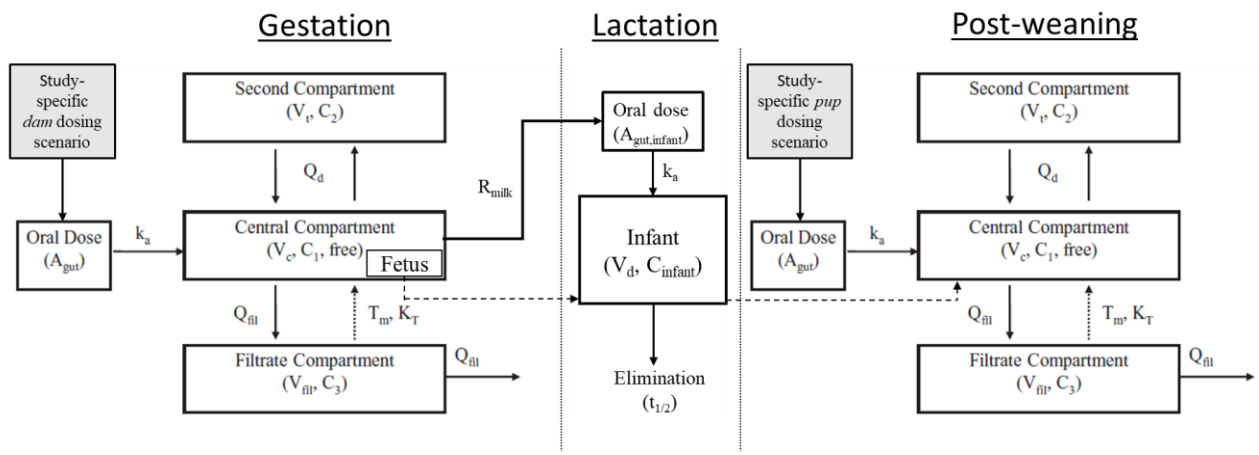


Figure 4-1. Model Structure for Life Stage Modeling

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. (2022, 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.
- Infant animal growth during the lactational period is governed by the infant growth curves outlined in Kapraun et al. (2022, 9641977).
- Rapid equilibrium between maternal serum PFOS and milk PFOS is assumed and modeled using a serum:milk partition coefficient.
- 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 4-5 below.
- Following the lactation period, infant time course concentrations are tracked using the more physiologically-based Wambaugh model to model post-weaning exposure and infant growth.

A simple one-compartment model for infant lactational exposure was chosen because of differences between PFOS Vd reported in the literature and Wambaugh et al. (2013, 2850932) model-predicted Vd following extrapolation to a relatively low infant body weights. Because V_d is assumed to be extracellular water in humans, 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 (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, EPA used the literature reported V_d {Kim, 2016, 3749289; Chang, 2012, 1289832} for the one compartment model to describe infant

toxicokinetics (Table 4-5). Finally, the Wambaugh et al. (2013, 2850932) model was not parameterized for a post-partum infant, and it was not possible to evaluate the mechanistic assumptions for renal elimination with postnatal toxicokinetic data. Therefore, the parameters listed in Table 4-5 in a one-compartment gestation/lactation model were used in conjunction with the parameters published in Wambaugh et al. (2013, 2850932) to predict developmental dose metrics for PFOS.

Table 4-5. Additional PK Parameters for Gestation/Lactation for PFOS

Parameter	Units	Rat	Mouse
Maternal Milk:Blood Partition Coefficient (P_{milk})	Unitless	0.13[a]	0.32[e]
Fetus:Mother Concentration Ratio (R_{fm})	Unitless	0.83[b]	0.41[f]
Elimination Half-Life ($t_{1/2}$)	Days	40[c]	36.87[g]
Volume of Distribution (V_d)	L/kg	0.28[d]	0.26[g]
Starting Milk Consumption Rate (r^0_{milk})	kg_{milk}/day	0.001[h]	0.0001[i]
Week 1 Milk Consumption Rate (r^1_{milk})	kg_{milk}/day	0.003[h]	0.0003[i]
Week 2 Milk Consumption Rate (r^2_{milk})	kg_{milk}/day	0.0054[h]	0.00054[i]
Week 3 Milk Consumption Rate (r^3_{milk})	kg_{milk}/day	0.0059[h]	0.00059[i]

Notes: PK = pharmacokinetic.
[a] Information obtained from Loccisano et al. (2013, 1326665) (derived from Kuklenyik et al. (2004, 1598132)).
[b] Information obtained from Lau et al. (2003, 757854).
[c] Average of male/female half-lives reported in Huang et al. (2019, 5387170), Kim et al. (2016, 3749289), and Chang et al. (2012, 1289832).
[d] Information obtained from Kim et al. (2016, 3749289).
[e] Assume same P_{milk} as PFOA (lack of mouse data).
[f] Information obtained from Wan et al. (2020, 7174720).
[g] Information obtained from Chang et al. (2012, 1289832).
[h] Information obtained from Kapraun et al. (2022, 9641977) (adapted from Lehmann et al. (2014, 2447276)).
[i] Information obtained from Kapraun et al. (2022, 9641977) (mouse value is 10% of rat based on assumption that milk ingestion rate is proportional to body mass).

These developmental-specific parameters include the maternal milk:blood PFOS partition coefficient (P_{milk}), the ratio of the concentrations in the fetus(es) and the mother during pregnancy (R_{fm}), the species-specific *in vivo* determined half-life ($t_{1/2}$) and V_d for PFOS, and the species-specific milk consumption rate during lactation (r^i_{milk}) for the i^{th} week of lactation. Milk rate consumptions are defined as:

- r^0_{milk}, the starting milk consumption rate in kg milk per day (kg/d);
- r^1_{milk}, the (average) milk consumption rate (kg/d) during the first week of lactation (and nursing);
- r^2_{milk}, the (average) milk consumption rate (kg/d) during the second week of lactation; and
- r^3_{milk}, the (average) milk consumption rate (kg/d) during the third week of lactation.

where R_{milk} used in the model is a piecewise linear function comprising each r^i_{milk} depending on the week of lactation.

Using this gestation/lactation model, EPA fit one study for PFOS exposure in rats to ensure the model predicted the time-course concentration curves for both the dam and the pup. For all gestation/lactation studies, time zero represents conception followed by a gestational window (21 days for the rat). Dosing prior to day zero represents pre-mating exposure to PFOS.

Figure 4-2 demonstrates the model's ability to predict gestation/lactation study design in the rat for dams exposed to 1.6 mg/kg/day PFOS giving birth to pups who are exposed through lactation {Luebker, 2005, 1276160}. For developmental PK simulations, the original Wambaugh et al. (2013, 2850932) model with increasing maternal weight predicts dam concentrations in female rats while the one-compartmental lactational transfer model predicts infant concentrations for pups exposed both *in utero* and through lactation only.

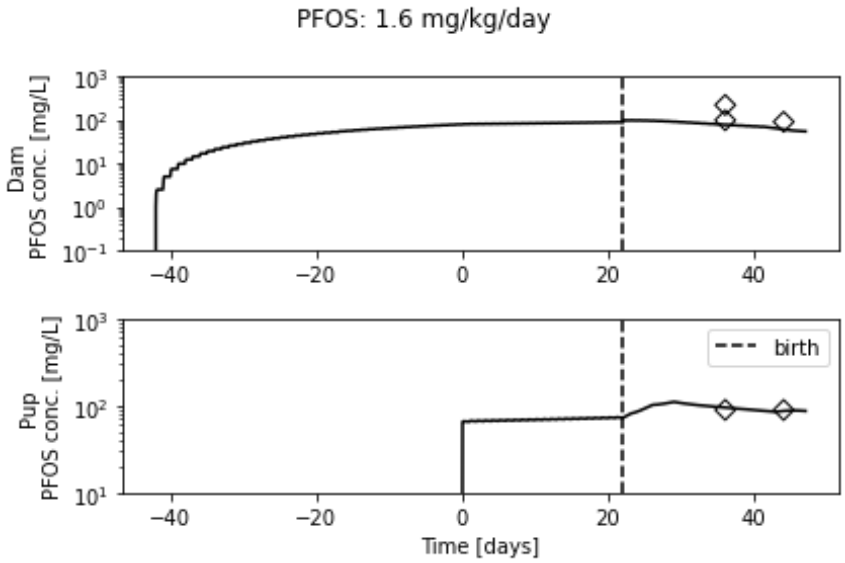


Figure 4-2. Gestation/Lactation Predictions of PFOS in the Rat

Top panel represents predicted dam concentrations with open diamonds (◊) representing the dam concentrations reported in Luebker et al. (2005, 1276160). Bottom panel represents predicted pup concentrations with open diamonds (◊) representing the reported pup concentrations in Luebker et al. (2005, 1276160) where the source of PFOS exposure is from the breast milk. Vertical dashed line represents birth.

The purpose of the animal PBPK model is to make predictions of internal dose in lab animals used in toxicity studies and extrapolate these internal dose points-of-departure to 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 PFOS 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 of the study (C_{last7}, 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 (C_{max}) and average blood concentrations (C_{avg}). In the dam, the C_{max} and C_{avg} were calculated over a range of life stages: during gestation ($C_{avg\_dam\_gest}$), during lactation ($C_{avg\_dam\_lact}$), or combined gestation and lactation ($C_{avg\_dam\_gest\_lact}$). In pups for C_{max}, 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} was calculated for during gestation, lactation or combined gestation and lactation ($C_{avg\_pup\_gest}$, $C_{avg\_pup\_lact}$ and $C_{avg\_pup\_gest\_lact}$).

4.1.3.2 Pharmacokinetic Model for Human Dosimetry

The key factors considered in model determination were to implement a human model from the literature that was able to model gestational and lactational exposure to infants, that was able to describe time course changes in serum concentration due to changes in bodyweight during growth, and that required minimal new development. Previous modeling efforts suggested 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 one compartment human developmental model published by Verner et al. (2016, 3299692). Several alternative models to EPA's updated version of the Verner et al. (2016, 3299692) model for the calculation of POD_{HED} from an internal POD were considered. This included consideration of full PBPK models (i.e., the Loccisano family of models {Loccisano, 2011, 787186; Loccisano, 2012, 1289830; Loccisano, 2012, 1289833; Loccisano, 2013, 1326665} and a developmental PBPK model in rats {Chou, 2021, 7542658}), as well as other one-compartment PK models (e.g., Goeden et al. (2019, 5080506)). Discussion on the justification for selection of the Verner et al. (2016, 3299629) model as the basis for the pharmacokinetic modeling approach used for PFOS is available in Sections 6.6.2 and 6.7.

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 for the code to be more accessible to others by updating it to a contemporary modeling language, as acslX software is no longer available or supported. The starting point for the conversion to R/MCSim was another model with a similar structure that was in development by EPA at that time {Kapraun, 2022, 9641977}. Second, 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}. Age-dependent breastmilk intake rates were based on the 95th percentile estimates from EPA's *Exposure Factors Handbook* and was defined relative to the infant's bodyweight {U.S. EPA, 2011, 786546}.

A third modification was the update of parameters: the half-life, V_d, the ratio of PFOS concentration in cord blood to maternal serum, and the ratio of PFOS concentration in breastmilk and maternal serum. Details for how these parameters were updated are given in the following paragraphs. In the model, half-life and V_d are used to calculate the clearance, which is used in the model directly and is also used for calculation of steady-state concentrations in adults. Other than half-life and, because of that, clearance, the updated parameters were similar to the original parameters (Table 4-6). The results of the new R model and updated acslX model with the original parameters were essentially identical (see PFOS Appendix). With the updated parameters, the predicted PFOS serum concentrations are approximately 60% of the original values during pregnancy, and the child's serum concentration is approximately 80% of the original values during the first year of life.

The use of the Verner model in humans presents a substantial advancement in approach for endpoints in children compared to the previous EPA assessment of PFOS {U.S. EPA, 2016, 3603365}. The previous assessment did not explicitly model children, but instead applied an uncertainty factor to an RfD based on long-term adult exposure to account for the potential for increased susceptibility. The current approach explicitly models PFOS exposure to infants during nursing and the rapid growth of children, who do not reach steady state until near adulthood. This allows for a more accurate estimation of exposures associated with either serum levels in children or dose metric from developmental animal toxicological studies. The Verner model also explicitly models the mother from her birth through the end of breastfeeding which allows for the description of accumulation in the mother prior to pregnancy followed by decreasing maternal levels during pregnancy. Detailed modeling of this period is important for dose metrics based on maternal levels during pregnancy, especially near term, and on cord blood levels.

Application of the updated Verner model to three cohorts with paired maternal measurements and subsequent samples in children between ages of 6 months and 6 years showed good agreement between reported and predicted serum levels in the children (See PFOS Appendix). This suggests that the assumptions made governing lactational transfer and the selected half-life value are reasonable. A local sensitivity analysis was also performed to better understand the influence of each parameter on model output (See PFOS Appendix).

Table 4-6. Updated and Original Chemical-Specific Parameters for PFOS in Humans

Parameter	Updated Value	Original Value[a]
Volume of Distribution (mL/kg)	230[b]	230
Half-life (yr)	3.4[c]	5.5
Clearance (mL/kg/d)	0.128[d]	0.079
Cord Serum:Maternal Serum Ratio	0.40[e]	0.42
Milk:Serum Partition Coefficient	0.016[f]	0.014

[a] Verner et al. (2016, 3299692).
[b] Thompson et al. (2010, 2919278).
[c] Li et al. (2018, 4238434).
[d] Calculated from half-life ($t_{1/2}$) and volume of distribution (V_d). Clearance (Cl) = $V_d * \ln(2)/t_{1/2}$.
[e] Average values for total PFOA Cord Serum:Maternal Serum ratios (see PFOS Appendix). This is a similar approach to that used by Verner et al. (2016, 3299692), but also includes studies made available after the publication of that model.
[f] Average value of studies as reported in Table 4-7. This is a similar approach to that used by Verner et al. (2016, 3299692), but also includes studies made available after the publication of that model.

EPA's approach for selection of half-life for this effort was to select a reported value from an exposure to the general population, with a clear decrease in exposure, a high number of individuals, and a long follow-up time. With these criteria, a half-life of 3.4 years for PFOS was selected {Li, 2018, 4238434}. This value for PFOS comes from a community with contaminated drinking water with serial samples of 106 individuals for a relatively short follow-up time of 2 years. A summary of PFOS half-life values is presented in the Appendix (See PFOS Appendix). Uncertainties related to EPA's selected half-life are discussed in Section 6.6.2.

The updated value for human V_d , 230 mL/kg, was sourced from Thompson et al. (2010, 2919278). To estimate the V_d for PFOS, Thompson et al. (2010, 2919278) scaled the value they obtained for PFOA by the ratio of V_ds obtained by Andersen et al. (2006, 818501) in the parameterization of that PK model using PK data in monkey. That is, V_d PFOA, human) = V_d

(PFOA, human*V_d (PFOS, monkey)/V_d (PFOA, monkey). V_d is a parameter that is relatively easily obtained from an analysis of PK data from a controlled experimental study, as it is related to the peak concentration observed after dosing and is expected to be similar between human and non-human primates {Mordenti, 1991, 9571900}. For comparison, the optimized V_d value from oral dosing in monkeys was 220 mL/kg for PFOS {Andersen, 2006, 818501}.

A summary of PFOS V_d values is presented in the Appendix (see PFOS Appendix). Uncertainties related to EPA's selected V_d are discussed in Section 6.6.2.

In the original model, the ratio of PFOS concentration in cord blood to maternal serum, and the ratio of PFOS concentration in breastmilk and maternal serum were based on an average of values available in the literature; here, EPA identified literature made available since the original model was published and updated those parameters with the averages of all identified values (Table 4-7). The values for cord blood to maternal serum ratio are presented in the Appendix (see PFOS Appendix). One restriction implemented on the measurements of the cord blood to maternal serum ratio was to only include reports where the ratio was reported, and not to calculate the ratio from reported mean cord and maternal serum values. This was due to potential bias that could be introduced if a greater proportion of cord blood measurements are below the limit of detection compared to maternal serum.

Table 4-7. Summary of Studies Reporting the Ratio of PFOS Levels in Breastmilk and Maternal Serum or Plasma

Source	HERO ID	Milk:Maternal Plasma Ratio	Included in Verner et al. (2016, 3299692) Analysis
Haug et al. (2011, 2577501)	2577501	0.014	No
Seung-Kyu Kim et al. (2011, 2919258)	2919258	0.011	No
Liu et al. (2011, 2919240)	2919240	0.020	No
Kärrman et al. (2007, 1290903)	1290903	0.010	No
Cariou et al. (2015, 3859840)[a]	3859840	0.011	Yes
Sunmi Kim et al. (2011, 1424975)[b]	1424975	0.030	Yes
Verner et al. (2016, 3299692)	3299692	0.014[c]	–
Additional Studies	–	0.016[d]	–

Whether studies were included in the analysis of Verner et al. (2016, 3299692) is noted. The reported values were based on the mean of ratios in the study populations except when noted otherwise.
[a] Median result based on the report of Pizzurro et al. (2019, 5387175).
[b] Median result as reported by the authors.
[c] Average value of milk:maternal plasma ratio used by Verner et al. (2016, 3299692).
[d] Average value of milk:maternal plasma ratio with the inclusion of additional studies not in the original analysis. This value was used in the human PK model.

This updated model was used to simulate the human equivalent doses (HED) from the animal PODs that were obtained from BMD modeling of the animal toxicological studies (see PFOS Appendix). It was also used to simulate selected epidemiological studies (Section 4.1.4) to obtain a chronic dose that would result in the internal POD obtained from dose-response modeling (see PFOS Appendix). For PODs resulting from chronic exposure, such as a long-term animal toxicological study or an epidemiological study on an adult cohort, the steady state approximation was used to calculate a POD_{HED} that would result in the same dose metric after chronic exposure. For PODs from exposure to animals in developmental scenarios, the updated

Verner model was used to calculate a POD_{HED} that results in the same dose metric during the developmental window selected. The updated Verner model was also used to calculate a POD_{HED} for PODs based on epidemiological observations of maternal serum concentration during pregnancy, cord blood concentration, and serum concentrations in children.

The pharmacokinetic modeling code for both the updated Wambaugh et al. (2013, 850932) and Verner et al. (2013, 299692) models that was used to calculate human equivalence doses is available in an online repository (https://github.com/USEPA/OW-PFOS-PFOA-MCLG-support-PK-models). The model code was thoroughly QA'd through the established EPA Quality Assurance Project Plan (QAPP) for PBPK models {U.S. EPA, 2018, 4326432}.

4.1.4 Application of Pharmacokinetic Modeling for Animal-Human Extrapolation of PFOS Toxicological Endpoints and Dosimetric Interpretation of Epidemiological Endpoints

Table 4-8 displays the POD and estimated internal and POD_{HED}s for immune, developmental, cardiovascular (serum lipids), and hepatic endpoints from animal and/or human studies selected for the derivation of candidate RfDs. The PODs from epidemiological studies (immune, developmental, hepatic, and serum lipid endpoints) were derived using benchmark dose modeling (see PFOS Appendix) which provided an internal serum concentration in mg/L. The internal dose PODs were converted to a POD_{HED} using the modified Verner model described in Section 4.1.3.1.3 to calculate the dose that results in the same serum concentrations. Specifically, reverse dosimetry was performed by multiplying an internal dose POD by a model predicted ratio of a standard exposure and the internal dose for that standard exposure. This expedited procedure can be performed because the human model is linear, that is, the ratio of external and internal dose is constant with dose. Additional details are provided below and in Table 4-8.

The PODs from the animal toxicological studies were derived by first converting the administered dose to an internal dose as described in Section 4.1.3.1.1. The rationale for the internal dosimetric selected for each endpoint is described in the Appendix (see PFOS Appendix). Because a toxicological endpoint of interest results from the presence of chemical at the organ-specific site of action, dose response modeling is preferentially performed on internal doses rather than administered doses and assumes the internal dose metric is proportional to the target tissue dose. In addition, the non-linear elimination described in Wambaugh et al. (2013, 2850932) requires conversion to an internal dose as the relationship between internal and external dose will not scale linearly. The internal doses were then modeled using the Benchmark Dose Software (BMDS) (see PFOS Appendix for additional modeling details). The internal dose animal PODs were converted to a POD_{HED} using the model described in Section 4.1.3.1.3. Reverse dosimetry for the animal PODs used the ratio of standard exposure and internal dose as was applied to PODs from epidemiological data. For animal toxicological studies using the average concentration over the final week of the study (C_{last7}), the POD_{HED} is the human dose that would result in the same steady-state concentration in adults. When a concentration internal dose metric in the pup during lactation and/or gestation was selected, the POD_{HED} is the dose to the mother that results in the same average concentration in the fetus/infant over that period.

Table 4-8. POD_{HED}s Considered for the Derivation of Candidate RfD Values

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Type, Model	POD	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)
Immunological Effects						
Decreased serum anti-tetanus antibody concentration in children	Budtz-Jørgensen (2018, 5083631)[b] Medium	Human, male and female; PFOS concentrations at age five years and anti-tetanus antibody serum concentrations at age seven years	$BMDL_{0.5SD}$, Linear		18.5 ng/mL	2.71×10^{-6}
	Budtz-Jørgensen and Grandjean (2018, 5083631)[b] Medium	Human, male and female; PFOS concentrations in the mother[c] and anti-tetanus antibody serum concentrations at age 5 years	$BMDL_{0.5SD}$, Linear		29.9 ng/mL	5.21×10^{-6}
	Timmerman et al. (2021, 9416315) Medium	Human, male and female; PFOS concentrations and anti-tetanus antibody concentrations at ages 7–10 years	$BMDL_{0.5SD}$, Linear		9.66 ng/mL	1.78×10^{-6}
Decreased serum anti-diphtheria antibody concentration in children	Budtz-Jørgensen (2018, 5083631)[b] Medium	Human, male and female; PFOS concentrations at age five years and anti-diphtheria antibody serum concentrations at age seven years	$BMDL_{0.5SD}$, Linear		12.5 ng/mL	1.83×10^{-6}
	Budtz-Jørgensen and Grandjean (2018, 5083631)[b] Medium	Human, male and female; PFOS concentrations in the mother[c] and anti-tetanus antibody serum concentrations at age 5 years	$BMDL_{0.5SD}$, Linear		20.0 ng/mL	3.48×10^{-6}
	Timmerman et al. (2021, 9416315) Medium	Human, male and female; PFOS concentrations and anti-diphtheria antibody	$BMDL_{0.5SD}$, Linear		5.61 ng/mL	1.03×10^{-6}

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Type, Model	POD	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)
		concentrations at ages 7–10 years				
Decreased PFC response to SRBC	Zhong et al. (2016, 3748828) Medium	C57BL/6 Mice, PNW 4 F_1 males	$BMDL_{1SD}$, Hill		3.3 mg/L $C_{avg\_pup\_gest\_lact}$	5.32×10^{-4}
Extramedullary Hematopoiesis in the Spleen	NTP (2019, 5400978) High	Sprague-Dawley Rats, female	$BMDL_{10RD}$, Multistage Degree 1		2.27 mg/L C_{last7}	2.91×10^{-4}
Extramedullary Hematopoiesis in the Spleen	NTP (2019, 5400978) High	Sprague-Dawley Rats, male	$BMDL_{10RD}$, Logistic		9.59 mg/L C_{last7}	1.23×10^{-3}
Developmental Effects						
Low Birth Weight	Chu et al. (2020, 6315711) High	Human, male and female; PFOS serum concentrations in third trimester	$BMDL_{5RD}$, Hybrid		7.3 ng/mL	1.27×10^{-6}
	Sagiv et al. (2018, 4238410) High	Human, male and female; PFOS serum concentrations in first trimester	$BMDL_{5RD}$, Hybrid		41.0 ng/mL	6.00×10^{-6}
	Starling et al. (2017, 3858473) High	Human, male and female; PFOS serum concentrations in second and third trimesters	$BMDL_{5RD}$, Hybrid		5.7 ng/mL	9.26×10^{-7}
	Wikström et al. (2020, 6311677) High	Human, male and female; PFOS serum concentrations in first and second trimesters	$BMDL_{5RD}$, Hybrid		7.7 ng/mL	1.13×10^{-6}
	Darrow et al. (2013, 2850966) High	Human, male and female: maternal PFOS serum concentrations taken at time of enrollment in C8 project[d]	$BMDL_{5RD}$, Hybrid		17.4 ng/mL	2.51×10^{-6}

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Type, Model	POD	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)
	Yao et al. (2021, 9960202) High	Human, male and female; PFOS serum concentrations in third trimester	$BMDL_{5RD}$, Hybrid		5.0 ng/L	8.70×10^{-7}
Decreased Fetal Body Weight	Lee et al. (2015, 2851075) Medium	CD-1 Mice, F_1 males and females	NOAEL[e]	0.5 mg/kg/day	8.75×10^{-1} mg/L $C_{avg\_pup\_gest}$	3.40×10^{-4}
Decreased Pup Body Weight	Luebker et al. (2005, 757857) Medium	Sprague-Dawley Rats, F_1 male and female	$BMDL_{5RD}$, Polynomial Degree 6		10.2 mg/L $C_{avg\_pup\_gest}$	3.96×10^{-3}
Increased Number of Dead Fetuses	Lee et al. (2015, 2851075) Medium	CD-1 Mice, females	LOAEL[e]	0.5 mg/kg/day	2.13 mg/L $C_{avg\_dam\_gest}$	3.32×10^{-4}
Cardiovascular Effects (Serum Lipids)						
Increased Total Cholesterol	Dong et al. (2019, 5080195) Medium	Human, male and female; excluding individuals prescribed cholesterol medication	$BMDL_{5RD}$, Hybrid		9.34 ng/mL	1.20×10^{-6}
	Steenland et al. (2009, 1291109) Medium	Human, male and female; excluding individuals prescribed cholesterol medication	$BMDL_{5RD}$, Hybrid		9.52 ng/mL	1.22×10^{-6}
	Lin et al. (2019, 5187597) Medium	Human, male and female	$BMDL_{5RD}$, Hybrid		66.5 ng/mL	8.51×10^{-6}
Hepatic Effects						
Elevated ALT	Gallo et al. (2012, 1276142) Medium	Human, female	$BMDL_{5RD}$, Hybrid		56.8 ng/mL	7.27×10^{-6}
	Nian et al. (2019, 5080307) Medium	Human, female	$BMDL_{5RD}$, Hybrid		15.1 ng/mL	1.94×10^{-6}
Increased individual Cell Necrosis in the Liver	Butenhoff et al. (2012, 1276144)/ Thomford (2002, 5029075)[f] High	Sprague-Dawley rats, females	$BMDL_{10RD}$, Log-logisitic		27.0 mg/L C_{last7}	3.45×10^{-3}

Notes: ALT = alanine aminotransferase; AUC = area under the curve; $BMDL_{0.5SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean response equal to 0.5 standard deviation from the control mean; $BMDL_{1SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean response equal to 1 standard deviation from the control mean; $BMDL_{5RD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 5% change in response; $BMDL_{10RD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit of a 10% change in response; Cavg_pup_gest = average blood concentration during gestation; C_{last7} = blood concentration over the last 7 days; F_1 = first generation; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PFC = plaque forming cell; PNW = postnatal week; POD = point of departure; POD_{HED} = point of departure human equivalent dose; RfD = reference dose; SRBC = sheep red blood cell.

[a] See PFOS Appendix for additional details on BMD modeling.

[b] Supported by Grandjean et al. (2012, 1248827); Grandjean et al. (2017, 3858518); Grandjean et al. (2017, 4239492).

[c] Maternal serum concentrations were taken either in the third trimester (32 weeks) or about two weeks after the expected term date.

[d] 99% of the pregnancies of participants in Darrow et al. (2013, 2850966) were within 3 years of the serum PFOS measurement.

[e] No models provided adequate fit; therefore, a NOAEL/LOAEL approach was selected.

[f] Butenhoff et al. (2012, 1276144) and Thomford et al. (2002, 5029075) reported the same data.

4.1.4.1 Hepatic Effects

Increased ALT in individuals aged 18 and older {Gallo, 2012, 1276142; Nian, 2019, 5080307}

The POD for increased ALT in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected from adults aged 18 years and older {Gallo, 2012, 1276142; Nian, 2019, 5080307}, which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day. Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation is simply the POD multiplied by the selected clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Individual Cell Necrosis in the Liver, Sprague-Dawley rats, females, C_{last7} {Butenhoff, 2012, 1276144}

Increased incidence of individual cell necrosis in the liver was observed in female Sprague-Dawley Crl:CD(SD)IGS BR rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen. The C_{last7} was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of individual cell necrosis in the liver. The BMDS produced a BMDL in mg/L. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis. This calculation is simply the POD multiplied by the selected clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

4.1.4.2 Immune Effects

Decreased Diphtheria and Tetanus antibody response in vaccinated children at age 7 {Budtz-Jorgensen, 2018, 5083631}

The POD for decreased antibody production at age 7 was derived by quantifying a benchmark dose (see PFOS Appendix) on the measured PFOS serum concentrations at age 5, which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in 4.1.3.1.3). For this, the model was run starting at the birth of the mother, with constant exposure relative to bodyweight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child is governed by the observed ratio between maternal serum and cord blood at delivery. Then the model is run through the 1 year breastfeeding period, where the exposure to the child is only through lactation, which is much greater than the exposure to the mother. After 1 year, the exposure to the child, relative to bodyweight, is set to the same value as the mother. The model provides predictions up to a child age of 5 years, when the serum concentrations used to determine the POD were collected, and reverse dosimetry was used to determine the POD_{HED} that results in the POD serum concentration. Because of different growth curves used for male and female children used in the model, the model predicted slightly different (less than 5%) serum concentrations for them. The lower HED was then selected as it was the most health protective.

Decreased Diphtheria and Tetanus antibody response in vaccinated children at age 5 {Budtz-Jorgensen, 2018, 5083631}

The POD for decreased antibody production at age 5 was derived by quantifying a benchmark dose (see PFOS Appendix) on the measured PFOS serum concentrations collected from the mother either in the third trimester (32 weeks) or about two weeks after the expected term date, which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). For this, the model was run similarly to the endpoint based on antibodies at age 7, except that the model was only run until the maternal age of 25 years, when delivery occurs in the model. As the POD was based on maternal serum concentrations taken before and after birth, the time of delivery was chosen as an average of the two. Reverse dosimetry was performed on model predicted maternal serum concentration at that time to calculate the POD_{HED}. This metric is independent of the sex of the child in the model.

Decreased Diphtheria and Tetanus antibody response in vaccinated children at ages 7–12 {Timmerman, 2021, 9416315}

The POD for decreased antibody production in children aged 7–12 was derived by quantifying a benchmark dose (see PFOS Appendix) on the measured PFOS serum concentrations at ages 7–12, which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). For this, the model was run similarly to the endpoint based on antibodies at age 7 {Budtz-Jorgensen, 2018, 5083631}, but the model was run until the median age of this cohort at blood collection, 9.9 years. Reverse dosimetry was used to calculate the POD_{HED} that resulted in a serum level equal to the POD at that age. Because of different growth curves used for male and female children, the model predicted slightly different serum concentrations for them. The lower HED was then selected as it was the most health protective.

Decreased plaque forming cell (PFC) response to SRBC, C57BL/6 Mice, PNW 4 F_1 males, $C_{avg\_pup\_gest\_lact}$ {Zhong, 2016, 3748828}

Decreased mean level of PFC response of splenic cells was observed in F_1 male C57BL/6 mice. Continuous models were used to fit dose-response data. Using the Wambaugh et al. (2013, 2850932) model, daily exposure to PFOS through oral gavage was simulated from GD1-GD17 days using female CD1 mice parameters (C57BL/6 mice parameters are not available for PFOS). An average concentration in the pup during gestation and lactation ($C_{avg\_pup\_gest\_lact}$) was calculated as the internal dose metric for each dose group. A benchmark response (BMR) of a change in the mean equal to 1 SD from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}. The $C_{avg\_pup\_gest\_lact}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of decreased plaque forming cell response of splenic cells from across the gestation and lactation lifestages. The BMDS produced a BMDL in mg/L. The internal serum POD, based on the predicted average serum concentration in the pup during gestation and lactation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). For this, the model was run starting at the birth of the mother, with constant exposure relative to bodyweight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in

the child is governed by the observed ratio between maternal serum and cord blood at delivery. Then the model is run through the 1 year breastfeeding period. The average serum concentration in the infant through gestation and lactation is determined for this scenario and reverse dosimetry is used to calculate the exposure that results in the same value as the POD. A male infant was used for this calculation to match the sex of the animals.

Extramedullary hematopoiesis in the spleen, Sprague-Dawley Rats, female and male, C_{last7} {NTP, 2019, 5400978}

Increased incidence of extramedullary hematopoiesis in the spleen was observed in male Sprague-Dawley rats. Using the Wambaugh et al. (2013, 2850932) model, daily exposure to PFOS through oral gavage was simulated for 28 days using Sprague-Dawley rat parameters. An average concentration over the last seven days of exposure (C_{last7}) was calculated as the internal dose metric for each dose group. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* {U.S. EPA, 2012, 1239433}. The C_{last7} was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration is expected to better correlate with an accumulation of extramedullary hematopoiesis in the spleen. The BMDS produced a BMDL in mg/L. A POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration in humans equal to the POD from the animal analysis. This calculation is simply the POD multiplied by the selected human clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

4.1.4.3 Cardiovascular Effects

Increased total cholesterol in individuals aged 20–80, excluding individuals prescribed cholesterol medication {Dong, 2019, 5080195}

The POD for increased TC in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected from adults aged 20–80 years not prescribed cholesterol medication through the NHANES, which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day. Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation is simply the POD multiplied by the selected human clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Increased total cholesterol in individuals aged 18 and older, excluding individuals prescribed cholesterol medication {Steenland, 2009, 1291109}

The POD for increased TC in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected from adults aged 18 years and older not prescribed cholesterol medication from the C8 study population, which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day. Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation is simply the POD multiplied by

the selected human clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

Increased total cholesterol in individuals aged 25 and older {Lin, 2019, 5187597}

The POD for increased TC in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected in adults 25 years and older who were at high risk of developing type 2 diabetes and hyperlipidemia from the Diabetes Prevention Program (DPP) and Outcomes Study (DPPOS), which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day. Specifically, the POD_{HED} was calculated as the external dose that would result in a steady-state serum concentration equal to the internal serum POD. This calculation is simply the POD multiplied by the selected human clearance value (0.128 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

4.1.4.4 Developmental Effects

Decreased birthweight using the mother's serum PFOS concentration collected in third trimester {Chu, 2020, 6315711}

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected from the mother in the third trimester (blood was collected within 3 days after delivery), which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). This calculation was performed similarly for each of the birthweight endpoints. The model was run starting at the birth of the mother, with constant exposure relative to bodyweight. Pregnancy began at 24.25 years maternal age. The model was stopped at a time to match the median gestational age of the cohort at sample time for samples taken during pregnancy, or at delivery (25 years maternal age) in the case of maternal samples at delivery or samples of cord blood. Reverse dosimetry was performed to calculate the POD_{HED} resulting in serum levels matching the POD at the model end time. For this study, maternal blood was drawn within a few days of the birth of the child, so delivery was chosen as the model end time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected in in first trimester {Sagiv, 2018, 4238410}

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected from the mother in the first trimester (median gestational age of 9 weeks), which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). This was performed as described for the Chu et al. (2020, 6315711) study. The model was stopped at the median gestational age of this cohort, 9 weeks. The time after conception was calculated as the fraction of pregnancy competed after 9 weeks (9/39 weeks), times the pregnancy duration of 0.75 year. Reverse dosimetry was performed to

calculate the POD_{HED} that resulted in the POD in maternal serum at that time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected in second and third trimesters {Starling, 2017, 3858473}

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected from the mother in the trimesters 2 and 3 (median gestational age of 27 weeks), which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). This was performed as described for the Chu et al. (2020, 6315711) study. The model was stopped at the median gestational age of this cohort, 27 weeks. The time after conception was calculated as the fraction of pregnancy completed after 27 weeks (27/39 weeks), times the pregnancy duration of 0.75 year. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected in first and second trimesters {Wikström, 2020, 6311677}

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected from the mother in the trimesters 1 and 2 (median gestational age of 10 weeks), which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). This was performed as described for the Chu et al. (2020, 6315711) study. The model was stopped at the median gestational age of this cohort, 10 weeks. The time after conception was calculated as the fraction of pregnancy completed at 10 weeks (10/39 weeks), times the pregnancy duration of 0.75 year. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected in third trimester {Yao, 2021, 9960202}

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected from the mother in the third trimester (blood was collected within 3 days of delivery, at hospital admittance), which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). This calculation was performed similarly for each of the birthweight endpoints. The model was run starting at the birth of the mother, with constant exposure relative to bodyweight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The model was stopped at a time to match the median gestational age of the cohort at sample time for samples taken during pregnancy, or at delivery in the case of maternal samples at delivery or samples of cord blood. Reverse dosimetry was performed to calculate the POD_{HED} resulting in serum levels matching the POD at the model

end time. For these studies, maternal blood was drawn withing a few days of the birth of the child, so delivery was chosen as the model end time. This metric is independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOS concentration collected at enrollment into the C8 study {Darrow, 2013, 2850966}

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see PFOS Appendix) on the measured PFOS serum concentrations collected from the mother at the time of enrollment in the C8 project, which provided an internal serum concentration POD in mg/L. The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in 4.1.3.1.3). This was performed as described for the Chu et al. (2020, 6315711) study. In this cohort, blood samples were taken from women before (52%), during (22%), and after (26%) pregnancy. Because most samples were drawn prior to pregnancy, the POD_{HED} was calculated based on a maternal age of 24.25 years, prior to any pharmacokinetic effects related to pregnancy. Reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD in maternal serum at that time.

Decreased Fetal Body Weight, CD-1 Mice, F_1 males and females, $C_{avg\_pup\_gest}$ {Lee, 2015, 2851075}

Decreased mean response of fetal body weight was observed in F_1 male and female CD-1 mice. Continuous models were used to fit dose-response data. A BMR of 5% extra risk was selected as described in Section 4.1.2, and a change in the mean equal to 0.5 standard deviations from the control mean was provided for comparison purposes (See PFOS Appendix). The average blood concentration of the pup during gestation ($C_{avg\_pup\_gest}$) was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration during gestation is expected to better correlate with an accumulation of effect resulting in decreased fetal body weight. The BMDS did not produce a model with adequate fit, so a NOAEL approach was taken. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). For this endpoint, the model was run starting at the birth of the mother, with constant exposure relative to bodyweight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The model was run up to the birth of the child. The average serum concentration in the infant during gestation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Before birth, model predictions for male and female children are equivalent.

Increased Number of Dead Fetuses, CD-1 Mice, females, $C_{avg\_dam\_gest}$ {Lee, 2015, 2851075}

Increased number of dead fetuses 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 0.5 standard deviations from the control mean was chosen. The average blood concentration of the dam during gestation ($C_{avg\_dam\_gest}$) and maximum maternal concentration during gestation (C_{max_dam}) were both considered (see PFOS Appendix) because fetal death could be a result of exposure during a sensitive window of development. The average blood concentration of the dam during gestation ($C_{avg\_dam\_gest}$) was ultimately selected because this metric is expected to better correlate

with an accumulation of effect resulting in decreased fetal survival. The BMDS did not produce a model with adequate fit, so a LOAEL approach was taken. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). For this, the model was run starting at the birth of the mother, with constant exposure relative to bodyweight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The model was run up to the birth of the child. The average serum concentration in the infant during gestation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Before birth, model predictions for male and female children are equivalent**.**

Decreased Pup Body Weight, Sprague-Dawley Rats, F_1 male and female, $C_{avg\_pup\_gest}$ {Luebker, 2005, 757857}

Decreased mean pup body weight relative to the litter at LD 5 was observed in F_1 male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of 5% extra risk was selected as described in Section 4.1.2, and a change in the mean equal to 0.5 standard deviations from the control mean was provided for comparison purposes (See PFOS Appendix). The $C_{avg\_pup\_gest}$ was selected for this model rather than alternate metrics such as C_{max} because the average blood concentration of the pup during gestation is expected to better correlate with an accumulation of effect resulting in decreased pup body weight. The BMDS produced a BMDL in mg/L. The internal serum POD, based on the predicted average serum concentration in the pup during gestation, was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.1.3). For this, the model was run starting at the birth of the mother, with constant exposure relative to bodyweight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The model was run up to the birth of the child. The average serum concentration in the infant during gestation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Before birth, model predictions for male and female children are equivalent.

4.1.5 Derivation of Candidate Chronic Oral Reference Doses (RfDs)

Though multiple POD_{HED}s were derived for multiple health systems from both epidemiological and animal toxicological studies, EPA selected the POD_{HED}s with the greatest strength of evidence and the lowest risk of bias represented by *high* or *medium* confidence studies for candidate RfD derivation, as described below. As presented in Table 4-1, epidemiological data representing the four prioritized health outcomes represented the most sensitive effects after PFOS exposure in the lower dose range. Four endpoints from epidemiological studies representing the four health outcomes were considered for candidate RfD derivation. These endpoints are decreased antibody response, low birth weight, increased total cholesterol, and elevated ALT. As described in the subsections below, EPA further evaluated studies within each endpoint to determine those most suitable for candidate RfD derivation.

EPA also further evaluated animal toxicological studies to determine which were the most suitable for candidate RfD derivation. Factors considered included study confidence (i.e., *high* confidence studies were prioritized over *medium* confidence studies), amenability to benchmark

dose modeling, and health effects observed after exposure in the lower dose range among the animal toxicological studies. As described in the subsections below, this examination led to the exclusion a number of studies considered for POD derivation, including both epidemiological and animal toxicological studies, from further consideration.

4.1.5.1 Hepatic Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination {Gallo, 2012, 1276142; Nian, 2019, 5080307}. EPA considered both studies as they represented the low-dose range of effects across hepatic endpoints and provided data from relatively large populations, including U.S. populations.

One *high* confidence animal toxicological study was carried forward for candidate RfD determination {Butenhoff, 2012, 1276144}. This study was prioritized for candidate RfD development because it was determined to be a *high* confidence study and it was the only study with a chronic exposure duration that histopathologically examined animals treated with PFOS.

4.1.5.2 Immune Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination {Budtz-Jørgensen, 2018, 5083631; Timmerman, 2021, 9416315}. EPA considered both studies as they both represented the low-dose range of effects across immunological endpoints and provided data regarding sensitive populations (i.e., children). Although EPA derived POD_{HED}s for two time points reported by Budtz-Jørgensen and Grandjean (2018, 5083631) (i.e., PFOS serum concentrations at age 5 and antibody concentrations at age 7; PFOS serum concentrations in the mother during the third trimester or approximately 2 weeks after the expected term date and antibody concentrations at age 5), EPA did not carry forward POD_{HED}s based on serum PFOS concentrations measured in the mother for candidate RfD derivation because of concerns surrounding bias due to pregnancy-related hemodynamic effects.

One *high* and one *medium* confidence animal toxicological studies were carried forward for candidate RfD determination {NTP, 2019, 5400978; Zhong, 2016, 3748828}. NTP (2019, 5400978) is a *high* confidence study reporting the effect of extramedullary hematopoiesis of the spleen in both male and female rats, female rats being marginally more sensitive than males. This effect was accompanied by increased bone marrow hypocellularity, suggesting that PFOS disrupts hematopoiesis in the bone marrow. As extramedullary hematopoiesis was observed in a *high* confidence study, in in both sexes, and was amenable to BMD modeling, this endpoint was carried forward for candidate RfD derivation. The endpoint of reduced PFC response as reported by Zhong et al. (2016, 3748828) was also selected for candidate RfD derivation because the effect was reported by multiple studies and represented effects in the low-dose range for immune effects reported by animal toxicological studies. In addition, Zhong et al. (2016, 3748828) reported this effect in pups exposed to PFOS during gestation and therefore encompasses a sensitive population that is coherent with the developmental immunotoxicity observed in humans.

4.1.5.3 Cardiovascular Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination {Dong, 2019, 5080195; Steenland, 2009, 1291109}. Of the three studies for which

POD_{HED}s were derived, Dong et al. (2019, 5080195) and Steenland et al. (2009, 1291109) exclude individuals who were prescribed cholesterol medication, minimizing concerns surrounding confounding due to the medical intervention altering serum total cholesterol levels. Therefore, these two studies were considered further for candidate RfD derivation.

4.1.5.4 Developmental Effects

Two *high* confidence epidemiological studies were carried forward for candidate RfD determination {Sagiv, 2018, 4238410; Wikström, 2019, 6311677}. Of the six epidemiological studies for which POD_{HED}s were derived, Sagiv et al. (2018, 4238410) and Wikström et al. (2019, 6311677) assessed maternal PFOS serum concentrations primarily or exclusively in the first trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Therefore, these two studies were considered further for candidate RfD derivation.

One *medium* confidence animal toxicological study was carried forward for candidate RfD determination {Luebker, 2005, 757857}. The endpoint of reduced pup weight from this study was amenable to benchmark dose modeling (i.e., BMD modeling produced viable model fits), unlike the endpoints of fetal death and fetal weight reported by Lee et al. (2015, 2851075), which had a LOAEL and NOAEL as the basis of the POD_{HED}s, respectively. As the endpoint of decreased pup weight reported Luebker et al. (2005, 757857) encompasses sensitive populations and is coherent with the observed effect of low birth weight in humans, this study was considered further for candidate RfD derivation. The selection of this study is consistent with critical study selection in the 2016 HESD {U.S. EPA, 2016, 3603365}.

4.1.5.5 Application of Uncertainty Factors (UFs)

To calculate the candidate RfD values, EPA applied UFs to the POD_{HED}s derived from selected epidemiological and animal toxicological studies (Table 4-9 and Table 4-10). 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 4-9. Uncertainty Factors for the Development of the Candidate Chronic RfD Values from Epidemiological Studies {U.S. EPA, 2002, 88824}

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied to effects observed in epidemiological studies as the study population is humans.
UF_H	10	A UF_H of 10 is applied when information is not available relative to variability in the human population.
UF_S	1	A UF_S of 1 is applied when effects are observed in adult human populations that are assumed to have been exposed to a contaminant over the course of many years. A UF_S of 1 is applied for developmental effects 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}.
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	A UF_D of 1 is applied when the database for a contaminant contains a multitude of studies of adequate quality that encompass a comprehensive array of endpoints in various life stages and populations and allow for a complete characterization of the contaminant's toxicity.

UF	Value	Justification
UF_C	10	Composite $UF_C = UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

Notes: UF_A = interspecies uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor; UF_S = uncertainty factor for extrapolation from a subchronic to a chronic exposure duration; UF_C = composite uncertainty factors.

An interspecies UF (UF_A) of 1 was applied to POD_{HED}s derived from epidemiological studies because the dose response information from these studies is directly relevant to humans. There is no need to account for uncertainty in extrapolating from laboratory animals to humans.

An intraspecies UF (UF_H) of 10 was applied to POD_{HED}s derived from epidemiological studies 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 quantitatively characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics.

A LOAEL-to-NOAEL extrapolation UF (UF_L) of 1 was applied to POD_{HED}s derived from epidemiological studies 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 was applied to POD_{HED}s derived from epidemiological studies. A UF_s of 1 was applied to the hepatic and cardiovascular endpoints because the effects were observed in adult populations that were assumed to have been exposed to PFOS over the course of many years. A UF_S of 1 was applied to the developmental endpoints 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 was 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 1 was applied to account for deficiencies in the database for PFOS. In animals, comprehensive oral short term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and developmental toxicity studies including assessment of immune effects following developmental exposure. Moreover, there is a robust epidemiological database which was used quantitatively in this assessment. Typically, the specific study types lacking in a chemical's database that influence the value of the 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.

The composite UFs applied to all epidemiological studies considered for candidate RfD derivation were the same value (UF_C = 10) (Table 4-9).

Increased uncertainty is associated with the use of animal toxicological studies as the basis of candidate RfDs. The composite UFs applied to animal toxicological studies considered for candidate RfD derivation were either one of two values, depending on the duration of exposure (i.e., chronic vs. subchronic) or exposure window (e.g., gestational) (Table 4-10).

Table 4-10. Uncertainty Factors for the Development of the Candidate Chronic RfD Values from Animal Toxicological Studies {U.S. EPA, 2002, 88824}

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied for the extrapolation from animal models to humans due to the implementation of a PK model for animal POD_{HED} derivation.
UF_H	10	A UF_H of 10 is applied when information is not available relative to variability in the human population.
UF_S	1 or 10	A UF_S of 10 is applied for the extrapolation of subchronic to chronic exposure durations. A UF_S of 1 is applied to studies with chronic exposure durations or that encompass a developmental period (i.e., gestation). 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	A UF_D of 1 is applied when the database for a contaminant contains a multitude of studies of adequate quality that encompass a comprehensive array of endpoints in various life stages and populations and allow for a complete characterization of the contaminant's toxicity.
UF_C	30 or 300	Composite $UF_C = UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

Notes: UF_A = interspecies uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL extrapolation uncertainty factor; UF_S = uncertainty factor for extrapolation from a subchronic to a chronic exposure duration; UF_C = composite uncertainty factors.

A UF_A of 3 was applied to POD_{HED}s derived from animal toxicological studies to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). The 3-fold factor is applied to account for toxicodynamic differences between the animals and humans. The HEDs were derived using a model that accounted for PK differences between animals and humans.

A UF_H of 10 was applied to POD_{HED}s derived from animal toxicological studies to account for variability in the responses within human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, life stage, and health status) and extrinsic (lifestyle) factors 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 UF_L of 1 was applied to POD_{HED}s derived from animal toxicological studies 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_S of 1 was applied to POD_{HED}s derived from chronic animal toxicological studies as well as animal toxicological studies that encompass a developmental period (i.e., gestation). A UF_s of 1 was applied to developmental endpoints 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 10 was applied to POD_{HED}s derived from studies that implemented a less-than-chronic exposure duration because extrapolation is required to translate from a subchronic POD_{HED} to a chronic RfD.

A UF_D of 1 was applied to account for deficiencies in the database for PFOS. In animals, comprehensive oral short term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and developmental toxicity studies including assessment of immune effects following developmental exposure. Moreover, there is a robust epidemiological database which was used quantitatively in this assessment. Typically, the specific study types lacking in a chemical's database that influence the value of the 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.

4.1.5.6 Candidate RfDs

Table 4-11 shows the UFs applied to each candidate study to subsequently derive the candidate RfDs.

Table 4-11. Candidate Reference Doses (RfDs)

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD_{HED} (mg/kg/day)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_{TOT}	Candidate RfD[a] (mg/kg/day)
Immune Effects										
Decreased Serum Anti-Tetanus Antibody Concentration in Children	Budtz-Jørgensen and Grandjean (2018, 5083631) Medium	Human, male and female	2.71×10^{-6}	1	10	1	1	1	10	$2.71\times10^{-7} = 3\times10^{-7}$
	Timmerman et al. (2021, 9416315) Medium	Human, male and female	1.78×10^{-6}	1	10	1	1	1	10	$1.78\times10^{-7} = 2\times10^{-7}$
Decreased Serum Anti-Diphtheria Antibody Concentration in Children	Budtz-Jørgensen and Grandjean (2018, 5083631) Medium	Human, male and female	1.83×10^{-6}	1	10	1	1	1	10	$1.83\times10^{-7} = 2\times10^{-7}$
	Timmerman et al. (2021, 9416315) Medium	Human, male and female	1.03×10^{-6}	1	10	1	1	1	10	$1.03\times10^{-7} = 1\times10^{-7}$
Decreased Plaque Forming Cell (PFC) Response to SRBC	Zhong et al. (2016, 3748828) Medium	C57BL/6 Mice, PNW 4 F_1 males	5.32×10^{-4}	3	10	1	1	1	30	$1.77\times10^{-5} = 2\times10^{-5}$
Extramedullary Hematopoiesis in the Spleen	NTP (2019, 5400978) High	Sprague-Dawley rats, female	2.91×10^{-4}	3	10	10	1	1	300	$9.70\times10^{-7} = 1\times10^{-6}$
Developmental Effects										
Low Birth Weight	Sagiv et al. (2018, 4238410) High	Human, male and female	6.00×10^{-6}	1	10	1	1	1	10	$6.00\times10^{-7} = 6\times10^{-7}$
	Wikström et al. (2019, 6311677) High	Human, male and female	1.13×10^{-6}	1	10	1	1	1	10	$1.13\times10^{-7} = 1\times10^{-7}$
Decreased Pup Body Weight	Luebker et al. (2005, 757857) Medium	Sprague-Dawley Rats, F_1 male and female	3.96×10^{-3}	3	10	1	1	1	30	$1.32\times10^{-4} = 1\times10^{-4}$
Cardiovascular Effects										

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD_{HED} (mg/kg/day)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_{TOT}	Candidate RfD[a] (mg/kg/day)
Increased Serum Total Cholesterol	Dong et al. (2019, 5080195) Medium	Human, male and female, excluding individuals prescribed cholesterol medication	1.20×10^{-6}	1	10	1	1	1	10	$1.20\times10^{-7} = 1\times10^{-7}$
	Steenland et al. (2009, 1291109) Medium	Human, male and female, excluding individuals prescribed cholesterol medication	1.22×10^{-6}	1	10	1	1	1	10	$1.22\times10^{-7} = 1\times10^{-7}$
Hepatic Effects										
Increased Serum ALT	Gallo et al. (2012, 1276142) Medium	Human, female	7.27×10^{-6}	1	10	1	1	1	10	$7.27\times10^{-7} = 7\times10^{-7}$
	Nian et al. (2019, 5080307) Medium	Human, female	1.94×10^{-6}	1	10	1	1	1	10	$1.94\times10^{-7} = 2\times10^{-7}$
Individual Cell Necrosis in the Liver	Butenhoff et al. (2012, 1276144)/Thomford (2002, 5029075)[b] High	Sprague-Dawley rats, females	3.45×10^{-3}	3	10	1	1	1	30	$1.15\times10^{-4} = 1\times10^{-4}$

Notes: ALT = alanine transaminase; UF_A = interspecies uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_S = subchronic-to-chronic extrapolation uncertainty factor; UF_L = extrapolation from a LOAEL to a NOAEL uncertainty factor; UF_{TOT} = composite uncertainty factor.

[a] RfDs were rounded to one significant figure.

[b] Butenhoff et al. (2012, 1276144) and Thomford et al. (2002, 5029075) reported data from the same experiment.

4.1.6 RfD Selection

As presented in Section 4.1.5 (Table 4-11), EPA derived and considered multiple candidate RfDs across the four non-cancer health outcomes that EPA determined had the strongest weight of evidence (i.e., immune, cardiovascular, hepatic, and developmental). EPA derived candidate RfDs based on both epidemiological and animal toxicological studies. As depicted in Figure 4-3 the candidate RfDs derived from epidemiological studies were all within 1 order of magnitude of each other (10^{-6} to 10^{-7} mg/kg/day), regardless of endpoint, health outcome, or study population.

Candidate RfDs derived from animal toxicological studies were generally 2-3 orders of magnitude higher than candidate RfDs derived from epidemiological studies. However, EPA does not necessarily expect concordance between animal and epidemiological studies in terms of the adverse effect(s) observed, as well as the dose level that elicits the adverse effect(s). For example, EPA's *Guidelines for Developmental Toxicity Risk Assessment* states that "the fact that every species may not react in the same way could be due to species-specific differences in critical periods, differences in timing of exposure, metabolism, developmental patterns, placentation, or mechanisms of action" {U.S. EPA, 1991, 732120}. Additionally, for developmental effects, the guidance says that "the experimental animal data were generally predictive of adverse developmental effects in humans, but in some cases, the administered dose or exposure level required to achieve these adverse effects was much higher than the effective dose in humans" {U.S. EPA, 1991, 732120}.

As shown in Table 4-11 and Figure 4-3, there is greater uncertainty associated with the use of animal toxicological studies as the basis of RfDs than human epidemiological studies. Though there are some uncertainties in the use of epidemiological studies for quantitative dose-response analyses (see Section 6.1), human data eliminate the uncertainties associated with interspecies extrapolation and the toxicokinetic differences between species which are major uncertainties associated with the PFOS animal toxicological studies due to the half-life differences between humans and other species. These uncertainties may explain why the candidate RfDs derived from animal toxicological studies were several orders of magnitude higher in value than the candidate RfDs derived from epidemiological studies. Moreover, the human epidemiological studies also have greater relevance of exposure to human exposure because they directly measure environmental or serum concentrations of PFOS. In accordance with EPA's current best practices for systematic review, "animal studies provide supporting evidence when adequate human studies are available, and they are considered to be the studies of primary interest when adequate human studies are not available" {U.S. EPA, 2022, 10367891}. For these reasons, EPA determined that candidate RfDs based on animal toxicological studies would not be further considered for health outcome-specific RfD selection or overall RfD selection. See Section 6.2 for further comparisons between toxicity values derived from epidemiological and animal toxicological studies.

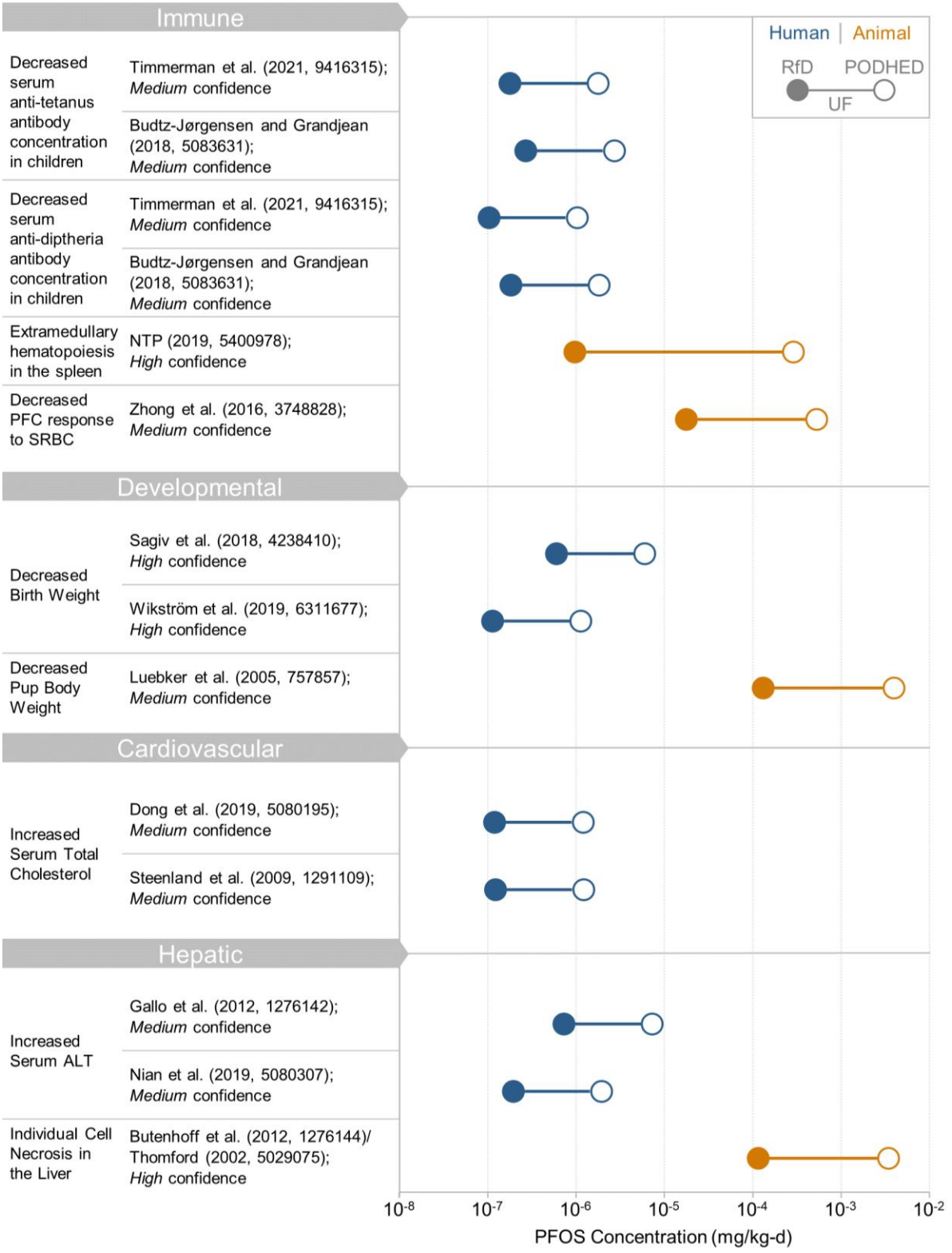


Figure 4-3. Comparison of Candidate RfDs Resulting from the Application of Uncertainty Factors to POD_{HED}s Derived from Epidemiological and Animal Toxicological Studies

As described in the subsections below, EPA selected amongst the candidate RfDs to identify an RfD representative of each of the four prioritized health outcomes (i.e., health outcome-specific RfDs), as well as an overall RfD that is protective of the effects of PFOS on all health outcomes and endpoints (Figure 4-4).

4.1.6.1 Health outcome-Specific RfDs

4.1.6.1.1 Hepatic Effects

Two *medium* confidence epidemiological studies were selected as candidates for RfD derivation for the endpoint of elevated ALT {Gallo, 2012, 1276142; Nian, 2019, 5080307}. The larger study of PFOS and ALT in adults {Gallo, 2012, 1276142} was conducted in over 30,000 adults from the C8 Study. The other study {Nian, 2019, 5080307} examined a large population of adults in Shenyang (one of the largest fluoropolymer manufacturing centers in China) as part of the Isomers of C8 Health Project and observed significant increases in lognormal ALT per each ln-unit increase in PFOS, as well significant increases in odds ratios of elevated ALT. The RfD for increased ALT from Nian et al. (2019, 5080307) was ultimately selected as the health outcome-specific RfD for hepatic effects because PFOS was the predominating PFAS in this study which reduces concern about potential confounding by other PFAS. The resulting health outcome-specific RfD is 2×10^{-7} mg/kg/day (Figure 4-4). Note that both candidate RfDs based on epidemiological studies for the hepatic outcome were within one order of magnitude of the selected health outcome-specific RfD.

4.1.6.1.2 Immune Effects

Two *medium* confidence epidemiological studies were considered for RfD derivation for the endpoint of decreased antibody production in response to various vaccinations in children {Budtz-Jørgensen, 2018, 5083631; Timmerman, 2021, 9416315}. These candidate studies offer a variety of PFOS exposure measures across various populations and various vaccinations. Budtz-Jørgensen and Grandjean (2018, 5083631) investigated anti-tetanus and anti-diphtheria responses in Faroese children aged 5–7 and Timmerman et al. (2021, 9416315) investigated anti-tetanus and anti-diphtheria responses in Greenlandic children aged 7–12. Though both are *medium* confidence studies, the study by Budtz-Jørgensen and Grandjean (2018, 5083631) has two features that strengthen the results: 1) the response reported by this study reached statistical significance, and 2) the analysis considered co-exposures of other PFAS. The RfDs for anti-diphtheria responses in 7-year-old Faroese children from Budtz-Jørgensen and Grandjean (2018, 5083631) was ultimately selected as the basis for the health outcome-specific RfD for immune effects because the response reported by this study reached statistical significance, this analysis considered co-exposures of other PFAS, and it was the more health-protective of the two vaccine-specific responses reported by Budtz-Jørgensen and Grandjean (2018, 5083631). The resulting health outcome-specific RfD is 2×10^{-7} mg/kg/day (Figure 4-4). Note that all candidate RfDs based on epidemiological studies for the immune outcome were within one order of magnitude of the selected health outcome-specific RfD.

4.1.6.1.3 Cardiovascular Effects

Two *medium* confidence epidemiological studies were considered for RfD derivation for the endpoint of increased total cholesterol {Dong, 2019, 5080195; Steenland, 2009, 1291109}. These candidate studies offer a variety of PFOS exposure measures across various populations. Dong et al. (2019, 5080195) investigated the NHANES population (2003–2014), while

Steenland et al. (2009, 1291109) investigated effects in a high-exposure community (the C8 Health Project study population). Both of these studies excluded individuals prescribed cholesterol medication which minimizes concerns of confounding due to medical intervention. The RfD for increased TC from Dong et al. (2019, 5080195) was ultimately selected for the health outcome-specific RfD for cardiovascular effects as there is marginally increased confidence in the modeling from this study. Steenland et al. (2009, 1291109) presented analyses using both PFOS and TC as categorical and continuous variables. The results using the natural log transformed TC and the natural log transformed PFOS were stated to fit the data slightly better than the ones using untransformed PFOS. However, the dramatically different changes in regression slopes between the two analyses by Steenland et al. (2009, 1291109) resulting in extremely different PODs raise concerns about the appropriateness of using the data for RfD derivation. Therefore, the resulting health outcome-specific RfD based on results from Dong et al. (2019, 5080195) is 1×10^{-7} mg/kg/day (Figure 4-4). Note that the candidate RfDs for the cardiovascular outcome were nearly identical.

4.1.6.1.4 Developmental Effects

Two *high* confidence epidemiological studies were considered for RfD derivation for the endpoint of low birth weight {Sagiv, 2018, 4238410; Wikström, 2019, 6311677}. These candidate studies assessed maternal PFOS serum concentrations primarily or exclusively in the first trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Both were *high* confidence prospective cohort studies with many study strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias. The RfD for low birth weight from Wikström et al. (2020, 6311677) was selected as the basis for the organ-specific RfD for developmental effects as it was the lowest and therefore most health protective candidate RfD from these two studies. The resulting health outcome-specific RfD is 1×10^{-7} mg/kg/day (Figure 4-4). Note that both candidate RfDs based on epidemiological studies for the developmental outcome were within one order of magnitude of the selected health outcome-specific RfD.

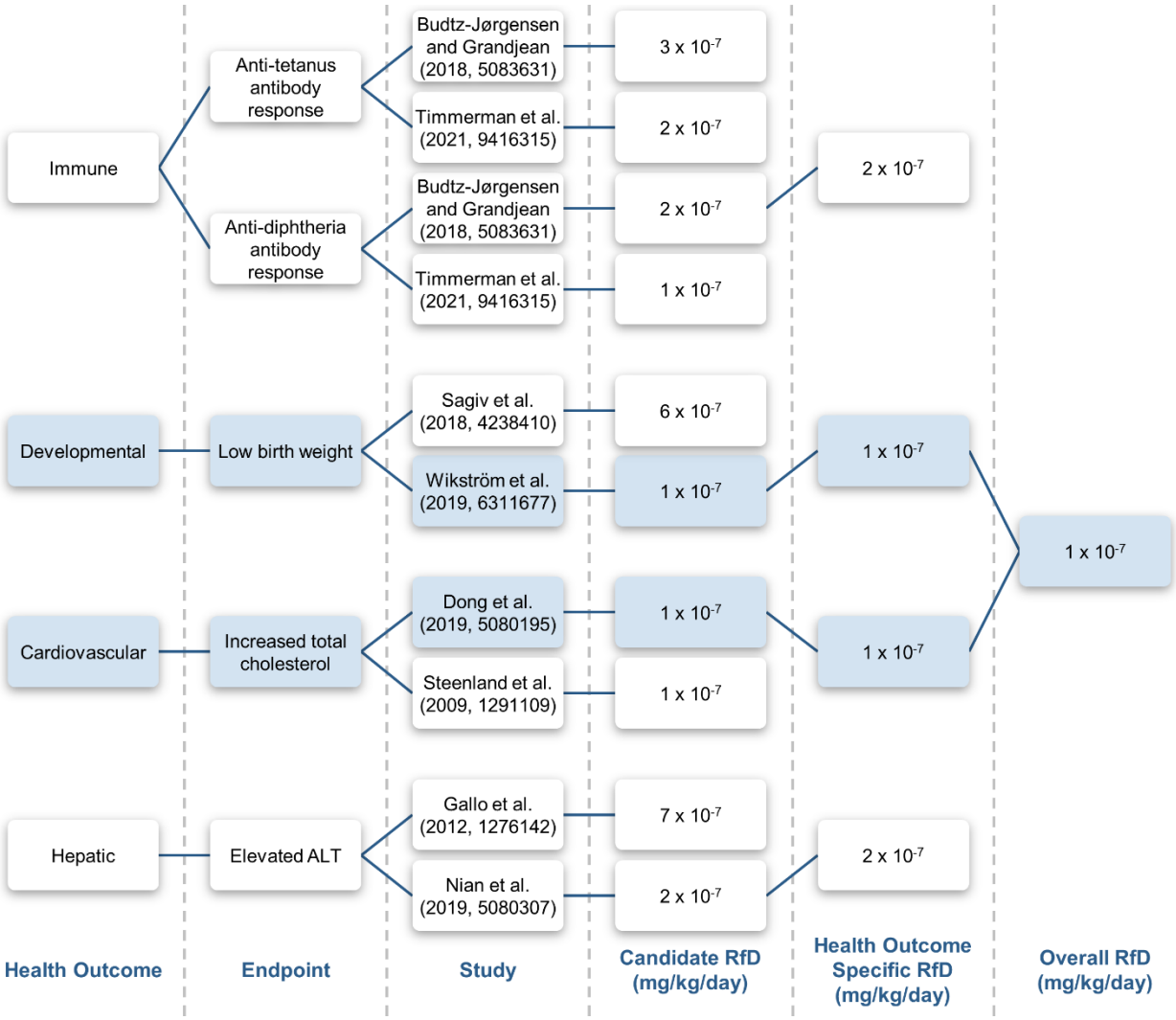


Figure 4-4. Schematic depicting selection of the overall RfD for PFOS

4.1.6.2 Overall RfD

The available evidence indicates there are effects across immune, developmental, cardiovascular, and hepatic organ systems at the same or approximately the same level of PFOS exposure. In fact, candidate RfDs within the developmental and cardiovascular outcomes are the same value (i.e., 1 x 10^{-7} mg/kg/day). Therefore, EPA has selected an overall RfD for PFOS of 1 x 10^{-7} mg/kg/day (Figure 4-4). The developmental and cardiovascular RfDs based on endpoints of low birth weight and increased total cholesterol, respectively, serve as co-critical effects for this RfD. Notably, the RfD is protective of effects that may occur in sensitive populations (i.e., infants and children; see Section 6.8), as well as immune and hepatic effects that may result from PFOS exposure. As one of the co-critical effects identified for PFOS is a developmental endpoint and can potentially result from a short-term exposure during critical periods of development, EPA concludes that the overall RfD for PFOS is applicable to both short-term and chronic risk assessment scenarios.

The critical studies that serve as the basis of the RfD are all *medium* or *high* confidence epidemiological studies. The critical studies are supported by multiple other *medium* or *high* confidence studies in both humans and animal models and have health outcome databases for which EPA determined that either *evidence indicates* or *evidence demonstrates* that oral PFOS exposure is associated with adverse effects. Additionally, the selected critical effects can lead to clinical outcomes in a sensitive lifestage (children) and/or yield the lowest POD_{HED} and candidate RfDs and therefore, is expected to be protective of all other health effects in humans.

4.2 Cancer

4.2.1 Study Selection

Several *medium* and *high* confidence epidemiological studies and a single *high* confidence animal chronic cancer bioassay comprise the evidence database for the carcinogenicity of PFOS. The available epidemiology studies report elevated risks of bladder, prostate, kidney, and breast cancers after chronic PFOS exposure. While there are reports of cancer incidence from epidemiological studies, the study designs, analyses, and mixed results preclude definitive conclusions about the relationship between PFOS exposure and cancer outcomes in humans and also limit the potential for quantitative assessment of these data (i.e., dose-response modeling for CSF derivation).

The sole animal chronic cancer bioassay study provides evidence of multi-site tumorigenesis in male and female rats. The Thomford (2002, 5029075)/Butenhoff et al. (2012, 1276144) chronic cancer study was determined to be *high* confidence and provides several multi-dose tumor incidence findings in male and female rats that are suitable for dose-response modeling and subsequent CSF derivation, further described below.

4.2.2 CSF Development

EPA derived PODs and candidate CSFs for four endpoints reported by Thomford (2002, 5029075)/Butenhoff et al. (2012, 1276144): hepatocellular adenomas in male rats; hepatocellular adenomas in female rats; combined hepatocellular adenomas and carcinomas in female rats; and pancreatic islet cell carcinomas in male rats (Table 4-12). As noted in Table 3-13, EPA expressed tumor incidence as the number of animals with reported tumors over the number of animals alive at the time of first occurrence of the tumor. Expressing incidence in this way quantitatively eliminates animals that died prior to the PFOS treatment duration plausibly required to result in tumor formation in the critical study. BMDLs were derived using the BMDS 3.2 program. Multistage models were used consistent with the long-standing practice of EPA to prefer multistage models to fit tumor dose-response data and a BMR of 10% extra risk was chosen per EPA's Benchmark Dose Technical Guidance {U.S. EPA, 2012, 1239433}. AUC averaged over study duration (AUC_{avg}), equivalent to mean serum concentration during the duration of the study, was selected for this model because the AUC accounts for the accumulation of effects expected to precede the increased incidence of adenomas and/or carcinomas. The BMDS produced a BMDL in mg/L. The animal POD was converted to a POD_{HED} by multiplying the POD by the human clearance value (Table 4-6). This POD_{HED} is equivalent to the constant exposure, per bodyweight, that would result in serum concentration equal to the POD at steady state. The CSF is then calculated by dividing the BMR of 10% by the POD_{HED}.

Table 4-12. Cancer Slope Factors (CSFs) derived from results reported by Butenhoff et al. (2012, 1276144)/Thomford (2002, 5029075)[a] in Sprague-Dawley rats

Tumor Type	Sex	POD Type, Model	POD Internal Dose /Internal Dose Metric[b]	POD_{HED}	Candidate CSF (BMR/POD_{HED})
Hepatocellular Adenomas	Male	$BMDL_{10}$ Multistage Degree 4 Model	25.6 mg/L (AUC normalized per day (AUC_{avg}))	3.28×10^{-3} mg/kg/day	30.5 $(mg/kg/day)^{-1}$
Hepatocellular Adenomas	Female	$BMDL_{10}$ Multistage Degree 1 Model	21.8 mg/L (AUC normalized per day (AUC_{avg}))	2.79×10^{-3} mg/kg/day	35.8 $(mg/kg/day)^{-1}$
Combined Hepatocellular Adenomas and Carcinomas[c]	**Female**	**$BMDL_{10}$ Multistage Degree 1 Model**	**19.8 mg/L (AUC normalized per day (AUC_{avg}))**	**2.53×10^{-3} mg/kg/day**	**39.5 $(mg/kg/day)^{-1}$**
Pancreatic Islet Cell Carcinomas	Male	$BMDL_{10}$ Multistage Degree 1 Model	26.1 mg/L (AUC normalized per day (AUC_{avg}))	3.34×10^{-3} mg/kg/day	29.9 $(mg/kg/day)^{-1}$

Notes: $BMDL_{10}$ = benchmark dose level corresponding to the 95% lower confidence limit of a 10% change.
[a] Butenhoff et al. (2012, 1276144) and Thomford (2002, 5029075) reported data from the same experiment.
[b] See PFOS Appendix for additional details on benchmark dose modeling.
[c] Endpoint is bolded to note that it was selected as the basis for the selected cancer slope factor.

4.2.3 CSF Selection

EPA selected the hepatocellular adenomas and carcinomas in female rats reported by Butenhoff et al. (2012, 1276144)/Thomford (2002, 5029075) as the basis of the CSF for PFOS. This endpoint was selected because: 1) there was a statistically significant increase in tumor incidence in the highest dose group; 2) a statistically significant trend of increased incidence with increasing PFOS concentrations across dose groups; and 3) it is representative of both hepatocellular tumor types observed in male and female rats. The resulting CSF is 39.5 $(mg/kg/day)^{-1}$.

Selection of hepatocellular adenomas and carcinomas in female rats is supported by statistically significant increases in hepatocellular tumor incidence in the high dose group as well as a statistically significant trend of this response observed in the male rats. The critical effect of pancreatic islet cell carcinomas was not selected as the basis of the CSF because the response of the high dose group was not statistically different from the control group, though the trend of response across dose groups was statistically significant. Regardless, the resulting CSF from this endpoint is relatively close in value to the CSFs derived from hepatocellular tumors and bolsters the confidence in the selected CSF.

4.2.4 Application of Age-Dependent Adjustment Factors

EPA's *Guidelines for Carcinogen Risk Assessment* and *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* require the consideration of applying age-dependent adjustment factors (ADAFs) to CSFs to address potential increased risk for cancer due to early life stage susceptibility to chemical exposure {U.S. EPA, 2005, 6324329; U.S. EPA, 2005, 88823}. ADAFs are only to be used for carcinogenic chemicals with a mutagenic MOA when chemical-specific data about early-life susceptibility are lacking. For carcinogens with any

MOA, including mutagens and non-mutagens, but with available chemical specific data for early-life exposure, those data should be used.

As described in Section 3.5.3.1.1, the limited number of *in vivo* and *in vitro* studies assessing mutagenicity following PFOS exposure were primarily negative. Therefore, EPA has determined that PFOS is unlikely to cause tumorigenesis via a mutagenic MOA. Given the lack of evidence of a mutagenic MOA, EPA does not recommend applying ADAFs when quantitatively determining the cancer risk for PFOS {U.S. EPA, 2011, 783747}.

Additionally, there is insufficient information available from epidemiological and animal toxicological studies to adequately determine whether PFOS exposure during early-life periods, per EPA's above-referenced supplemental guidance, may increase incidence or reduce latency for cancer compared with adult-only exposure. No current studies allow for comparisons of cancer incidence after early-life vs. adult-only PFOS exposure.

5 MCLG Derivation

Consistent with the *Guidelines for Carcinogen Risk Assessment* {U.S. EPA, 2005, 6324329}, EPA reviewed the weight of the evidence and determined that PFOS is *Likely to Be Carcinogenic to Humans*, as "the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor *Carcinogenic to Humans.*" This determination is based on the evidence of hepatocellular tumors in male and female rats, pancreatic islet cell carcinomas in male rats, and mixed but plausible evidence of bladder, prostate, kidney, and breast cancers in humans as outlined in Section 3.5.4. As previously noted, the results reported by one chronic cancer bioassay in rats exceeds the descriptor of *Suggestive Evidence of Carcinogenic Potential* as it provides evidence of multi-site and multi-sex tumorigenesis {Thomford, 2002, 5029075; Butenhoff, 2012, 1276144}.

Unless a non-linear mode of action is determined, EPA establishes MCLGs of zero for carcinogens classified as *Carcinogenic to Humans* or *Likely to be Carcinogenic to Humans* consistent with the statutory definition of MCLG, which requires EPA to establish MCLGs at a level where there are "no known or anticipated adverse effects" on public health and with "an adequate margin of safety." Under SDWA, where there is insufficient information to determine that a carcinogen has a threshold below which there are no carcinogenic effects, EPA takes the health-protective approach of assuming that there is no such threshold and that carcinogenic effects should therefore be extrapolated linearly to zero {U.S. EPA, 1985, 9207; U.S. EPA, 1991, 5499; U.S. EPA, 2016, 6557097}. This approach, known as the linear default extrapolation approach, ensures that the MCLG is set at a level where there are no adverse health effects with a margin of safety. EPA has determined that PFOS is *Likely to be Carcinogenic to Humans* based on sufficient evidence of carcinogenicity in humans and animals, that there is not sufficient evidence of a threshold for PFOS, and that therefore a linear default extrapolation approach is appropriate {U.S. EPA, 2005, 6324329}. Based upon a consideration of the best available peer reviewed science and a consideration of an adequate margin of safety, EPA proposes a MCLG of zero for PFOS in drinking water.

6 Effects Characterization

6.1 Addressing Uncertainties in the Use of Epidemiological Studies for Quantitative Dose-Response Analyses

In the 2016 PFOS HESD and Drinking Water Health Advisory {U.S. EPA, 2016, 3982043; U.S. EPA, 2016, 3603365}, EPA qualitatively considered epidemiological studies as a supporting line of evidence but did not quantitatively consider them for POD derivation, citing the following as reasons to exclude the epidemiological data that were available at that time from quantitative analyses:

- inconsistencies in the epidemiological database,
- the use of mean serum PFOS concentrations rather than estimates of exposure,
- declining serum PFOS values in the U.S. general population over time {CDC, 2017, 4296146},
- uncertainties related to potential exposure to additional PFAS, telomer alcohols that metabolically break down into PFOS, and other bio-persistent contaminants, and
- uncertainties related to the clinical significance of effects observed in epidemiological studies.

Since 2016, EPA has identified many additional epidemiology studies that have increased the database of information for PFOS (see Sections 3.1.1, 3.4, and 3.5). Further, new tools that have facilitated the use of study quality evaluation as part of systematic review have enabled EPA to systematically assess study quality in a way that includes consideration of confounding. As a result, EPA is now in a position to be able to quantitatively consider epidemiological studies for POD derivation in this assessment.

In this assessment EPA has assessed the strength of epidemiological and animal evidence systematically, a process that was not followed in 2016. By performing an updated assessment using systematic review methods, EPA determined that four health outcomes and four epidemiological endpoints within these outcomes (i.e., decreased antibody response to vaccination in children, decreased birthweight, elevated total cholesterol and elevated ALT) have sufficient weight of evidence to consider quantitatively. Each endpoint quantified in this assessment has consistent evidence from multiple *medium* and/or *high* confidence epidemiological and animal toxicological studies supporting an association between PFOS exposure and the adverse effect. Each of the endpoints were also specifically supported by multiple epidemiological studies in different populations, including general and highly exposed populations. Several of these supporting studies have been published since 2016 and have strengthened the weight of evidence for this assessment.

As described in Section 4.1.1.3, EPA has improved upon the pharmacokinetic modeling technique used in 2016. Though there are challenges in estimations of human dosimetry from measured or modeled serum concentrations (see Section 6.6.2), EPA has evaluated the available literature and developed a pharmacokinetic model that estimates PFOS exposure concentrations

from the serum PFOS concentrations provided in epidemiological studies, which reduces uncertainties related to exposure estimations in humans. This new approach is supplemented with the UF accounting for intraspecies variation of 10x applied to each POD_{HED}, which accounts for the sensitivities of specific populations, including those that may have increased susceptibility to PFOS toxicity due to differential toxicokinetics.

An additional source of uncertainty in using epidemiological data for POD derivation is the documented declined in human serum PFOS levels over time, which raises concerns about whether one-time serum PFOS measurements are a good representation of lifetime peak exposure. Because of PFOS's long half-life in serum, however, one-time measurements likely reflect several years of exposure. Importantly, EPA considered multiple time periods when estimating PFOS exposure, ranging from the longest period with available data on PFOS serum levels within the U.S. population (1999-2018) to the shortest and most recent period (2017-2018) (see PFOS Appendix E), when performing dose-response modeling of the ALT and TC endpoints in the epidemiological data. EPA selected PODs for these two endpoints using PFOS exposure estimates based on the serum PFOS data for 1999–2018, which is likely to capture the peak PFOS exposures in the U.S. which occurred in the 1990s {Dong, 2019, 5080195}. The modeling results show that the BMDL estimates for increased TC derived using these exposure data are consistently lower than those based on the 2017–2018 PFOS exposure data whereas for ALT, the BMDL estimates using data from the longest exposure period are consistently higher than those based on the 2017–2018 PFOS exposure data. Based on these analyses, it appears that selection of one exposure time-period over another does not predictably impact the modeling results. Therefore, for this assessment, EPA decided to consistently select the time periods more likely to capture peak PFOS exposures (e.g., 1999-2018) as the basis of BMDL estimates for all endpoints of interest (see PFOS Appendix E).

It is plausible that observed associations between adverse health effects and PFOS exposure could be explained in part by confounding from other PFAS exposures, including the metabolism of precursor compounds to PFOS in the human body. However, for four of the five priority health outcomes, at least one available study performed multi-pollutant modeling. For example, for the decreased antibody production endpoint, Budtz-Jorgensen and Grandjean (2018, 5083631) performed a follow-up analysis of the study by Grandjean et al. (2012, 1248827) in which results were additionally adjusted for PFOA, and there was no notable attenuation of the observed association between PFOS exposure and decreased antibody response. For an extended review of the uncertainties associated with PFAS co-exposures, see *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* {U.S. EPA, 2020, 8642427}.

Additionally, there is uncertainty about the magnitude of the contribution of PFAS precursors to PFOS serum concentrations, especially as biotransformation efficiency appears to vary depending on the precursor of interest {Mcdonough, 2022, 10412593; Vestergren, 2008, 2558842; D'eon, 2011, 2903650}. The contributions of PFAS precursors to serum concentrations also varies between populations with differing PFAS exposure histories (i.e., individuals living at or near sites with AFFF use may have different precursor PFOS contributions than the general population).

In addition, some populations may be disproportionately exposed to other contaminants, such as polychlorobiphenyls and methylmercury. To address this, EPA quantified associations between

PFOS serum concentrations and endpoints of interest in populations with varying exposure histories, including the general population and high-exposure communities. EPA observed associations for endpoints in populations known to have been predominantly exposed to PFOS (e.g., Isomers of C8 Health Project participants), reducing the uncertainty related to potential confounding of other contaminants, including PFAS precursor compounds. These sensitivity analyses are supportive of EPA's conclusions regarding the effects of PFOS reported across many epidemiological studies.

In this assessment, studies were not excluded from consideration based primarily on lack of or incomplete adjustments for potential confounders including socioeconomic status (SES) or race/ethnicity. A small number of studies examining PFAS serum levels across SES and racial/ethnic groups were identified. These studies (most with sampling from the early-mid 2000s) reported conflicting results regarding the relationship between race/ethnicity and serum PFOS concentrations, with studies differing depending on locations sampled, further stratification of results by age, cohort characteristics, etc. {Kato, 2014, 2851230; Nelson, 2012, 4904674; Calafat, 2007, 1290899; Park, 2019, 5381560}. EPA acknowledges that in observational epidemiological studies, potential residual confounding may result from SES and racial/ethnic disparities. Additional racially and ethnically diverse studies in multiple U.S. communities are needed to fill this important data gap. The PFOS Appendix provides detailed information on the available epidemiological studies and identifies the study-specific confounding variables that were considered, such as SES.

Lastly, the potential uncertainty related to the clinical significance of effects observed in the PFOS epidemiological studies is sometimes cited for dismissing the epidemiological data quantitatively. However, as described in section 4.1.1, increased ALT levels, decreased antibody responses in children, increased serum cholesterol levels, and decreased birthweight are clinically meaningful effects, and EPA's *A Review of the Reference Dose and Reference Concentration Processes*, states that a RfD should be based on an adverse effect or a precursor to an adverse effect (e.g., increased risk of an adverse effect occuring) {U.S. EPA, 2002, 88824}.

Briefly, evidence from both human epidemiological and animal toxicological studies indicates that increased serum ALT is associated with increased risk for liver disease {Ioannou, 2006, 10473853; Ioannou, 2006, 10473854; Kwo, 2017, 10328876; Roth, 2021, 9960592}. Human epidemiological studies have also demonstrated that even low magnitude increases in serum ALT can be clinically significant (See section 4.1.1.1). It is also important to note that while evaluation of direct liver damage is possible in animal studies, it is difficult to obtain biopsy-confirmed histological data in humans. Therefore, liver injury is typically assessed using serum biomarkers of hepatotoxicity {Costello et al, 2022, 10285082}. The SAB's PFAS review panel noted that reduction in the level of antibodies produced in response to a vaccine represents a failure of the immune system to respond to a challenge and is considered an adverse immunological health outcome {U.S. EPA, 2022, 10476098}. Further, a review by Selgrade (2007, 736210) suggests that specific immunotoxic effects, such as antibody response, observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards.

Additionally, increased serum cholesterol is associated with changes in incidence of cardiovascular disease events such as myocardial infarction (MI, i.e., heart attack), ischemic stroke (IS), and cardiovascular mortality occurring in populations without prior CVD events

{D'Agostino, 2008, 10694408; Goff, 2014, 3121148; Lloyd-Jones, 2017, 10694407}. Moreover, disturbances in cholesterol homeostasis contribute to the pathology of non-alcoholic fatty liver disease (NAFLD) and to accumulation of lipids in hepatocytes {Malhotra, 2020, 10442471}, providing further evidence of effects in the liver. Finally, substantial evidence links low birth weight to a variety of adverse health outcomes at various stages of life. It has been shown to predict prenatal mortality and morbidity {Cutland, 2017, 10473225; U.S. EPA, 2013, 4158459; WHO, 2014, 10473141} and is a leading cause of infant mortality in the United States {CDC, 2020, 10473144}.Low birth weight is also associated with increased risk for diseases in adulthood, including obesity, diabetes, and cardiovascular disease {Gluckman, 2008, 10473269; Osmond, 2000, 3421656; Risnes, 2011, 2738398; Smith, 2016, 10474151; Ong, 2002, 10474127, as reported in Yang et al. (2022, 10176603).

There are challenges associated with quantitative use of epidemiological data for risk assessment {Deener, 2018, 6793519} as described above; however, improvements such as methodological advancements that minimize bias and confounding, strengthened methods to estimate and measure exposure, and updated systematic review practices facilitate the use of epidemiological studies to quantitatively inform risk.

6.2 Comparisons Between Toxicity Values Derived from Animal Toxicological Studies and Epidemiological studies

As recommended by the SAB {U.S. EPA, 2022, 10476098}, EPA derived candidate RfDs and CSFs for multiple health outcomes using data from both epidemiological and animal toxicological studies. Candidate RfDs from epidemiological and animal toxicological studies within a health outcome differed by approximately two to three orders of magnitude (see Figure 4-3), with epidemiological studies producing lower values. EPA does not necessarily expect concordance between animal and epidemiological studies in terms of the adverse effect(s) observed, as well as the dose level that elicits the adverse effect(s). For example, EPA's *Guidelines for Developmental Toxicity Risk Assessment* states that "the fact that every species may not react in the same way could be due to species-specific differences in critical periods, differences in timing of exposure, metabolism, developmental patterns, placentation, or mechanisms of action" {U.S. EPA, 1991, 732120}. EPA further describes these factors in relation to this assessment below.

First, there are well-established differences in the toxicokinetics between humans and animal models such as rats and mice. As described in Section 3.3.1.4.5, PFOS half-life estimates vary considerably by species, being lowest in rodents (hours to days) and several orders of magnitude higher in humans (years). All candidate toxicity values based on animal toxicological studies were derived from studies conducted in rats or mice, adding a potential source of uncertainty related to toxicokinetic differences in these species compared to humans. To address this potential source of uncertainty, EPA utilized a PK model to estimate the internal dosimetry of each animal model and convert the values into predicted levels of human exposure that would result in the corresponding observed health effects. However, the outputs of these models are *estimates* and may not fully account for species-specific toxicokinetic differences, particularly differences in excretion. The application of uncertainty factors (i.e., UF_A) also may not precisely reflect animal-human toxicokinetic differences.

Second, candidate toxicity values derived from epidemiological studies are based on responses associated with actual environmental exposure levels, whereas animal toxicological studies are limited to the tested dose levels which are often several orders of magnitude higher than the ranges of exposure levels in humans. Extrapolation from relatively high experimental doses to environmental exposure levels introduces a potential source of uncertainty for toxicity values derived from animal toxicological studies; exposures at higher dose levels could result in different responses, perhaps due to differences in mechanisms activated, compared to responses to lower dose levels. One example of this is the difference between epidemiological and animal toxicological studies in the effect of PFOS exposure on serum lipid levels (i.e., potential non-monotonic dose-response relationships that are not easily assessed in animal studies due to low dose levels needed to elicit the same response observed in humans).

Third, there may be differences in mechanistic responses between humans and animal models. Two examples of this is the PPARα and CAR responses. It is unclear to what extent PPARα and CAR influence the responses to PFOS exposure observed in humans, though the rodent PPARα and CAR responses may differ from those observed in humans (see Section 3.4.1.3.1). Mechanistic differences could influence dose-response relationships and subsequently result in differences between toxicity values derived from epidemiological and animal toxicological studies. There may be additional mechanisms that differ between humans and animal models that could contribute to the magnitude of responses and doses required to elicit responses across species.

The factors described above represent some but not all potential contributors that may explain the differences between toxicity values derived from epidemiological and animal toxicological studies. In this assessment, EPA prioritized epidemiological studies of *medium* or *high* confidence for the selection of health outcome-specific and overall RfDs and CSFs (see Section 4.1.6). The use of human data to derive toxicity values removes uncertainties and assumptions about human relevance inherent in extrapolating from and interpreting animal toxicological data in quantitative risk assessment.

6.3 Updated Approach to Animal Toxicological RfD Derivation Compared to the 2016 PFOS HESD

For POD derivation in this assessment, EPA considered the studies identified in the recent literature searches and also re-examined the candidate RfDs derived in the 2016 PFOS HESD {U.S. EPA, 2016, 3603365} and the animal toxicological studies and endpoints on which they were based. The updated approach used for hazard identification and dose response in the current assessment as compared to the 2016 HESD led to some differences between animal toxicological studies and endpoints used as the basis of candidate RfDs for each assessment. These updates and the resulting differences are further described below.

For the 2016 PFOS HESD, EPA did not use BMD modeling to derive PODs, and instead relied on the NOAEL/LOAEL approach for all candidate studies and endpoints {U.S. EPA, 2016, 3603365}. The NOAEL/LOAEL approach allows for the incorporation of multiple endpoints from a single study to derive a single POD, if the endpoints have the same NOAEL and/or LOAEL. For example, in the 2016 PFOS HESD, EPA derived a candidate RfD based on the endpoints of increased ALT and increased blood urea nitrogen (BUN) reported by Seacat et al. (2003, 1290852), both of which shared a common POD (NOAEL). For the current assessment,

EPA preferentially used BMD modeling to derive PODs because it allows for greater precision than the NOAEL/LOAEL approach and considers the entirety of the dose-response curve. This approach requires the consideration of endpoints on an individual basis and further examination of the weight of evidence for particular endpoints, as well as the dose-response trend reported for each endpoint, in order to derive a BMDL. When considering an effect on a standalone basis rather than grouped with other effects occurring at the same exposure level, EPA sometimes determined the weight of evidence was not sufficient to consider an individual endpoint for POD derivation. For the current assessment, EPA used a systematic review approach consistent with the IRIS Handbook {U.S. EPA, 2022, 10367891} to consider the weight of evidence for both the health outcomes as well as for individual endpoints of interest when selecting endpoints and studies for dose-response modeling. In the case of the endpoints selected in the 2016 PFOS HESD from the Seacat et al. (2003, 1290852) study, renal effects such as increased BUN were reevaluated and determined to have *evidence suggestive* of an association with PFOS exposure. As described in Section 4.1.1, in this assessment, EPA only derived PODs for endpoints from health outcomes with *evidence indicating* or *evidence demonstrating* an association with PFOS exposure.

Additionally, for the current assessment, EPA preferentially selected endpoints that were amenable to BMD modeling, had dose-dependent trends in responses, were supported by at least one other study in the available literature, and were direct/specific measures of toxicity for POD derivation. For some studies considered in the 2016 PFOS HESD and reevaluated during the current assessment, EPA attempted BMD modeling for specific endpoints but the efforts did not result in viable model fits. For the current assessment, EPA elected to derive a candidate RfD for hepatic effects based on histopathological lesions observed in the liver as reported by Butenhoff et al. (2012, 1276144)/Thomford (2002, 5029075) rather than serum ALT reported by Seacat et al. (2003, 1290852), as the Butenhoff et al. (2012, 1276144)/Thomford (2002, 5029075) studies were rated as *high* confidence (vs. the *medium* confidence Seacat et al. (2003, 1290852)), used a chronic study design (vs. the 14-week exposure used by Seacat et al. (2003, 1290852)), and histopathological lesions reflect direct damage to the liver whereas ALT is an indicator of liver damage. In animal studies, evaluation of direct liver damage is possible, however in humans, it is difficult to obtain biopsy-confirmed histological data. Therefore, liver injury is typically assessed using serum biomarkers of hepatotoxicity {Costello et al, 2022, 10285082}.

For some health outcomes, new studies have been published since 2016 that improve upon the weight of evidence determined in the 2016 PFOS HESD. For example, in 2016, EPA did not derive a candidate RfD based on immune effects. Since that time, several *high* and *medium* confidence studies (both animal toxicological and epidemiological) have been published that increased the strength of evidence for this health outcome. As described in Section 3.4.2.4, *evidence indicates* that PFOS exposure is associated with immune effects and therefore, in this assessment, EPA derived candidate RfDs for the immune health outcome.

For transparency, EPA has provided a comparison of studies and endpoints used to derive candidate RfDs for both the 2016 PFOS HESD and the present assessment in Table 6-1.

Table 6-1. Comparison of Candidate RfDs Derived from Animal Toxicological Studies for Priority Health Outcomes[a]

Studies and Effects Used in 2016 for Candidate RfD Derivation[b]	Studies and Effects Used in 2023 for Candidate RfD Derivation
Immune	
NA	Zhong et al. (2016, 3748828), *medium* confidence – decreased PFC response to SRBC NTP (2019, 5400978), *high* confidence – extramedullary hematopoiesis
Developmental	
Luebker et al. (2005, 757857) *medium* confidence – decreased pup body weight Luebker et al. (2005, 1276160), *medium* confidence – decreased pup survival Lau et al. (2003, 757854), *medium* confidence – decreased pup survival	Luebker et al. (2005, 757857), *medium* confidence – decreased pup body weight
Hepatic	
Seacat et al. (2003, 1290852), *medium* confidence – increased ALT (and increased BUN)	Butenhoff et al. (2012, 1276144)/Thomford (2002, 5029075), *high* confidence – individual cell necrosis in the liver

Notes: RfD = reference dose; NA = not applicable; PFC = plaque forming cell; SRBC = sheep red blood cell; NTP = National Toxicology Program; ALT = alanine aminotransferase; BUN = blood urea nitrogen.

[a] Note that candidate RfDs for the fourth priority health outcome (i.e., cardiovascular) are not presented in this table because candidate RfDs based on animal toxicological studies representing this health outcome were not derived in the 2016 HESD or the current assessment.

[b] Candidate RfDs from the 2016 HESD that correspond to non-prioritized health outcomes (e.g., nervous) are not presented here.

6.4 Reevaluation of the PFOS Carcinogenicity Database

In November 2021, EPA published the draft *Proposed Approaches to the Derivation of a Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* for review by the SAB PFAS Review Panel {U.S. EPA, 2021, 10428576}. As part of the review process, EPA charged the SAB panel with providing comment on the rationale and conclusion for the PFOS cancer classification. Prior to SAB review, EPA had concluded that the weight of evidence supported the determination of PFOS as having *Suggestive Evidence of Carcinogenicity*, similar to the conclusions of the 2016 PFOS HESD {U.S. EPA, 2016, 3603365}. This was, in part, because no new animal toxicological studies have been published since publication of the HESD and the new epidemiological literature continue to provide mixed results.

As part of the final report, the SAB noted, "[s]everal new studies have been published that warrant further evaluation to determine whether the "likely" designation is appropriate" {U.S. EPA, 2022, 10476098}. The SAB recommended EPA reevaluate several aspects of the carcinogenicity database for PFOS to confirm or update the draft *Proposed Approaches* conclusion that PFOS has *Suggestive Evidence of Carcinogenic Potential*, including epidemiological studies reporting kidney cancer (i.e., Shearer et al. (2021, 7161466) and Li et al. (2022, 9961926)), mechanistic data (e.g., Benninghoff et al. (2012, 1274145)), and "overly

conservative" conclusions about animal toxicological data in rats (i.e., Butenhoff et al. (2012, 1276144)). EPA has reevaluated these aspects of the database and relevant discussions of the recommended studies are provided in Section 3.5.

Upon reassessment of the PFOS carcinogenicity database, including the epidemiological, animal toxicological, and mechanistic databases, EPA has now determined the available data for PFOS surpass many of the descriptions for *Suggestive Evidence of Carcinogenic Potential* according to the *Guidelines for Carcinogen Risk Assessment* {U.S. EPA, 2005, 6324329}. The examples for which the PFOS database exceeds the descriptions outlined in the *Guidelines for Carcinogen Risk Assessment* include:

- "a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor '*Likely to Be Carcinogenic to Humans*;'
- a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed;
- evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion; and
- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend" {U.S. EPA, 2005, 6324329}.

The strongest evidence for the carcinogenicity of PFOS is primarily from one chronic animal bioassay which presents findings surpassing several of these criteria. {Thomford, 2002, 5029075/Butenhoff, 2012, 1276144}. The Thomford/Butenhoff et al. (2002, 5029075; 2012, 1276144) study is a *high* confidence study that observed statistically significant increases at individual dose levels and/or statistically significant trends in two tumor types and in one or more sexes, even with the relatively low dose levels used. The background incidence of these tumor types was low or negligible.

In the draft *Proposed Approaches* document, EPA relied upon the tumor incidences provided in Butenhoff et al. (2012, 1276144), which is the peer-reviewed manuscript of an unpublished industry report - Thomford (2002, 5029075). Upon further review of the Thomford (2002, 5029075) report, EPA recognized two factors that influenced previous qualitative and quantitative interpretations of the data: 1) the Butenhoff et al. (2012, 1276144) study reported combined incidences of neoplastic lesions in the control and high dose groups from the interim time point (52 weeks of dietary exposure; n = 10) and terminal time point (104 weeks of dietary exposure; n = 50); and 2) the Butenhoff et al. (2012, 1276144) study did not report incidences for pancreatic islet cell neoplasms. The first factor resulted in statistical dilutions of tumor incidence in the high dose group as many of the tumor types observed in the study, including hepatocellular neoplasms, were not reported until approximately 70 weeks of treatment or later. Therefore, EPA excluded animals sacrificed at the interim time point from statistical analyses as it was biologically implausible for the 10 animals from the interim time point to have presented with neoplasms. The second factor resulted in a previous lack of recognition by EPA that a statistically significant trend in a second tumor site/type (pancreatic islet cell carcinomas) was

observed in the chronic cancer bioassay. This factor importantly results in PFOS meeting an additional characteristic for the designation of *Likely to be Carcinogenic to Humans:* "an agent that has tested positive in animal experiments in more than one species, sex, strain, **site**, or exposure route, with or without evidence of carcinogenicity in humans" {U.S. EPA, 2005, 6324329}.

Although the study design (i.e., low dose levels and relatively large gap between the highest and next highest dose group levels (5 and 20 ppm)) may limit the ability to interpret the dose-response relationship presented by Thomford/Butenhoff et al. (2002, 5029075; 2012, 1276144), these results are quantifiable and have been used to derive CSFs within this assessment. Overall, the Thomford/Butenhoff et al. (2002, 5029075; 2012, 1276144) report, along with plausible associations between PFOS exposure and carcinogenicity reported by epidemiological studies, provides substantive evidence that PFOS exceeds the designation of *Suggestive Evidence of Carcinogenic Potential* and is consistent with *Likely Evidence of Carcinogenic Potential in Humans* (see Section 3.5.5 for more information on the *Likely* determination).

Table 6-2. Comparison of the PFOS Carcinogenicity Database with the *Suggestive* Cancer Descriptor as Described in the Guidelines for Carcinogen Risk Assessment {U.S. EPA, 2005, 6324329}

Suggestive Evidence of Carcinogenic Potential	
A small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "Likely to Be Carcinogenic to Humans." The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system	**PFOS data exceed this description**. Observed statistically significant increases in hepatic tumors (adenomas in males and adenomas and carcinomas in females) at the high dose and a statistically significant trend overall in both sexes.
A small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed.	**This description is not applicable to the tumor types observed after PFOS exposure.**
Evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence (such as structure-activity relationships)	**PFOS data exceed this description**. The study from which carcinogenicity data are available was determined to be *high* confidence during study quality evaluation.
A statistically significant increase at one dose only, but no significant response at the other doses and no overall trend	**PFOS data exceed this description**. Observed statistically significant increases in hepatic tumors (adenomas in males and adenomas and carcinomas in females) at the high dose and a statistically significant trend overall. Also observed statistically significant trend of increased pancreatic islet cell tumors with increasing dose.

6.5 Health Outcomes with Evidence Integration Judgments of *Evidence Suggests* Bordering on *Evidence Indicates*

EPA evaluated sixteen non-cancer health outcomes as part of this assessment. In accordance with recommendations from the SAB {U.S. EPA, 2022, 10476098} and the IRIS Handbook {U.S. EPA, 2022, 10367891}, for both quantitative and qualitative analyses in the current assessment, EPA prioritized health outcomes with either *evidence demonstrating* or *evidence indicating* associations between PFOS exposure and adverse health effects. Health outcomes reaching these tiers of judgment were the hepatic, immune, developmental, cardiovascular, and cancer outcomes. Some other health outcomes were determined to have *evidence suggestive* of associations between PFOS and adverse health effects as well as some characteristics associated with the *evidence indicates* tier, and EPA made judgments on these health outcomes as described below.

For PFOS, two health outcomes that had characteristics of both *evidence suggests* and *evidence indicates* were the endocrine and nervous system outcomes. Endpoints relevant to these two health outcomes had been previously considered for POD derivation in the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water.* However, upon further examination using the protocols for evidence integration outlined in the PFOS Appendix and Section 2.1.5, EPA concluded that the available epidemiological and animal toxicological evidence did not meet the criteria necessary for subsequent quantitative dose-response analyses. Although these health outcomes were not prioritized in the current assessment, based on the available data, EPA concluded that PFOS exposure may cause adverse endocrine or nervous system effects.

Epidemiological studies considered for evidence integration for adverse endocrine effects include many *high* and *medium* confidence studies. There was *slight evidence* to suggest human endocrine toxicity, including associations between PFOS exposure and thyroid disease. However, this evidence was limited to one *high* confidence study {Kim, 2020, 6833758}. In addition, the available evidence supports the relationship between PFOS exposure and thyroid stimulating hormone (TSH) in children and, to a lesser extent, adults. Similar to what was concluded in the 2016 PFOS HESD, evidence supporting adverse endocrine effects was inconsistent among epidemiological studies. Animal toxicological studies considered for evidence integration consisted of 13 *high* or *medium* confidence studies. The animal evidence for an association between PFOS exposure and effects on the endocrine system was considered *moderate*, based on observed disruptions of normal thyroid function (i.e., decreased free thyroxine (T4), total T4 and total triiodothyronine (T3)). In addition, reductions in hormones associated with the hypothalamic-pituitary-adrenal axis were observed, although the corresponding histopathological data was inconsistent. Overall, the available human and animal evidence was *suggestive* but not *indicative* of, adverse endocrine effects due to PFOS exposure. Therefore, EPA did not prioritize this outcome for dose-response modeling. See Appendix C for a detailed description of endocrine evidence synthesis and integration.

Similar endocrine effects are observed among the family of PFAS chemicals. For example, the thyroid was identified as a target for oral exposure to PFBS {U.S. EPA, 2021, 7310530}. Additionally, the draft IRIS *Toxicological Review of PFBA* concluded that the available *evidence indicates* the observed thyroid effects were likely due to PFBA exposure {U.S. EPA, 2021,

10064222}. Given the similarities across PFAS, these findings support potential associations between PFOS and adverse endocrine effects.

There was also *slight* evidence from epidemiological studies that supported a relationship between PFOS exposure and adverse nervous system effects, but study results were mostly mixed or limited. For example, studies evaluating neurodevelopmental, neuropsychological, and cognitive outcomes were limited with only one study supporting an adverse effect of PFOS exposure on hearing {Li, 2020, 6833686}. Although multiple studies examining associations between PFOS and ADHD were available, only one study reported a significant relationship between PFOS and ADHD {Lenters, 2019, 5080366}. There was an indication of a potential relationship between PFOS and autistic behaviors or ASD diagnosis in some studies {Braun, 2014, 2345999; Oulhote, 2016, 3789517; Shin, 2020, 6507470}, however there were methodology concerns associated with these studies. Animal studies considered for evidence integration suggest a relationship between PFOS exposure and nervous system effects, specifically in relation to learning and memory and neurotransmitter concentrations. Although there is *moderate* evidence to support adverse effects on the nervous system following exposure to PFOS from animal toxicological studies, EPA concluded there is considerable uncertainty in the results due to inconsistency across studies and limited number of studies. Overall, the available human and animal evidence was *suggestive* but not *indicative* of adverse nervous system effects due to PFOS exposure. Therefore, EPA did not prioritize this outcome for dose-response modeling. See Appendix C for a detailed description of endocrine evidence synthesis and integration.

As the databases for endocrine and nervous system outcomes were *suggestive* of human health effects resulting from PFOS exposure, they were not prioritized during the updated literature review conducted in February 2022. However, EPA acknowledges that future studies of these currently "borderline" associations could impact the strength of the association and the weight of evidence for these health outcomes. The currently available studies indicate the potential for endocrine and nervous system effects after PFOS exposure. Studies on endocrine and nervous system health outcomes represent two important research needs.

6.6 Challenges and Uncertainty in Modeling

6.6.1 Modeling of Animal Internal Dosimetry

There are several limitations and uncertainties associated with using pharmacokinetic models in general and estimating animal internal dosimetry. In this assessment, EPA utilized the Wambaugh et al. (2013, 2850932) animal internal dosimetry model because it had availability of model parameters across almost all species of interest, agreement with out-of-sample datasets (see PFOS Appendix), and flexibility to implement life-course modeling (see Section 4.1.3.1). However, there were some limitations to this approach.

First, posterior parameter distributions summarized in Table 4-3 for each sex/species combination were determined using a single study. Therefore, uncertainty in these parameters represents only uncertainty in fitting that single study; any variability between studies or differences in study design were not accounted for in the uncertainty of these parameters. Second, issues with parameter identifiability for some sex/species combinations resulted in

substantial uncertainty for some parameters. For example, filtrate volume (Vfil) represents a parameter with poor identifiability when determined using only serum data due to lack of sensitivity to serum concentrations (See PFOS Appendix). Measurements in additional matrices, such as urine, would help inform this parameter and reduce the uncertainty reflected in the wide credible intervals of the posterior distribution. These parameters with wide posterior CIs represent parameters that are not sensitive to the concentration-time datasets on which the model was trained (See PFOS Appendix). However, these uncertain model parameters will not impact the median prediction used for BMD modeling and simply demonstrate that the available data are unable to identify all parameters across every species over the range of doses used for model calibration. Finally, the model is only parameterized using adult, single dose, PFOS study designs. Gestational and lactational PK modeling parameters were later identified from numerous sources (Table 4-5) to allow for the modeling of these life stages with a more detailed description of the life-course modeling in Section 4.1.3.1.3.

The Wambaugh et al. (2013, 2850932) model fit the selected PFOS developmental study data well, though there are several limitations to using this method to model developmental life stages. 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. Second, clearance in the pup during lactation is assumed to be a first-order process governed by a single half-life. At low doses, this assumption is in line with adult clearance, but it is unclear how physiological changes during development impact the infant half-life. Finally, PFOS concentrations in breast milk are assumed to partition passively from the maternal blood. This assumption does not account for the presence of active transport in the mammary gland or time-course changes for PFOS uptake to the milk. Despite these limitations, the incorporation of model parameters related to developmental life stages is a significant improvement over the model used in the 2016 HESD which did not implement life course modeling {U.S. EPA, 2016, 3603365}.

6.6.2 Modeling of Human Dosimetry

Uncertainties may stem from efforts to model human dosimetry. One limitation is that the clearance parameter, which is a function of the measured half-life and V_d values, is difficult to estimate in the human general population. Specifically for PFOS, the measurement of half-life is hindered by slow excretion and ongoing exposure. Additionally, it is unclear whether some of the variability in measured half-life values reflects actual variability in the population, as opposed to 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 PFOS in children and infants is not well understood. The ontogeny of renal transporters, age-dependent changes in overall renal function, and the amount of protein binding (especially in serum) could all play a role in PFOS excretion and could vary between children and adults. It is even difficult to predict the overall direction of change in excretion in children (higher or lower than in adults) without a clear understanding of

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 for pharmaceuticals, but PFOS is unlike most pharmaceuticals 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 PFOS.

The updated half-life value was developed based upon a review of recent literature (see Section 3.3.1.4.5). Many half-life values have been reported for the clearance of PFOS in humans (see PFOS Appendix). The slow excretion of PFOS requires measurement of a small change in serum concentration over a long time; the difficulties associated with making these measurements may represent one reason for the variance in reported values. Another challenge is the ubiquity of PFOS exposure. Ongoing exposure will result in a positive bias in observed half-life values if not considered {Russell, 2015, 2851185}. In studies that calculate the half-life in a population with greatly decreased PFOS exposures, typically due to the end of occupational exposure or the introduction of drinking water filtration, the amount of bias due to continuing exposure will be related to the ratio of the prior and ongoing exposure. That is, for a given ongoing exposure, a higher prior exposure may be less likely to overestimate half-life compared to a lower prior exposure. However, a half-life value determined from a population with very high exposure may not be informative of the half-life in typical exposure scenarios 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, which are more representative of the general population, due to saturation of renal resorption and increased urinary clearance in the study population.

Because the derivation of the V_d for PFOS relied on the value for PFOA, it is important to consider alternate values for V_d for PFOA. For PFOA, the V_d calculation depended on the half-life. Thompson et al. (2010, 2919278) used 2.3 years, which was estimated within their population. If EPA chosen half-life of 2.7 years was used instead, the V_d for PFOA would be 200 mL/kg, which results in a PFOS value of 271 mL/kg. EPA did not update the V_d values based on the updated half-life because the value of 2.3 years was calculated based on the same data as the V_d and this half-life may be more representative of that population at that specific time. Gomis et al. (2017, 3981280) also calculated V_d by taking the average of reported animal and human values and estimated values of 235 mL/kg for PFOS. This calculation included the value from Thompson et al. (2010, 2919278) and did not include additional values derived from human data. This average value shows that the value from Thompson et al. (2010, 2919278), which was selected based on the fact that it was derived only from human and non-human primate data, is reasonable.

Lastly, the description of breastfeeding in the updated Verner et al. (2016, 3299692) model relied on a number of assumptions: that infants were exclusively breastfed for one year, that there was a constant relationship between maternal serum and breastmilk PFOS concentrations, and that weaning was an immediate process with the infant transitioning from a fully breastmilk diet to the background exposure at one year. This is a relatively long duration of breastfeeding, only 27% of children in the U.S. are being breastfed at one year of age {CDC, 2013, 1936457}. Along with using the 95th percentile of breastmilk consumption, this provides a scenario of high but

realistic lactational exposure. Lactational exposure to the infant is much greater than background exposure so the scenario of long breastfeeding is a conservative approach and will result in a lower POD_{HED} than a scenario with earlier weaning. Children in the U.S. are very unlikely to be exclusively breastfed for up to one year, and this approach does not account for potential PFOS exposure via the introduction of solid foods. However, since lactational exposure is much greater than exposure after weaning, a breastfeeding scenario that does not account for potential PFOS exposure from introduction of infants to solid foods is not expected to introduce substantial error.

6.6.3 Approach of Estimating a Benchmark Dose from a Regression Coefficient

EPA identified epidemiological studies (e.g., Steenland et al. (2009, 1291109)) that reported associations between PFOS exposure and diseases or clinical outcomes as regression coefficients. BMD modeling of regression coefficients results in a non-traditional BMD, where the BMR is associated with a change in the regression coefficient of the response variable rather than the measured biological response variable. As a result, there is some uncertainty about the biological relevance of this non-traditional BMD associated with a regression coefficient. However, as this regression coefficient is associated with a change in the biological response variable, it is biologically meaningful and EPA concluded that it can therefore be used for POD derivation. EPA modeled these regression coefficients using the same approach that EPA used to model for studies that reported measured response variables which is similar to the approach followed by CalEPA in their draft Public Health Goal for PFOS {CalEPA, 2021, 9416932}.

To evaluate this potential uncertainty, EPA obtained the measured dose response data across exposure deciles from Steenland et al. (2009, 1291109) (kindly provided to EPA on June 30, 2022 via email communication with the corresponding study author) and conducted sensitivity analyses to compare BMDs produced by the reported regression coefficients with the measured response variable (i.e., mean total cholesterol and odds ratios of elevated total cholesterol). These analyses are presented in detail in the PFOS Appendix.

For PFOS, $BMDL_5$ values estimated using the regression coefficient and using the measured response variable were 9.52 ng/L and 26.39 ng/L, respectively. The two BMDL estimates from the two approaches are within an order of magnitude, less than a 3-fold difference, and the RfD allows for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate. Therefore, EPA is confident in its use regression coefficients as the basis of POD_{HED}s.

6.7 Human Dosimetry Models: Consideration of Alternate Modeling Approaches

PBPK models are typically preferred over a one-compartment approach because they can provide individual tissue information and have a one-to-one correspondence with the biological system that can be used to incorporate additional features of pharmacokinetics, including tissue-specific internal dosimetry and local metabolism. In addition, though PBPK models are more complex than one-compartment models, 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 toxicological studies when parameterizing a model for humans, where data are typically scarcer.

The decision to select a non-physiologically based model as opposed to one of the PBPK models was influenced in part by past issues identified during evaluation of the application of PBPK models to other PFAS for the purpose of risk assessment. During the process of adapting a published PBPK model for EPA needs, models are subjected to an extensive EPA internal QA review. During initial review of the Loccisano family of models {Loccisano, 2011, 787186; Loccisano, 2012, 1289830; Loccisano, 2012, 1289833; Loccisano, 2013, 1326665}, an unusual implementation of PFOS plasma binding appeared to introduce a mass balance error. Due to the stated goal of minimizing new model development (see Section 4.1.3.2), EPA did not pursue resolution of the discrepancies, which would have required modifications to one of these models for application in this assessment.

A new publication describing a developmental PBPK model in rats and humans was also evaluated for this effort {Chou, 2021, 7542658}. This model used the *in vitro* extrapolation that was previously developed by Worley et al. (2015, 3981311) for PFOA as an initial point for parameter optimization for PFOS. The complex nature of this renal model, with processes for resorption, secretion, and passive diffusion presented multiple competing options for parameterization based on the available human data. Specifically, the set of available model parameters can take numerous values that fit the human observations equally well. However, when the model is applied within similar conditions to the human observations, predicting the exact values of the parameters may not impact the model's ability to predict the targeted biomarkers (i.e., human milk, fetal serum, and maternal serum). For our purposes, it was not clear, whether the exposure and internal doses that needed modeling would be within the bounds of the doses used to parameterize the Chou et al. (2021, 7542658) model.

Due to the previous issues that EPA encountered for other PFAS when implementing PBPK models, the known issue with the Loccisano model and the models based upon it, and the concerns about application of the Chou et al. (2021, 7542658) model outside its original parameterization space, EPA concluded that a one-compartment model was the strongest approach to predict blood (or serum/plasma) concentrations. Serum/plasma is a good biomarker for exposure, because a major proportion of the PFOS in the body is found in serum/plasma due to albumin binding {Forsthuber, 2020, 6311640}. There were no other specific tissues that were considered essential to describe the dosimetry of PFOS. A full PBPK model can predict serum concentrations equally well, but with many more parameters, many of which are difficult to predict for PFOS due to parameter identifiability issues. PFOS presents an unusually high barrier in this regard because much of its PK is dependent on the interaction between PFOS and proteins in the form of binding {Frosthuber, 2020, 6311640} and active transport {Zhao, 2017, 3856461}. These protein interactions are more difficult to extrapolate from animal toxicological studies to humans than PK that is dependent on blood flow and passive diffusion.

The only one-compartment approach identified in the literature for PFOS was the model of Verner et al. (2016, 3299692). EPA also considered the model developed by the Minnesota Department of Health (MDH model), which was published as a PFOA model, but has been applied to other PFAS, including PFOS {Goeden, 2019, 5080506}. These two models are structurally very similar, with a single compartment each for mother and child, first-order excretion from those compartments, and a similar methodology for describing lactational transfer from mother to child. The following paragraphs describe the slight differences in model implementations, but it is first worth emphasizing the similarity in the two approaches. The

overall agreement in approach supports its validity for the task of human health risk assessment for PFOS.

One advantage of the Verner model is that it explicitly models the mother from birth through the end of breastfeeding. The MDH model, however, is limited to predictions for the time period after the birth of the child with maternal levels set to an initial steady-state level. An explicit description of maternal blood levels allows for the description of accumulation in the mother prior to pregnancy followed by decreasing maternal levels during pregnancy, as has been observed for serum PFOS in serial samples from pregnant women {Glynn, 2012, 1578498}. This decrease occurs due to the relatively rapid increase in body weight during pregnancy (compared to the years preceding pregnancy) and the increase in blood volume that occurs to support fetal growth {Sibai, 1995, 1101373}. Detailed modeling of this period is important for dose metrics based on maternal levels during pregnancy, especially near term, and on cord blood levels.

Another distinction of the Verner model is that it is written in terms of rates of change in mass rather than concentrations, as in the MDH model. This approach includes the effect of dilution of PFOS during childhood growth, without the need for an explicit term in the equations. Not accounting for growth will result in the overprediction of serum concentration in individuals exposed during growth. Despite this, PFOS concentration in infants at any specific time is driven more by recent lactational exposure than by earlier exposure (either during pregnancy or early breastfeeding), which tends to minimize the impact of growth dilution. Additionally, this structural consideration best matches the approach taken in our animal model, presenting a harmonized approach. These structural considerations favor the application of the updated Verner model over the MDH model.

EPA evaluated two other factors that were present in the MDH model: the application of a scaling factor to increase the V_d in children and the treatment of exposure as a drinking water intake rather than a constant exposure relative to bodyweight. After testing these features within the updated Verner model structure, EPA determined that neither of these features were appropriate for this assessment, primarily because they did not meaningfully improve the comparison of model predictions to validation data.

In the MDH model, V_d in children starts at 2.4 times the adult V_d and decreases relatively quickly to 1.5 times the adults V_d between 6 and 12 months, reaching the adult level at 10 years of age. These scaling values originated from measurements of body water content relative to weight compared to the adult value. There is no chemical-specific information to suggest that V_d is larger in children compared to adults for PFOS. However, it is generally accepted in pharmaceutical research that hydrophilic chemicals have greater V_d in children {Batchelor, 2015, 3223516}, which is attributed to increased body water. Still, PFOS is amphiphilic, not simply hydrophilic, and its distribution is driven by interactions with binding proteins and transporters, not by passive diffusion with body water. While it is plausible that V_d is larger in children, it is unknown to what degree.

Since increased V_d in children is plausible, but neither supported nor contradicted by direct evidence, EPA evaluated the effect of variable V_d by implementing this change in the updated Verner model and comparing the results with constant and variable V_d (see PFOS Appendix). This resulted in reduced predictions of serum concentrations, primarily during their peak in early childhood. The model with variable V_d did not decrease the average relative error or the average

absolute value of relative error compared to the model with constant V_d (with PFOA and PFOS results combined). Since the model with constant V_d had marginally better performance and was an overall simpler solution, EPA did not implement variable V_d in the application of the model for POD_{HED} calculation.

The other key difference between the MDH model and the updated Verner model is that instead of constant exposure relative to body weight, exposure in the MDH model was based on drinking water consumption, which is greater relative to bodyweight in young children compared to adults. Drinking water consumption is also greater in lactating women. To evaluate the potential impact of calculating a drinking water concentration directly, bypassing the RfD step, EPA implemented drinking water consumption in the modified Verner model (see PFOS Appendix). EPA evaluated this decision for PFOA and PFOS together because the choice of units used for human exposure represents a substantial difference in risk assessment methodology. For reasons explained below, EPA ultimately decided to continue to calculate an RfD in terms of constant exposure, with an MCLG calculated thereafter using life-stage specific drinking water consumption values.

When comparing exposure based on drinking water consumption to the traditional RfD approach, the impact on the serum concentrations predicted by the updated Verner model differed between PFOA and PFOS. For PFOA, the predicted serum concentration in the child was qualitatively similar, with the main effect seen in overprediction of timepoints that occur later in childhood. These timepoints are more susceptible to changes in exposure as early childhood exposure is dominated by lactational exposure. Lactational exposure is slightly increased in this scenario, because of increased drinking water consumption during lactation. However, the main source of PFOA or PFOS in breastmilk in the model with exposure based on drinking water consumption is that which accumulated over the mother's life prior to childbirth, not that which was consumed during lactation. For PFOS, the increased exposure predicted based on children's water intake results in much greater levels in later childhood compared to the model with constant exposure relative to bodyweight. Use of water ingestion rates to adjust the dose in the Verner model fails to match the decrease in PFOS concentration present in the reported data with multiple timepoints and overestimates the value for the Norwegian Mother, Father, and Child Cohort Study (MoBa) cohort with a single timepoint. There is a much greater effect on PFOS model results relative to PFOA. This comparison suggests that incorporating variations in drinking water exposure in this way is not appropriate for the updated Verner model.

In addition to the comparison with reported data, EPA's decision to use the Verner model was also considered in the context of the effect on the derivation of MCLGs. The epidemiological endpoints can be placed into three categories based on the age of the individuals: adults, children, and pregnant women. Because increased drinking water exposure is only applied to children and lactating women, the group of endpoints in children are the only ones that would be affected. While the RfD estimated using the updated Verner model assumed constant exposure, the MCLG is an algebraic calculation that incorporates the RfD, RSC, and drinking water intake. The drinking water intake used for the MCLG calculation is chosen based on the target population relevant to the critical effect that serves as the basis of the RfD. Therefore, even if the RfD does not incorporate increased drinking water intake in certain lifestages, the subsequent

MCLG calculation does take this into account. Furthermore, derivation of an RfD is useful for general assessment of risk and not limited to drinking water exposure.

For these reasons and based on EPA's analyses, EPA determined that the updated Verner model was the most appropriate available model structure for POD_{HED} calculation for PFOS. Including the determination that assuming V_d in children equal to the adult values was appropriate, and that calculating a RfD assuming a constant dose (mg/kg/day) was appropriate for this assessment.

6.8 Sensitive Populations

Some populations may be more susceptible to the potential adverse health effects of toxic substances such as PFOS. These potentially susceptible populations include populations exhibiting a greater response than others despite similar PFOS exposure due to increased biological sensitivity, as well as populations exhibiting a greater response due to higher PFOS exposure and/or exposure to other chemicals or non-chemical stressors. Populations with greater biological sensitivity may include pregnant women and their developing fetuses, lactating women, the elderly, and people with certain underlying medical conditions (see Section 6.8.1). Populations that could exhibit a greater response to PFOS exposure due to higher exposures to PFOS or other chemicals include communities overburdened by chemical exposures or non-chemical stressors such as communities with environmental justice concerns (see Section 6.8.2).

The potential health effects after PFOS exposure have been evaluated in some sensitive populations (e.g., pregnant women, children) and a small number of studies have assessed differences in exposure to PFOS across populations to assess whether racial/ethnic or socioeconomic differences are associated with greater PFOS exposure. However, the available research on PFOS's potential impacts on sensitive populations is limited and more research is needed. Health effects differences in sensitivity to PFOS exposure have not allowed for the identification or characterization of all potentially sensitive subpopulations. This lack of knowledge about susceptibility to PFOS represents a potential source of uncertainty in the assessment of PFOS.

6.8.1 Fetuses, Infants, Children

One of the more well-studied sensitive populations to PFOS exposure is developing fetuses, infants, and children. Both animal toxicological and epidemiological data suggest that the developing fetus is particularly sensitive to PFOS-induced toxicity. As described in Section 3.4.4.1, results of some epidemiological studies indicate an association between PFOS exposure during pregnancy and adverse birth outcomes such as low birth weight, and studies of PFOS exposure during early childhood, which may also reflect *in utero* exposure, suggest an association between PFOS exposure and effects on development, including immune system development (Section 3.4.2.1). The available animal toxicological data lend support to these findings; as described in Section 3.4.4.2, numerous studies in rodents report effects similar to those seen in humans (e.g., decreased body weights in offspring exposed to PFOS during gestation). Additionally, PFOS exposure during certain life stages or exposure windows (e.g., prenatal or early postnatal exposure windows) may be more consequential than others. For example, as described in Section C.7.2 of the PFOS Appendix, Grasty et al. (2003, 1332670; 2005, 2951495) identified GD 19-21 as a critical exposure window for neonatal lung development and subsequent neonatal mortality in rats. These potentially different effects in

different populations and/or exposure windows have not been fully characterized. More research is needed to fully understand the specific critical windows of exposure during development.

With respect to the decreased antibody production endpoint, children who have autoimmune diseases (e.g., juvenile arthritis) or are taking medications that weaken the immune system would be expected to be more likely to mount a low antibody response and would therefore represent potentially susceptible populations for PFOS exposure. There are also concerns about declines in vaccination status {Smith, 2011, 9642143; Bramer, 2020, 9642145} for children overall, and the possibility that diseases which are considered eradicated (such as diphtheria or tetanus) could return to the United States {Hotez, 2019, 9642144}. As noted by Dietert et al. (2010, 644213), the risks of developing infectious diseases may increase if immunosuppression occurs in the developing immune system.

6.8.2 Other Susceptible Populations

As noted in the SAB PFAS review panel's final report {U.S. EPA, 2022, 10476098}, there is uncertainty about whether there are susceptible populations, such as certain racial/ethnic groups, that might be more sensitive to the health effects of PFOS exposure because of either greater biological sensitivity or higher exposure to PFOS and/or other environmental chemicals. Although some studies have evaluated differences in PFAS exposure levels across SES and racial/ethnic groups (see Section 6.1), studies of differential health effects incidence and PFOS exposure are limited. To fully address equity and environmental justice concerns about PFOS, these data gaps regarding differential exposure and health effects after PFOS exposure need to be addressed. In the development of the proposed PFAS NPDWR, EPA conducted an analysis to evaluate potential environmental justice impacts of the proposed regulation (See Chapter 8 of the Economic Analysis for the Proposed PFAS National Primary Drinking Water Regulation {U.S. EPA, 2023, 10692765}). EPA acknowledges that exposure to PFOS, and PFAS in general, may have a disproportionate impact on certain communities (e.g., low SES communities; tribal communities; minority communities; communities in the vicinity of areas of historical PFOS manufacturing and/or contamination) and that studies of these communities are high priority research needs.

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