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

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

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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

Executive Summary

The U.S. Environmental Protection Agency (EPA) is issuing final toxicity values for *perfluorooctane sulfonic acid (PFOS), including all isomers and nonmetal salts.* The toxicity assessment for PFOS is a scientific report that describes the evaluation of the available animal toxicity and human epidemiology data in order to characterize the noncancer and cancer human health hazards. This assessment also includes f*inal toxicity values* associated with noncancer health effects (i.e., oral reference doses, or RfDs) and cancer effects (i.e., cancer slope factors, or CSFs) following oral PFOS exposure. It is not a risk assessment, as it does not include an exposure assessment or an overall risk characterization nor does it address the legal, policy, social, economic, or technical considerations involved in risk management. The PFOS toxicity assessment can be used by EPA, states, Tribes, and local communities, along with specific exposure and other relevant information, to determine, under the appropriate regulations and statutes, the potential risk associated with human exposures to PFOS, its isomers, and its nonmetal salts.

This final toxicity assessment was peer reviewed by the EPA Science Advisory Board (SAB) per- and polyfluoroalkyl substances (PFAS) Review Panel in November 2021 and underwent public comment in March 2023. It incorporated expert scientific recommendations received from the SAB in 2022 (U.S. EPA, 2022e) as well as feedback from the public comment period (U.S. EPA, 2024c). This final assessment builds upon the literature review presented in the *2016 Health Effects Support Document for Perfluorooctane Sulfonic Acid (PFOS)* (hereafter referred to as the 2016 PFOS HESD) (U.S. EPA, 2016b) and is an update of the *SAB review draft, Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (U.S. EPA, 2022b) and the subsequent *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023).

PFOS is a member of the PFAS group. These manufactured chemicals have a history of industrial and consumer use in the United States and are considered persistent chemicals based on their physicochemical properties. Some of the human health concerns about exposure to PFOS and other PFAS stem from their resistance to hydrolysis, photolysis, metabolism, and microbial degradation in the environment and in the human body. PFAS are not naturally occurring; they are manmade compounds that have been used widely over the past several decades in industrial applications and consumer products since many PFAS have repellant and surfactant properties. Frequently used as emulsifiers and as stain-, oil-, or water-repellents, PFAS are found in a variety of environmental media and in tissues of organisms, including humans.

Most PFOS production in the United States was voluntarily phased out by its primary manufacturer (3M) between 2000 and 2002. In 2002 and 2007, EPA took regulatory action under the Toxic Substances Control Act (TSCA) to require that EPA be notified prior to any future domestic manufacture or importation of PFOS and 270 related PFAS (U.S. EPA, 2016a). Manufacturers have since shifted to alternative short-chain PFAS, such as perfluorobutane sulfonic acid (PFBS) (3M, 2002). However, PFOS remains persistent in environmental media because it is resistant to environmental degradation processes.

The purpose of this human health toxicity assessment is to derive toxicity values pertaining to oral exposure for PFOS. The development of this toxicity assessment relied on a robust systematic review process, based on the EPA peer-reviewed human health risk assessment methodology outlined in the EPA *ORD Staff Handbook for Developing IRIS Assessments* (U.S. EPA, 2022d), to identify human epidemiological, animal toxicological, mechanistic, and toxicokinetic data relevant to oral exposure. The PFOS systematic review protocol (see Appendix A, (U.S. EPA, 2024a)) was developed prior to the initiation of this assessment largely mirrors the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (Anionic and Acid Forms) IRIS Assessments* (U.S. EPA, 2020b). The protocol outlines the scoping and problem-formulation efforts and describes the systematic review, including study quality evaluation, and the dose-response methods used to conduct this assessment. The final assessment incorporates peer-reviewed studies captured from: EPA's 2016 PFOS HESD (U.S. EPA, 2016b), literature searches of scientific databases and gray literature from 2013 through February 2023, the SAB PFAS Review Panel recommendations, and public comment. Consistent with the analysis provided in the peer-reviewed draft assessment (U.S. EPA, 2022b) and with recommendations from external peer review (i.e., the SAB PFAS Review Panel; (U.S. EPA, 2022e)), this final assessment focused on qualitative and quantitative assessments of five "priority" health outcome categories based on those with the strongest weight of evidence. These five priority health outcomes are cancer, hepatic, developmental, cardiovascular, and immune. The results of the systematic literature reviews and qualitative assessments for the remaining "nonpriority" health outcomes are presented in the Appendix accompanying this final assessment (U.S. EPA, 2024a).

Qualitative Assessment of Noncancer Effects

Overall, the available ***evidence indicates*** that PFOS exposure is likely to cause hepatic, immunological, cardiovascular, and developmental effects in humans given sufficient exposure conditions (e.g., at measured levels in humans as low as 0.57 to 5.0 ng/mL and at administered doses in animals as low as 0.0017 to 0.4 mg/kg/day). These judgments are based on data from epidemiological studies of infants, children, adolescents, pregnant individuals, and nonpregnant adults, as well as short-term (28-day), subchronic (90-day), developmental (gestational), and chronic (2-year) oral-exposure studies in rodents. For hepatic effects, the primary support is evidence of increased serum liver enzyme levels (i.e., alanine transaminase (ALT)) in humans and coherent evidence of hepatotoxicity in animals, including increased liver weights and hepatocellular hypertrophy accompanied by necrosis, inflammation, or increased liver enzyme levels that indicate liver injury. For immunological effects, the primary support is evidence of developmental immunosuppression in humans, specifically decreased antibody response to vaccination against tetanus, diphtheria, and rubella in children, and evidence of immunosuppression and other types of immunotoxicity in studies of adult animals, including decreased plaque forming cell response to sheep red blood cells, extramedullary hematopoiesis in the spleen, reduced spleen and thymus weights, changes in immune cell populations, and decreased splenic and thymic cellularity. For cardiovascular effects, the primary support is evidence of increased serum lipids levels in humans and alterations to lipid homeostasis in animals. For developmental effects, the primary evidence is decreased birth weight in human infants and decreased fetal and maternal weight in animal studies. According to the protocol described in Appendix A (U.S. EPA, 2024a) and aligned with EPA peer-reviewed human health risk assessment methodology (U.S. EPA, 2022d), selected quantitative data in medium and high

confidence studies from these identified hazards were used to derive toxicity values (see Table ES-1). Specific criteria for data and study selection are provided in Appendix A (U.S. EPA, 2024a) and Section 4.1.

Quantitative Assessment of Noncancer Effects and Oral RfD Derivation

EPA followed agency guidelines and methodologies for risk assessment in determining points of departure (PODs) for the derivation of the RfDs for PFOS (U.S. EPA, 2022d, 2014, 2012a, 2011b, 2002b) and performed modeling following EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012a). For data from epidemiological studies, the dose-response modeling approach was selected based on the health outcome and available data. A hybrid modeling approach, which estimated the probability of responses at specified exposure levels above the control, was conducted when clinically adverse outcome levels could be defined (i.e., for developmental, hepatic, and cardiovascular effects) following EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012a). For other outcomes (i.e., immune effects), study results from multivariate models were used to define a benchmark response (BMR). For data from animal toxicological studies, EPA conducted benchmark dose modeling, when possible, to empirically model the dose-response relationship in the range of observed data. When BMDLs could not be derived, EPA used a no-observed-adverse-effect level/lowest-observed-adverse-effect level (NOAEL/LOAEL) approach.

PODs were converted to external POD human equivalent doses (POD_{HEDS}) using pharmacokinetic modeling (see Section 4.1.3). Consistent with the recommendations presented in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b), EPA considered the database of information to inform the application of uncertainty factors (UFs) to POD_{HEDS} to address intraspecies variability, interspecies variability, extrapolation from a LOAEL to NOAEL, extrapolation from a subchronic to a chronic exposure duration, and database deficiencies. EPA derived and considered multiple candidate RfDs from both human epidemiological and animal toxicological studies across the four priority noncancer health outcomes that EPA determined had the strongest weight of evidence (i.e., immune, cardiovascular, hepatic, and developmental) (see Figure ES-1 for candidate RfD values). Additional details on candidate RfD derivation for PFOS are available in Section 4.1.

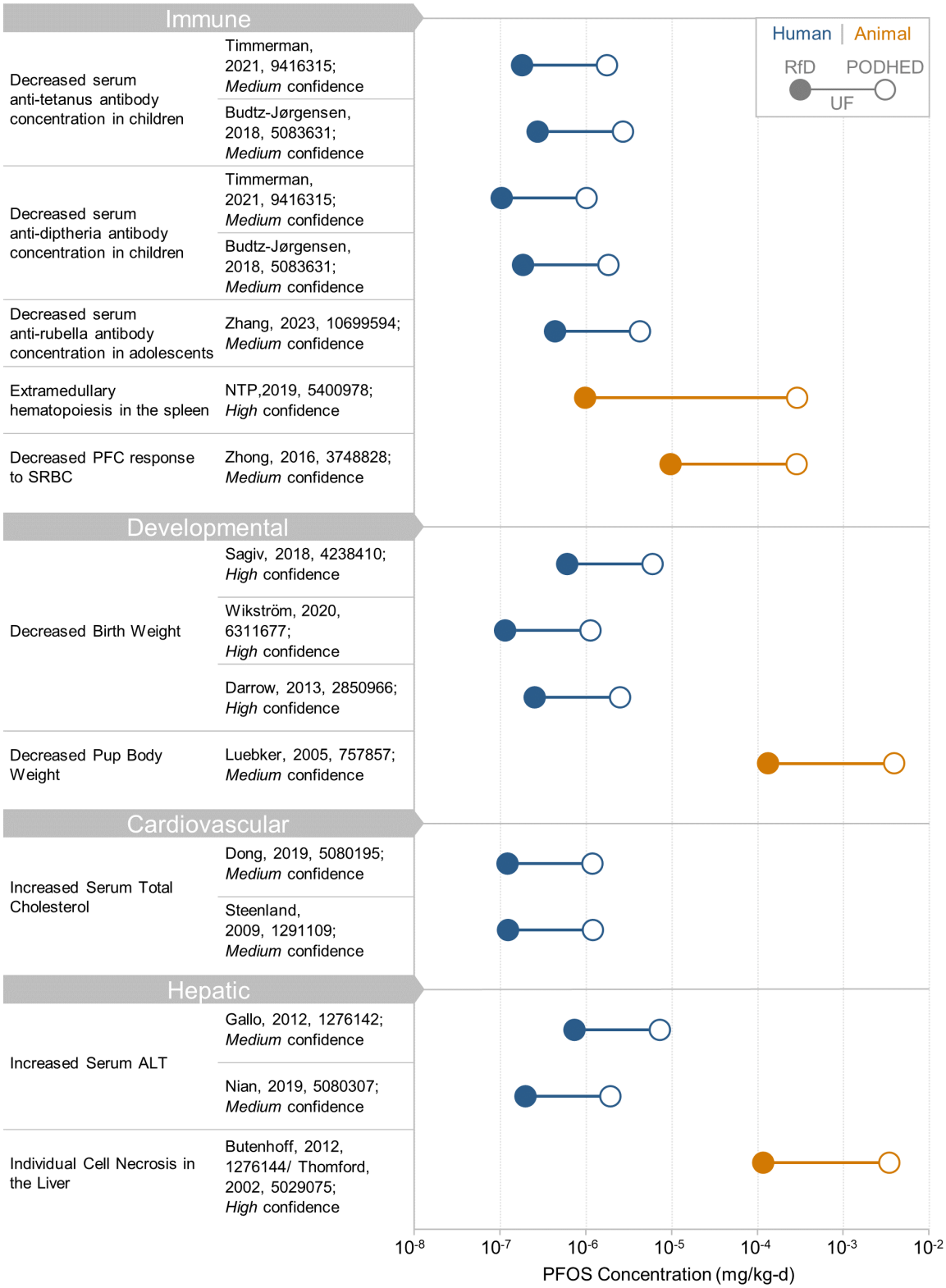

Figure ES-1. Schematic Depicting Candidate RfDs Derived From Epidemiological and Animal Toxicological Studies of PFOS

See text and Figure 4-3 in Section 4.1 for additional detail on dose-response modeling for PFOS studies.

The co-critical effects for the oral RfD of 1 x 10^{-7} mg/kg/day were decreased infant birth weight (Wikström et al., 2020) and increased total cholesterol in adults (Dong et al., 2019) (see Table ES-1). These co-critical effects were selected based on the procedures outlined in the protocol (see Appendix A, (U.S. EPA, 2024a)) and were consistent with EPA peer-reviewed human health risk assessment methodology (U.S. EPA, 2022d). The RfD was derived by using a total UF of 10 to account for intraspecies variability (UF_H). Notably, the RfD is protective of effects that may occur in sensitive populations (i.e., embryo and fetus, infants, and young children), as well as hepatic effects in adults 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.

Qualitative Carcinogenicity Assessment

Consistent with EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), EPA reviewed the available data and conducted a weight of evidence evaluation across the human epidemiological and animal toxicological studies and concluded that PFOS is *Likely to Be Carcinogenic to Humans* via the oral route of exposure (see Section 3.5). Epidemiological studies provided evidence of bladder, prostate, liver, kidney, and breast cancers in humans, although evidence was limited or mixed for some cancer types. Animal toxicological studies supported findings from human studies. Bioassays conducted in Sprague-Dawley rats reported hepatocellular tumors, pancreatic islet cell tumors, and thyroid follicular cell tumors after chronic oral exposure. Some studies observed multisite tumorigenesis (liver and pancreas) in male and female rats. PFOS exposure is associated with multiple key characteristics of carcinogenicity (Smith et al., 2016b). Available mechanistic data suggest that multiple modes of action (MOAs) play a role in pancreatic and hepatic tumorigenesis associated with PFOS exposure in animal models. A full MOA analysis, including in-depth discussions on the potential MOAs for kidney and testicular tumors, as well as discussions on the potential MOAs and human relevance for pancreatic and liver tumors observed in rats, is presented in Section 3.5.4.2.

Quantitative Cancer Assessment and CSF Derivation

EPA followed agency guidelines for risk assessment in deriving CSFs for PFOS (U.S. EPA, 2022d, 2012a, 2005a). EPA selected *medium* and *high* confidence studies for derivation that met criteria outlined in the protocol (see Appendix A, (U.S. EPA, 2024a)) and Section 4.1.1, conducted benchmark dose modeling (U.S. EPA, 2012a), and used the same pharmacokinetic modeling approach as described for the derivation of noncancer RfDs above (see Section 4.2.2). Data from epidemiological studies were not suitable for CSF derivation. From the studies that met the criteria, EPA used multistage models to derive and consider multiple candidate CSFs from animal toxicological studies across multiple tissue types or organ systems (i.e., liver and pancreas). Multistage cancer models were used to predict the doses at which the selected BMR for tumor incidence would occur. BMDLs for each tumor type served as the PODs, which were then converted to POD_{HED}s by applying the human clearance value. Candidate CSFs were then calculated by dividing the selected BMR by the POD_{HED}s for each tumor type.

The oral slope factor of 39.5 $(mg/kg/day)^{-1}$ for hepatocellular adenomas and carcinomas in female rats from Butenhoff et al. (2012)/Thomford (2002b) was selected as the basis of the overall CSF for PFOS (see Table ES-1; rationale in Section 4.2). Per EPA's *Guidelines for*

Carcinogen Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA, 2005a, b), age-dependent adjustment factors were not applied during CSF derivation because there was a lack of information to support a mutagenic MOA for PFOS, and the available evidence was insufficient to assess susceptibility to cancer following PFOS exposure during early life. Additional detail on candidate CSF derivation and CSF selection is provided in Table 4-12 in Section 4.2.

Final Toxicity Values for PFOS

Table ES-1. Final Toxicity Values for PFOS

Toxicity Value Type	Critical Effect(s)	Study, Confidence	Strain/Species, Sex, Age	Toxicity Value[a]
Reference Dose	Co-critical effects: decreased birth weight in infants; increased serum total cholesterol in adults	Wikström et al. (2020), *High*; Dong et al. (2019), *Medium*	Human, male and female, PFOS concentrations in first and second trimesters; Human, male and female, 20–80 years	1×10^{-7} (mg/kg/d)
Cancer Slope Factor	Combined hepatocellular adenomas and carcinomas	Butenhoff et al. (2012)/Thomford (2002b)[b], *High*	Sprague-Dawley rats, female	39.5 $(mg/kg/d)^{-1}$

Notes:
[a] Reference doses were rounded to one significant figure.
[b] Butenhoff et al. (2012) and Thomford (2002b) reported data from the same experiment.

1 Background

1.1 Purpose of This Document

The primary purpose of this toxicity assessment for perfluorooctane sulfonic acid (PFOS) is to describe the best available science on the human health effects associated with PFOS exposure and the derivation of toxicity values (i.e., noncancer reference doses (RfDs) and cancer slope factors (CSFs)). The latest health science on PFOS was identified, evaluated using systematic review methods, and described, and subsequently, a cancer classification was assigned and toxicity values were developed. The final cancer classification and cancer and noncancer toxicity values in this assessment build on the work described in the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023), *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (U.S. EPA, 2021b), and the *Health Effects Support Document for Perfluorooctane Sulfonate (PFOS)* (U.S. EPA, 2016b). This final toxicity assessment for PFOS reflects expert scientific recommendations from the U.S. Environmental Protection Agency (EPA) Science Advisory Board (SAB) (U.S. EPA, 2022e) and public comments received on the draft assessment (https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114; U.S. EPA (2024c)).

In addition to documenting EPA's basis for the cancer classification and toxicity values, this document serves to:

- Describe and document transparently the literature searches conducted and systematic review methods used to identify health effects information (epidemiological and animal toxicological studies and physiologically based pharmacokinetic models) in the literature (Sections 2 and 3; Appendices A and B, (U.S. EPA, 2024a)).
- Describe and document literature screening methods, including use of the Populations, Exposures, Comparators, and Outcomes (PECO) criteria and the process for tracking studies throughout the literature screening (Section 2; Appendix A, (U.S. EPA, 2024a)).
- Identify epidemiological and animal toxicological literature that reports health effects after exposure to PFOS (and its related salts) as outlined in the PECO criteria (Section 3).
- Describe and document the study quality evaluations conducted on epidemiological and animal toxicological studies considered potentially useful for point-of-departure (POD) derivation (Section 3).
- Describe and document the data from all epidemiological studies and animal toxicological studies that were considered for POD derivation (Section 3).
- Synthesize and document the adverse health effects evidence across studies. The assessment focuses on synthesizing the available evidence for five priority health outcomes that were found to have the strongest weight of evidence, as recommended by the SAB – developmental, hepatic, immune, and cardiovascular effects, and cancer (Section 3) – and also provides supplemental syntheses of evidence for dermal, endocrine, gastrointestinal, hematologic, metabolic, musculoskeletal, nervous, ocular, renal, and respiratory effects, reproductive effects in males or females, and general toxicity (Appendix C, (U.S. EPA, 2024a)).

- Evaluate and document the available mechanistic information (including toxicokinetic understanding) associated with PFOS exposure to inform interpretation of findings related to potential health effects in studies of humans and animals, with a focus on five priority health outcomes (developmental, hepatic, immune, and cardiovascular effects, and cancer) (Section 3).
- Develop and document strength of evidence judgments across studies (or subsets of studies) separately for epidemiological, animal toxicological, and mechanistic lines of evidence for the five priority health outcomes (Section 3).
- Develop and document integrated expert judgments across evidence streams (i.e., epidemiological, animal toxicological, and mechanistic streams) as to whether and to what extent the evidence supports that exposure to PFOS has the potential to be hazardous to humans (Section 3).
- Determine the cancer classification for PFOS using a weight-of-evidence approach (Section 3.5.5).
- Describe and document the attributes used to evaluate and select studies for derivation of toxicity values. These attributes are considered in addition to the study confidence evaluation domains and enable extrapolation to relevant exposure levels (e.g., studies with exposure levels near the range of typical environmental human exposures, broad exposure range, or multiple exposure levels) (Section 4).
- Describe and document the dose-response analyses conducted on the studies identified for POD derivation (Section 4).
- Derive candidate RfDs (Section 4.1) and CSFs (Section 4.2), select the final RfD (Section 4.1.6) and CSF (Section 4.2.3) for PFOS, and describe the rationale.
- Characterize hazards (e.g., uncertainties, data gaps) (Sections 3, 4, and 5).

1.2 Background on Per-and Polyfluoroalkyl Substances

Per- and polyfluoroalkyl substances (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. There is no consensus definition of PFAS as a class of chemicals (OSTP, 2023). Consistent with three related structural definitions associated with EPA's identification of PFAS included in the fifth Contaminant Candidate List[1] (CCL), the universe of environmentally relevant PFAS – including parent chemicals, metabolites, and degradants – is approximately 15,000 compounds.[2] The 2018 Organisation for Economic Co-operation and Development (OECD) *New Comprehensive Global Database of Per- and Polyfluoroalkyl Substances (PFASs)* includes over 4,700 PFAS (OECD, 2018).

PFAS have been manufactured and used in a wide variety of industries around the world, including in the United States since the 1950's. PFAS have strong, stable carbon-fluorine (C-F) bonds, making them resistant to hydrolysis, photolysis, microbial degradation, and metabolism (Ahrens, 2011; Buck et al., 2011; Beach et al., 2006). The chemical structures of PFAS enable

[1] The CCL is a list, published 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, and might require regulation under SDWA.
[2] See the EPA List of PFAS Structures available at: https://comptox.epa.gov/dashboard/chemical-lists/PFASSTRUCT.

them to repel water and oil, remain chemically and thermally stable, and exhibit surfactant properties. These properties make PFAS useful for commercial and industrial applications and make many PFAS extremely persistent in the human body and the environment (Kwiatkowski et al., 2020; Calafat et al., 2019; Calafat et al., 2007). Because of their widespread use, physicochemical properties, persistence, and bioaccumulation potential, many different PFAS co-occur in exposure media (e.g., air, water, ice, sediment) as well as in tissues and blood of aquatic and terrestrial organisms, including humans.

With regard to 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 et al., 2011). 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. PFOS belongs to the PFAA family of the non-polymer PFAS category and is among the most researched PFAS in terms of human health toxicity and biomonitoring studies (for review, see Podder et al. (2021)).

1.3 Chemical Identity

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

PFOS is stable in environmental media because it is resistant to environmental degradation processes, such as biodegradation, photolysis, and hydrolysis. In water, no natural degradation has been demonstrated, and 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, oceangoing birds, and fish found in remote areas (Lindstrom et al., 2011; Smithwick et al., 2006).

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} (Nguyen et al., 2020b; Li et al., 2018c), octanol-water partition coefficient (K_{ow}), and Henry's Law Constant (K_H) (NCBI, 2022; ATSDR, 2021). For example, for K_{ow}, the Agency for Toxic Substances and Disease Registry (ATSDR) (2021) 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 and Contaminant Background Support Document for the Final PFAS National Primary Drinking Water Regulation*(U.S. EPA, 2024e), the 2016 *PFOS Health Effects Support Document* (U.S. EPA, 2016b), and the *Draft Aquatic Life Ambient Water Quality Criteria for Perfluorooctane Sulfonate (PFOS)* (U.S. EPA, 2022a).

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)
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)
Molecular Weight	500.13 g/mol	NLM (2022)
Color/Physical State	Liquid	NLM (2022)
Boiling Point	249°C	NLM (2022)
Melting Point	>400°C	ATSDR (2021) (potassium salt)
Vapor Pressure	0.002 mm Hg at 25°C	NLM (2022) (estimated)
Henry's Law Constant (K_H)	4.1E-04 atm-m^3/mol at 25°C	NLM (2022) (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) (converted from log K_{oc} to K_{oc})
Log K_{ow}	4.49	NLM (2022) (estimated)
Solubility in Water	0.0032 mg/L at 25°C; 570 mg/L	NLM (2022) (estimated) ATSDR (2021) (potassium salt in pure water)

Notes: CASRN = Chemical Abstracts Service Registry Number; 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.

1.4 Occurrence Summary

1.4.1 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 has 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 PFOS declined by >85% between 1999 and 2018, presumably because of restrictions on its commercial usage in the United States (CDC, 2017). However, studies of residents in locations of suspected PFAS contamination show higher serum levels of PFAS, including PFOS, compared with the general U.S. population as reported by NHANES (ATSDR, 2022; Table 17-6 in ITRC, 2020; Kotlarz et al., 2020; Yu et al., 2020).

Most PFOS production in the United States was voluntarily phased out by its primary manufacturer (3M) between 2000 and 2002. In 2002 and 2007, EPA took regulatory action under the Toxic Substances Control Act (TSCA) to require that EPA be notified prior to any future domestic manufacture or importation of PFOS and 270 related PFAS (U.S. EPA, 2016a). Manufacturers have since shifted to alternative short-chain PFAS, such as perfluorobutane sulfonic acid (PFBS) (3M, 2002). 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.

1.4.2 Ambient Water

Among the PFAS with established analytical methods for detection, PFOS is one of the dominant PFAS compounds detected in ambient water both in the United States and worldwide (Remucal, 2019; Dinglasan-Panlilio et al., 2014; Zareitalabad et al., 2013; Benskin et al., 2012; Ahrens, 2011; Nakayama et al., 2007). Although 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 et al., 2021). Additional monitoring has been conducted in areas of known aqueous film-forming foam use.

In a recent review, Jarvis et al. (2021) 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). Although 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. Figure 1-1 (excerpted from Jarvis et al. (2021)) shows the distribution of PFOA concentrations (ng/L) measured in surface waters for each U.S. state or waterbody (excluding the Great Lakes) with reported data in the publicly available literature.

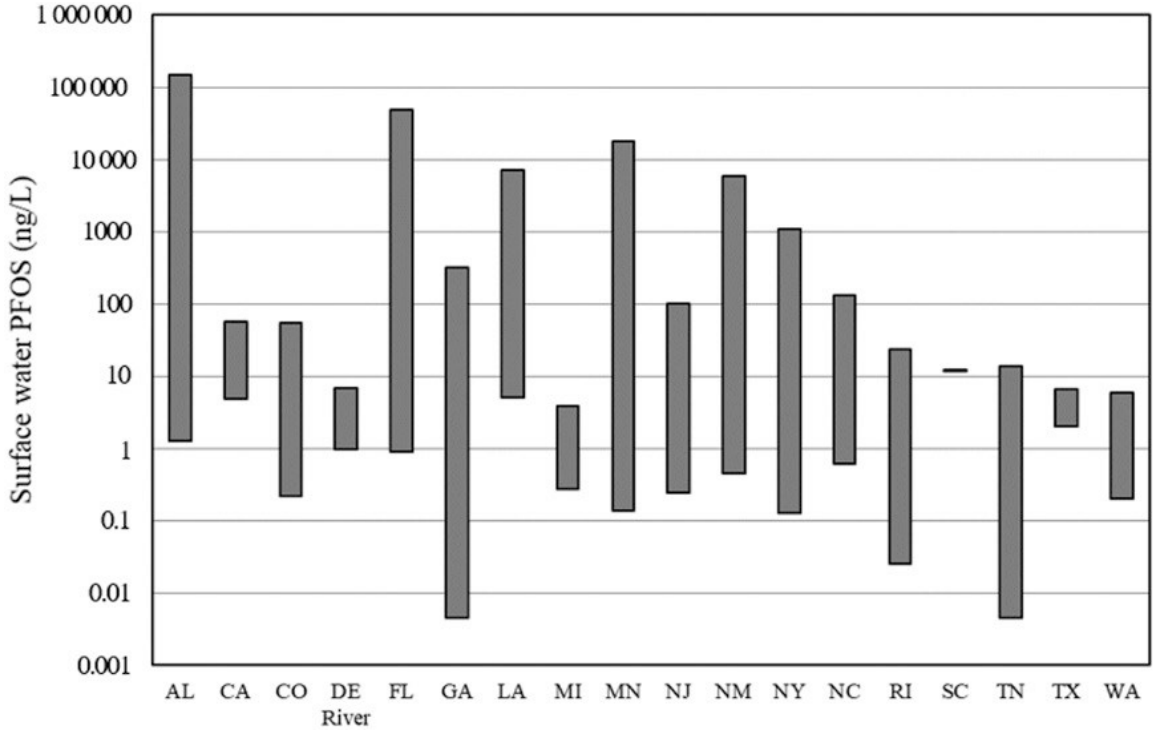

Figure 1-1. Distribution of PFOS Concentrations in Surface Waters by State/Waterbody (Excluding Great Lakes) in the United States (Jarvis et al., 2021)

1.4.3 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.

EPA uses the Unregulated Contaminant Monitoring Rule (UCMR) to collect data for contaminants that are suspected to be present in drinking water and do not have health-based standards set under the Safe Drinking Water Act (SDWA). Under the UCMR, drinking water is monitored from public water systems (PWSs), specifically community water systems and non-transient, non-community water systems. The UCMR improves EPA's understanding of the frequency and concentrations of contaminants of concern occurring in the nation's drinking water systems. The first four UCMRs collected data from a census of large water systems (serving more than 10,000 people) and from a statistically representative sample of small water systems (serving 10,000 or fewer people). UCMR 3 monitoring occurred between 2013 and 2015 and is currently the most comprehensive nationally representative finished water dataset for PFOS (U.S. EPA, 2024d, e). Under UCMR 3, 36,972 samples from 4,920 PWSs were analyzed. PFOS was found in 292 samples at 95 systems above the UCMR 3 minimum reporting level (40 ng/L). These systems serve a population of approximately 10.4 million people located in 28 states, Tribes, or U.S. territories (U.S. EPA, 2024d, e).

More recent state data were collected using newer EPA-approved analytical methods and some state results reflect lower reporting limits than those in the UCMR 3. State data are available from 32 states: Alabama, Arizona, California, Colorado, Delaware, Georgia, Idaho, Illinois, Indiana, Iowa, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri,

New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oregon, Pennsylvania, South Carolina, Tennessee, Vermont, Virginia, West Virginia, and Wisconsin (U.S. EPA, 2024d, e). State results show continued occurrence of PFOS in multiple geographic locations. These data also show PFOS occurrence at lower concentrations and significantly greater frequencies than were measured under the UCMR 3, likely because the more recent monitoring was able to rely on more sensitive analytical methods (U.S. EPA, 2024d, e). More than one-third of states that conducted nontargeted monitoring detected PFOA and/or PFOS at more than 25% of systems (U.S. EPA, 2024d, e). Among the detections, PFOS concentrations ranged from 0.24 to 650 ng/L with a range of median concentrations from 1.21 to 12.1 ng/L (U.S. EPA, 2024d, e). Monitoring data for PFOA and PFOS from states that conducted targeted monitoring efforts, including 15 states, demonstrate results consistent with the nontargeted state monitoring. Within the 20 states that conducted nontargeted monitoring, there are 1,260 systems with results above 4.0 ng/L and 1,577 systems with results above 4.0 ng/L (U.S. EPA, 2024d, e). These systems serve populations of 12.5 and 14.4 million people, respectively. Monitoring data for PFOS from states that conducted targeted sampling efforts showed additional systems exceeding 4 ng/L (U.S. EPA, 2024d, e).

Finally, the fifth UCMR (UCMR 5) was published in December 2021 and requires sample collection and analysis for 29 PFAS, including PFOS, between January 2023 and December 2025 using drinking water analytical methods developed by EPA (U.S. EPA, 2021e). The UCMR 5 defined the minimum reporting level at 4 ng/L for PFOS using EPA Method 533, which is lower than the 40 ng/L used in the UCMR 3 with EPA Method 537 (U.S. EPA, 2021e). Therefore, the UCMR 5 will be able to provide nationally representative occurrence data for PFOS at lower detection concentrations. While the complete UCMR 5 dataset is not currently available, the small subset of data released (7% of the total results that EPA expects to receive) as of July 2023 is consistent with the results of UCMR 3 and the state data described above (U.S. EPA, 2024d, e).

Likewise, Glassmeyer et al. (2017) 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.

1.5 History of EPA's Human Health Assessment for PFOS

EPA developed an HESD for PFOS after it was listed on the third CCL (CCL 3) in 2009 (U.S. EPA, 2009). An HESD is synonymous with a toxicity assessment in that they both describe the assessment of cancer and noncancer health effects and derive toxicity values. The 2016 PFOS HESD was peer reviewed in 2014 and revised based on consideration of peer reviewers' comments, public comments, and additional studies published through December 2015. The resulting *Health Effects Support Document for Perfluorooctane Sulfonic Acid (PFOS)* (U.S. EPA, 2016b) was published in 2016 and described the assessment of cancer and noncancer health effects and the derivation of a noncancer RfD for PFOS.

EPA initiated an update to the 2016 PFOS HESD in 2021 when the agency made a determination to regulate PFOS with a national primary drinking water regulation (NPDWR) (U.S. EPA,

2021c). The initial update of the 2016 PFOS HESD was the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021b). This assessment described the systematic review of cancer and noncancer health effects, the derivation of candidate oral cancer and noncancer toxicity values, a relative source contribution (RSC), and cancer classification, which would subsequently be used to prepare draft and final toxicity assessments. The agency sought peer review from the EPA SAB PFAS Review Panel on key scientific issues, including the systematic review approach for evaluating health effects studies, the derivation of oral toxicity values, the RSC, and the cancer classification for PFOS.

The SAB provided draft recommendations on June 3, 2022, and final recommendations on August 23, 2022 (U.S. EPA, 2022e). To be responsive to the SAB recommendations, EPA developed a detailed response to comment document (USEPA OOW, 2023) and addressed every recommendation from the SAB in the development of the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023). Briefly, EPA:

- 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 across health outcomes;
- 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 discussion for cancer effects and rationale for the cancer classification;
- strengthened the rationales for selection of PODs for the noncancer health outcomes; and
- clarified language related to the RSC determination, including the relevance of drinking water exposures and the relationship between the RfD and the RSC.

EPA then released the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* for a 60-day public comment period. These assessments described the systematic review of cancer and noncancer health effects, the derivation of candidate oral cancer and noncancer toxicity values, an RSC, and cancer classification for PFOS.

EPA incorporated feedback from public comment into the assessment and developed a detailed response to public comment document (U.S. EPA, 2024c). Briefly, EPA has improved descriptions of rationale and added clarifications related to the systematic review protocol used for this assessment, study and endpoint selection for POD derivation, and the modeling choices related to toxicity value derivation. Therefore, this *Final Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts* incorporates feedback from external peer review and public comment and supersedes all other health effects documents produced by the EPA Office of Water for PFOS.

2 Summary of Assessment Methods

This section summarizes the methods used for the systematic review of the health effects literature for 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). The purposes of this systematic review were to identify the best available and most relevant health effects literature, to evaluate studies for quality, and to subsequently identify health effects and studies for dose-response assessment. A detailed description of these methods is provided as a protocol in Appendix A, (U.S. EPA, 2024a).

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

For this updated PFOS toxicity assessment, systematic review methods were consistent with those in the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b) for the steps of literature search; screening; study quality evaluation; data extraction; display of study evaluation results; synthesis of human and experimental animal data; and evidence integration for all health outcomes through the 2020 literature searches, as presented in the preliminary analyses of the 2021 *Proposed Approaches To The Derivation Of A Draft Maximum Contaminant Level Goal For Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) In Drinking Water* draft document that was reviewed by the Science Advisory Board (SAB) (U.S. EPA, 2022d, 2021b). The EPA then focused the remaining steps of the systematic review process (synthesis and integration of mechanistic data; derivation of toxicity values) on health outcomes with the strongest weight of evidence based on the conclusions presented in the 2021 draft documents, and consistent with the recommendations of the SAB (U.S. EPA, 2022e). These five "priority" health outcomes are developmental, hepatic, immune, cardiovascular, and cancer. The updated systematic review focused on the priority health outcomes was published in

2023 as the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023).

The following subsections provide a summary of methods used to search for and screen identified literature, evaluate the identified studies to characterize study quality, extract data, and select studies for dose-response analysis. Extracted data are available in interactive visual formats (see Section 3) and can be downloaded in open access, interactive formats. The full systematic review protocol (see Appendix A, (U.S. EPA, 2024a)) provides a detailed description of the systematic review methods that were used. The protocol also includes the description of the problem formulation and key science issues guiding this assessment.

2.1.1 Literature Database

The EPA assembled a database of epidemiological, animal toxicological, mechanistic, and toxicokinetic studies for this PFOS toxicity assessment based on three main data streams: 1) literature published from 2013 through February 6, 2023 identified via literature searches conducted in 2019, 2020, 2022 and 2023 of a variety of publicly available scientific literature databases, 2) literature identified via other sources (e.g., searches of the gray literature, studies shared with EPA by the SAB, studies submitted through public comment), and 3) literature identified in EPA's 2016 *Health Effects Support Document for Perfluorooctane Sulfonic Acid (PFOS)* (U.S. EPA, 2016b). All of these streams are described in detail below.

For the literature searches, the search strings focused on the chemical name (PFOS and its related salts) with no limitations on lines of evidence (i.e., human/epidemiological, animal, *in vitro*, *in silico*) or health outcomes. The EPA conducted a 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 prior to SAB review of the draft assessment (2020 literature search), a third search covering September 2020 through February 3, 2022 prior to release of the draft assessment for public comment (2022 literature search), and a final supplemental search covering February 4, 2022 through February 6, 2023.

The publicly available databases listed below were searched for literature containing the chemical search terms outlined in Appendix A (U.S. EPA, 2024a):

- 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).

The search strings and literature sources searched are described in Appendix A (U.S. EPA, 2024a).

For the second data stream, other review efforts and searches of publicly available sources were used to identify relevant studies (see Appendix A, (U.S. EPA, 2024a)), as listed below:

- studies cited in assessments published by other U.S. federal, international, and/or U.S. state agencies (this included assessments by ATSDR (ATSDR, 2021) and California Environmental Protection Agency (CalEPA, 2021)),

- studies identified during mechanistic or toxicokinetic evidence 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),
- studies identified by the SAB in their final report dated August 23, 2022 (U.S. EPA, 2022e), and
- studies submitted through public comment by May 2023 (https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114).

For the third data stream, EPA relied on epidemiological and animal toxicological literature synthesized in the 2016 PFOS HESD to identify studies relevant to the five priority health outcomes, as recommended by SAB and consistent with preliminary conclusions from EPA's 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 PFOS HESD contained a summary of all relevant literature identified in searches conducted through 2013. EPA's 2016 PFOS HESD relied on animal toxicological studies for quantitative analyses whereas epidemiology studies were considered qualitatively, as a supporting line of evidence. This updated assessment includes epidemiological studies that were identified and presented in the 2016 PFOS HESD for the five priority health outcomes. It also includes "key" animal toxicological studies from the 2016 PFOS HESD, which includes studies that were selected in 2016 for dose-response modeling. The details of the studies included from the 2016 PFOS HESD are described in Appendix A (U.S. EPA, 2024a).

All studies identified through the data streams outlined above were uploaded into the publicly available Health and Environmental Research Online (HERO) database (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2608).

EPA has continued to monitor the literature published since February 2023 for other potentially relevant studies. Potentially relevant studies identified after February 2023 that were not recommended by the SAB in their final report or via public comment 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 Appendix A, (U.S. EPA, 2024a)).

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, the EPA used populations, exposures, comparators, and outcomes (PECO) criteria to screen the literature identified from the literature sources outlined above in order to prioritize studies for dose-response assessment and to identify studies containing supplemental information such as mechanistic studies that could inform the mode of action analyses. The PECO criteria used for screening the health effects, toxicokinetic, and mechanistic literature are provided in Appendix A (U.S. EPA, 2024a).

Consistent with the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b), 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 independent reviewers using screening tools consistent with the IRIS Handbook ((U.S. EPA, 2022d); DistillerSR or SWIFT ActiveScreener software), and studies that passed this screening underwent full-text review by at least two independent reviewers. Health effects studies that met PECO inclusion criteria following both title and abstract screening and full-text review underwent study quality evaluation as described below (Section 2.1.3). Studies that were tagged as containing relevant PBPK models were sent to the modeling technical experts for scientific and technical review. 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 Appendix A, (U.S. EPA, 2024a)). Supplemental studies that were identified as mechanistic or ADME during screening did not undergo study quality evaluation.

For the supplemental literature search conducted in 2023 and literature received through public comment, studies were screened for relevancy and considered for potential impact on the toxicity assessments for PFOS. Consistent with the IRIS Handbook (U.S. EPA, 2022d), the studies identified after February 3, 2022, including studies recommended via public comment, were "considered for inclusion only if they [were] directly relevant to the assessment PECO criteria and [were] expected to potentially impact assessment conclusions or address key uncertainties" (U.S. EPA, 2022d). For the purposes of this assessment, the EPA defined impacts on the assessment conclusions as data from a study (or studies) that, if incorporated into the assessment, have the potential to significantly affect (i.e., by an order of magnitude or more) the final toxicity values (i.e., RfDs and CSFs) or alter the cancer classification for PFOS (see Appendix A, (U.S. EPA, 2024a)).

2.1.3 Study Quality Evaluation for Epidemiological Studies and Animal Toxicological Studies

Study quality evaluations were performed consistent with the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b). For study quality evaluation of the PECO-relevant human epidemiological and animal toxicological studies (i.e., studies identified in the four literature searches (all health outcomes for the 2019 and 2020 searches; the five priority health outcomes for the 2022 search; studies impacting assessment conclusions within the five priority health outcomes for the 2023 search (see Appendix A, (U.S. EPA, 2024a))), studies recommended by the SAB, studies recommended via public comment that reported potentially significant results on one or more of the five priority health outcomes, epidemiological studies from the 2016 PFOS HESD that reported results on one or more of the five priority health outcomes, and key animal toxicological studies from the 2016 PFOS HESD), two independent primary reviewers followed by a quality assurance (QA) reviewer assigned ratings about the reliability of study results (*good, adequate, deficient* (or *"not reported"*), or *critically deficient*) for different evaluation domains as described in the IRIS Handbook (U.S. EPA, 2022d) (see Appendix A, (U.S. EPA, 2024a)). These study quality evaluation domains are listed below and

details about the domains, including prompting questions and suggested considerations, are described in Appendix A (U.S. EPA, 2024a).

- 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 quality; 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 consistent with the IRIS Handbook (U.S. EPA, 2022d). A study can be given an overall *mixed* confidence rating if different PECO-relevant endpoints within the study receive different confidence ratings (e.g., *medium* and *low* confidence ratings).

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. Due to the abundance of *medium* and *high* confidence studies in this database, data were only 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, 2022d). Studies evaluated as being *uninformative* for an endpoint were not considered further when characterizing that endpoint 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 summary to a full extraction of dose-response effect size information).

Extractions were conducted using DistillerSR for epidemiological studies and HAWC for animal toxicological studies. An initial reviewer conducted the extraction, followed by a second reviewer conducting an independent QA 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 Appendix A (U.S. EPA, 2024a).

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 outcome (i.e., an organ- or organ system-level category of related health effects and endpoints). 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. Consistent with the IRIS Handbook, groups of related outcomes within a health outcome category were considered together as a unit of analysis during evidence synthesis and evidence integration (U.S. EPA, 2022d). For example, birth weight, birth length, and head circumference were all considered under the unit of analysis of the fetal growth restriction.

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, 2022e). Consistent with the IRIS Handbook (U.S. EPA, 2022d), *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 in order to capture all of the available data for PFOS in the weight of evidence analyses. As described above, *uninformative* studies were not extracted or included in the evidence syntheses. Results from epidemiological studies were discussed within sections organized by population type, including children, general population adults, pregnant women, and occupational populations. Childhood was defined as the effect of environmental exposure during early life: from conception, infancy, early childhood and through adolescence until 21 years of age (U.S. EPA, 2021a). 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 on the same health outcome as another study were considered overlapping evidence, and, to avoid duplication or overrepresentation of results from the same group of participants, these additional studies were not discussed in the evidence synthesis narrative. In cases of overlapping studies, the study with the largest number of participants and/or the most accurate outcome measures was given preference. For the five priority health outcomes, EPA also developed mechanistic syntheses.

For evidence integration, conclusions regarding the strength of evidence were drawn for each health outcome across human and animal evidence streams. For the five priority health outcomes, this included consideration of epidemiological studies identified in the 2016 PFOS HESD, as well as mechanistic 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 judgments were 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).

The evidence integration was conducted according to guidance outlined in the IRIS Handbook (U.S. EPA, 2022d) and the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b). 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.*** Considerations for each evidence integration judgment are summarized within corresponding evidence integration sections in an evidence profile table (EPT). EPTs were organized by evidence stream (i.e., human, animal, and mechanistic, respectively), and, within evidence streams, units of analysis with the strongest evidence were presented first.

Additional details about evidence synthesis and integration are summarized in Appendix A (U.S. EPA, 2024a).

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 within those health outcomes. Dose-response modeling was performed for endpoints within health outcomes with data warranting evidence integration conclusions of *evidence demonstrates* and *evidence indicates (likely)* for noncancer endpoints and carcinogenicity descriptors of *Carcinogenic to Humans* and *Likely to be Carcinogenic to Humans*. EPA identified specific studies for dose-response modeling and POD derivation following attributes described in Table 7-2 of the IRIS Handbook (U.S. EPA, 2022d). Examples of study attributes evaluated included study design characteristics, study confidence, and data availability, among others (see Appendix A, (U.S. EPA, 2024a)). Human epidemiological and animal toxicological studies that were consistent with the overall weight of evidence for a specific endpoint were considered for dose-response. Additionally, for human evidence, all *high* or *medium* confidence studies pertaining to a specific endpoint were considered; for animal evidence, only animal toxicological studies with at least two PFOS exposure groups that were of *high* or *medium* confidence were considered. Relevance of the endpoint or species reported by animal toxicological studies to human health effects was also considered. Additional information on study selection is provided in Appendix A (U.S. EPA, 2024a).

2.2.1 Approach to POD and Candidate RfD Derivation for Noncancer Health Outcomes

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

For PFOS animal toxicological studies, EPA attempted benchmark dose (BMD) modeling on all studies considered for dose-response to refine the POD. BMD modeling was performed after converting the administered dose reported by the study to an internal dose using a pharmacokinetic model (see Section 4.1.3 for additional details). This approach resulted in dose levels corresponding to specific response levels near the low end of the observable range of the data and identified the lower limits of the BMDs (BMDLs) which serve as potential PODs (U.S. EPA, 2012a). EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). BMDS fits mathematical models to the data and determines the dose (i.e., BMD) that corresponds to a predetermined level of response (i.e., benchmark response or BMR). For dichotomous data, the BMR is typically set at either 5% or 10% above the background or the response of the control group. For continuous data, a BMR of one-half or one standard deviation from the control mean is typically used when there are no outcome-specific data to indicate what level of response is biologically significant (U.S. EPA, 2012a). For dose-response data for which BMD modeling did not produce an adequate model fit, a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) was used as the POD. However, a POD derived using a BMD approach typically provides a higher level of confidence in the conclusions for any individual case, as the BMDL takes into account all the data from the dose-response curve, incorporates the evaluation of the uncertainty in the BMD, and is related to a known and predefined potential effect size (i.e., the BMR) (U.S. EPA, 2022d, 2012a). For noncancer endpoints, there were several factors considered when selecting the final model and BMD/BMDL, including the type of measured response variable (i.e., dichotomous or continuous), experimental design, and covariates (U.S. EPA, 2012a). However, as there is currently no prescriptive hierarchy, selection of model types was often based on the goodness-of-fit and was judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. The *Benchmark Dose Technical Guidance* provides a "BMD Decision Tree" to assist in model selection (U.S. EPA, 2012a). See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

For the epidemiological studies considered for dose-response assessment, EPA used multiple modeling approaches to determine PODs, depending upon the health outcome and the data provided in the studies. For the developmental, hepatic, and serum lipid dose-response studies,

EPA used a hybrid modeling approach that involves estimating the incidence of individuals above or below a level considered to be adverse and determining the probability of responses at specified exposure levels above the control (U.S. EPA, 2012a) because the EPA was able to define a level considered clinically adverse for these outcomes (see Appendix E, (U.S. EPA, 2024a)). As sensitivity analyses for comparison purposes, EPA also performed BMD modeling and provided study LOAELs/NOAELs as PODs for the epidemiological hepatic and serum lipid dose-response studies. For the immune studies, for which a clinically defined adverse level is not established, EPA used multivariate models provided in the studies and determined a BMR according to EPA guidance to calculate BMDs and BMDLs (U.S. EPA, 2012a). See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

After POD derivation, EPA used a pharmacokinetic model for human dosimetry to estimate human equivalent doses (HEDs) from both animal and epidemiological studies. A pharmacokinetic model for human dosimetry is used to simulate the HED from the animal PODs and is also used to simulate selected epidemiological studies to obtain a chronic dose that would result in the internal dose POD obtained from dose-response modeling (Section 4.1.3). Based on the available data, a serum PFOS concentration was identified as a suitable internal dosimetry target for the human and animal endpoints of interest. Next, reference values are estimated by applying relevant adjustments to the point-of-departure human equivalent doses (POD_{HEDS}) to account for five possible areas of uncertainty and variability: human variation, extrapolation from animals to humans, extrapolation to chronic exposure duration, the type of POD being used for reference value derivation, and extrapolation to a minimal level of risk (if not observed in the data set). UFs used in this assessment were applied according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b). For additional detail on UFs, see Appendix A (U.S. EPA, 2024a). The POD_{HED} for a particular candidate RfD is divided by the composite UFs.

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 confidence, 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, magnitude of response, 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) 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, or monkey. Concentrations of PFOS in blood are considered for all the internal dose-metrics.

Step 2: Conduct dose-response modeling. See above and Appendix E (U.S. EPA, 2024a) 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 (e.g., BMDL, NOAEL) is converted to an HED following the method described in Section 4.1.3.

Step 4: Select appropriate UFs and provide rationale for UF selection. UFs are applied in accordance with EPA methodology 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 (U.S. EPA, 2002b).

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

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

where:

POD_{HED} = calculated from the internal dose POD 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 methodology (U.S. EPA, 2002b).

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, 2005a). A narrative is developed to provide a complete description of the weight of evidence and conditions of carcinogenicity. The potential carcinogenicity descriptors (presented in the 2005 guidelines) are:

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

More than one carcinogenicity descriptor can be applied if a chemical's carcinogenic effects differ by dose, exposure route, or mode of action (MOA)[3]. For example, a chemical may be

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

carcinogenic to humans above but not below a specific dose level if a key event in tumor formation does not occur below that dose. MOA information informs both the qualitative and quantitative aspects of the assessment, including the human relevance of tumors observed in animals. The MOA analysis must be conducted separately for each target organ/tissue type (U.S. EPA, 2005a).

2.2.2.2 Derivation of Candidate Cancer Slope Factors

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

For animal toxicological studies, EPA used the publicly available Benchmark Dose Software (BMDS) program developed and maintained by EPA (https://www.epa.gov/bmds). First, a PK model converted the administered dose reported by the study to an internal dose (see Section 4.1.3 for additional details). Then, BMDS fits multistage models, the preferred model type (U.S. EPA, 2012a), to the data and the model is used to identify a POD for extrapolation to the low-dose region based on the BMD associated with a significant increase in tumor incidence above the control. According to the 2005 guidelines, the POD is the lowest dose that is adequately supported by the data. The BMD_{10} (the dose corresponding to a 10% increase in tumors) and the $BMDL_{10}$ (the 95% lower confidence limit for that dose) are also reported and are often used as the POD. Similar to noncancer PODs, selection of model types is often based on the goodness-of-fit (U.S. EPA, 2012a). For PFOS, after a POD was determined, a PK model was used to calculate the HED for animal oral exposures (POD_{HED}). The CSF is derived by dividing the BMR by the POD_{HED}. See Appendix E (U.S. EPA, 2024a) for additional details on the study-specific modeling.

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

In cases for which a chemical is shown to cause cancer via an MOA that is not linear at low doses, and the chemical does not demonstrate mutagenic or other activity consistent with

linearity at low doses, a nonlinear extrapolation is conducted. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* state that "where tumors arise through a nonlinear MOA, an oral RfD or inhalation reference concentration, or both, should be developed in accordance with EPA's established practice of developing such values, taking into consideration the factors summarized in the characterization of the POD" (U.S. EPA, 2005a). In these cases, an RfD-like value is calculated based on the key event[4] for carcinogenesis or the tumor response.

2.2.3 Selecting Health Outcome-Specific and Overall Toxicity Values

The next step is to select a health outcome-specific toxicity value for each hazard (cancer and noncancer) identified in the assessment. This selection can be based on the study confidence considerations, the most sensitive outcome, a clustering of values, or a combination of such factors; the rationale for the selection is presented in the assessment. Key considerations for candidate value selection are described in the IRIS Handbook (U.S. EPA, 2022e) and include: 1) the weight of evidence for the specific effect or health outcome; 2) study confidence; 3) sensitivity and basis of the POD; and 4) uncertainties in modeling or extrapolations. The value selected as the organ/system-specific toxicity value is discussed in the assessment.

The selection of final toxicity values for noncancer and cancer effects involves the study preferences described above, consideration of overall toxicity, study confidence, and confidence in each value, including the strength of various dose-response analyses and the possibility of basing a more robust result on multiple data sets. The values selected as the overall RfD and CSF are discussed in Section 4.

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

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 7,160 unique records (combined for PFOA and PFOS) prior to running SWIFT Review. Table 3-1 shows the results from database searches conducted in April 2019, September 2020, and February 2022, and February 2023.

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 2/6/2023: 966 results
PubMed	4/10/2019: 2,191 results 9/3/2020: 811 results 2/2/2022: 1,728 results 2/6/2023: 719 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 2/2023: 1,153 results
Total number of references after running SWIFT Review[a]	4/2019: 1,977 results 9/2020: 867 results 2/2022: 1,370 results 2/2023: 881 results
Total number of unique references moved to screening[b]	**4,802**

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, studies identified by the Science Advisory Board (SAB), and EPA's 2016 Health Effects Support Documents (HESDs) for perfluorooctanoic acid (PFOA) (U.S. EPA, 2016c) and perfluorooctane sulfonate (PFOS) (U.S. EPA, 2016b)) yielded 238 unique records (combined for PFOA and PFOS).

The 4,802 studies captured with the SWIFT Review evidence streams filters and the 238 records identified from additional sources yield a total of 5,011 unique studies. These 5,011 studies were moved to the next stage of screening (title and abstract screening using either DistillerSR or

SWIFT ActiveScreener). Of the 5,011 unique studies, 1,062 moved on to full-text level review, 1,697 were excluded during title and abstract screening, and 2,252 were tagged as containing potentially relevant supplemental material. Of the 1,062 screened at the full-text level, 760 were considered to meet PECO eligibility criteria (see Appendix A, (U.S. EPA, 2024a)) and included relevant information on PFOS. The 760 studies that were determined to meet PECO criteria after full-text level screening included 429 epidemiological (human) studies, 45 animal toxicological studies, 11 physiologically based pharmacokinetic (PBPK) studies, and 275 studies that were not extracted (e.g., *low* confidence studies, meta-analyses, studies from the 2022 and 2023 searches 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 27 PBPK studies. Details of the literature search and screening process are shown in Figure 3-1.

The 429 epidemiological studies and 45 animal toxicological studies relevant to PFOS underwent study quality evaluation and were subsequently considered for data extraction as outlined in Sections 2.1.3 and 2.1.4 (see Appendix A, (U.S. EPA, 2024a)). The results of the health outcome-specific study quality evaluations and data extractions are described in Sections 3.4 and 3.5.

Additionally, the 27 studies tagged as containing relevant PBPK models for PFOS were reviewed by pharmacokinetic (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 104 toxicokinetic and 305 mechanistic studies identified as relevant for PFOS moved on to a limited data extraction as described in the Appendix (U.S. EPA, 2024a). The toxicokinetic studies pertaining to ADME are synthesized in Section 3.3.1. The mechanistic studies relevant to the five priority health outcomes are synthesized in Sections 3.4 and 3.5 and were considered as part of the evidence integration.

In addition to the studies identified through database searches and the other sources outlined above, public comments submitted in response to the *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) in Drinking Water* (USEPA, 2023) included 944 studies, relevant to PFOA and/or PFOS, which were reviewed for relevance to the toxicity assessment. Of the 944 studies, 297 were duplicates of studies included in the toxicity assessment and 31 were duplicates of studies included in the 2016 PFOA or PFOS HESD assessment. The 599 studies that were not identified in the 2016 HESDs and were not included in the toxicity assessments underwent additional review identify studies with that could impact assessment conclusions as outlined in Appendix A.3 (U.S. EPA, 2024a). Ultimately, none of the 599 studies were incorporated in the toxicity assessments upon further screening. The submitted references were either deemed not relevant after secondary review, were supplemental studies (e.g., PFOA or PFOS assessments published by other scientific bodies, mechanistic, ADME, etc), or addressed non- priority health outcomes. The results of this screening can be found in the docket ("Review of Public Comment References Related to PFOA and PFOS Health Effects;" https://www.regulations.gov/docket/EPA-HQ-OW-2022-0114).

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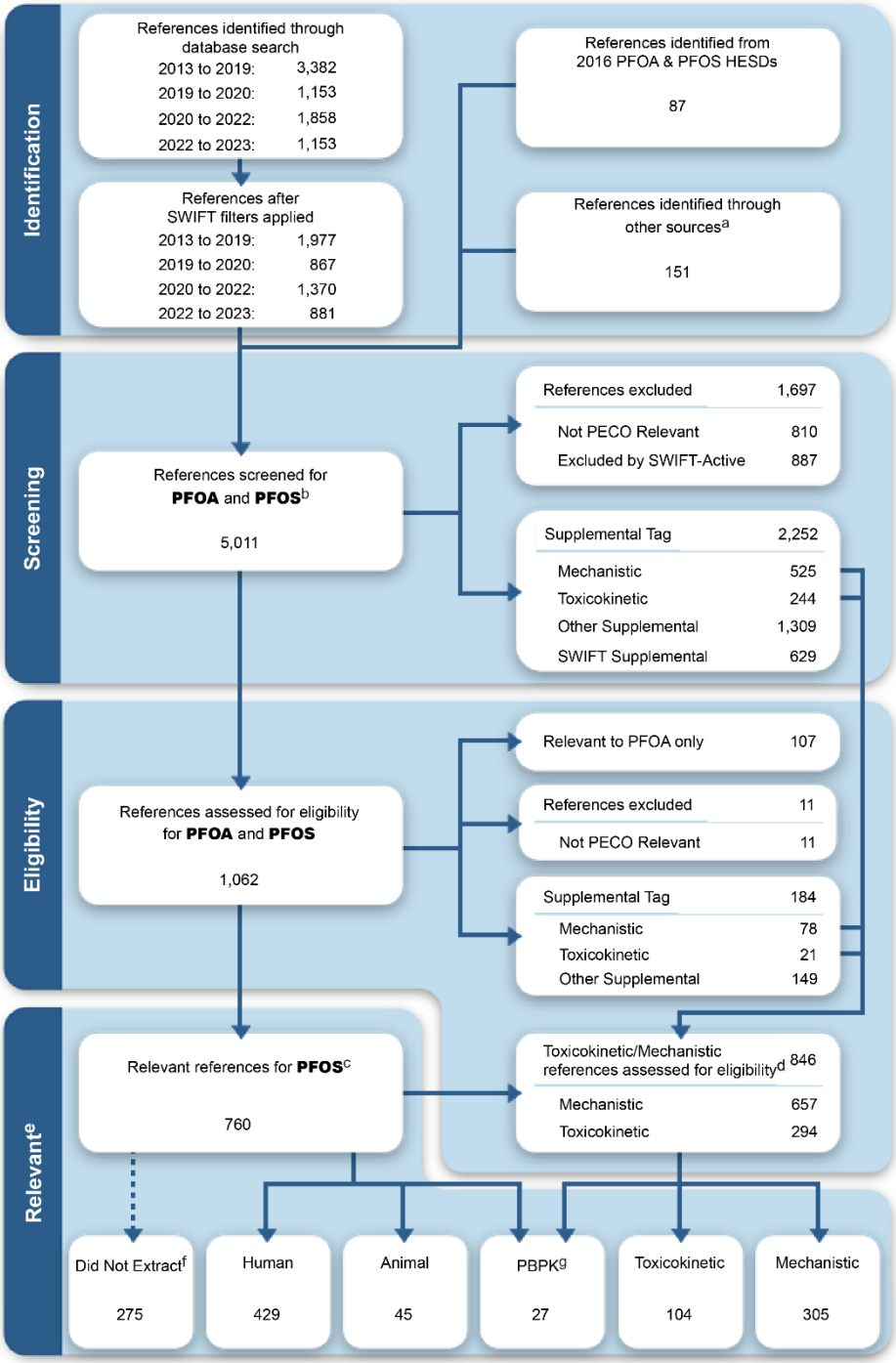


Figure 3-1. Summary of Literature Search and Screening Process for PFOS

Interactive figure and additional study details available on HAWC.
Interactive figure based on work by Magnuson et al. (2022).
"Other sources" include assessments published by other agencies, studies identified during mechanistic or toxicokinetic syntheses, and studies identified by the SAB.
[a] References identified by SAB and through database searches were counted as identified through database search only.
[b] Includes number of unique references after deduplication of studies captured with the SWIFT Review evidence streams filters and records identified from additional sources.
[c] Includes number of unique references considered to meet PECO eligibility criteria at the full-text level and include relevant information on PFOS.

[d] Includes number of unique references identified during title/abstract screening, full-text screening, and data extraction assessed for toxicokinetic and/or mechanistic eligibility.
[e] Only includes references with relevant information on PFOS.
[f] References tagged to 'Not a priority human health system' include those identified in the 2019 search that overlap with 2016 PFOS HESD references or those identified in 2022 and 2023 searches.
[g] Includes 11 PBPK references determined to meet PECO criteria plus an additional 16 PBPK references identified during the toxicokinetic screening.

3.1.1 Results for Epidemiology Studies of PFOS by Health Outcome

Of the 429 epidemiological studies that met the inclusion criteria and underwent extraction, 181 studies had a cohort study design, 169 had a cross-sectional design, 42 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 = 86), metabolic (n = 74), or immune systems (n = 66). 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	7	3	3	5	18
Cardiovascular	5	19	56	6	86
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	4	18	2	25
Immune	6	32	19	9	66
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	42	181	169	37	429

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 HAWC.
[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 and underwent extraction, most studies had either short-term (n = 19) or developmental (n = 15) study designs. Approximately equal numbers of studies were conducted in rats (n = 23) and mice (n = 21). The rat studies had short-term (n = 12), developmental (n = 7), chronic (n = 2), reproductive (n = 2), and subchronic (n = 1) study designs. The mouse studies had developmental (n = 8), short-term (n = 7), subchronic (n = 5), or reproductive (n = 1) study designs. The single monkey study used a chronic study design and the single rabbit study used 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 body weight), 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 HAWC.

[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

All data from this project are available in the public HAWC (https://hawc.epa.gov/assessment/100500248/) site displayed as exposure-response arrays, forest plots, and evidence maps. Data extracted from the 429 epidemiological studies are available here. Data extracted from the 45 animal toxicological studies are available here. 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 here and here, respectively.

3.3 Toxicokinetic Synthesis

As described in Section 3.1, EPA identified 104 and 27 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 (U.S. EPA, 2024a).

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. Given the transcriptional activation of many genes in PPARα-null mice, however, other gene products likely modify toxicokinetics of PFOS (Andersen et al., 2008).

3.3.1.1 Absorption

Absorption data are available in laboratory animals for oral (Chang et al., 2012) and inhalation (Rusch, 1979) exposures, and extensive data are available demonstrating the presence of PFOS in human serum. Limited in vitro absorption data are available (see Appendix B, (U.S. EPA, 2024a)).

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) 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 et al., 2018). 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 (Huang et al., 2019; Kim et al., 2016).

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 B (U.S. EPA, 2024a). 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 et al., 2016). 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 et al., 2020; Poothong et al., 2017; Jin et al., 2016). Poothong et al. (2017) 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 et al., 2007) 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) (Liu et al., 2017b; D'Alessandro et al., 2013; Salvalaglio et al., 2010; Chen and Guo, 2009; Zhang et al., 2009). In vitro analyses found that plasma proteins can bind PFOS in plasma from humans, cynomolgus monkeys, and rats (Kerstner-Wood et al., 2003). 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 (Chen and Guo, 2009; Zhang et al., 2009). Beesoon and Martin (2015) 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 et al., 2017b), 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 et al., 2013), and may interfere with PFOS uptake into cells (Sheng et al., 2020).

Binding to albumin and other serum proteins may affect transfer of PFOS from maternal blood to the fetus (Gao et al., 2019). Since there is effectively a competition between PFOS binding in maternal serum versus cord blood, lower cord blood albumin levels compared with maternal blood albumin levels are likely to reduce transfer from maternal serum across the placenta. Consistent with this hypothesis, Pan et al. (2017) found that 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) (Yang et al., 2020a; Zhang et al., 2013b; Luebker et al., 2002). L-FABP is an intracellular lipid carrier protein that reversibly binds long-chain fatty acids, phospholipids, and an assortment of peroxisome proliferators (Erol et al., 2004) and constitutes 2%–5% of the cytosolic protein in hepatocytes.

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) 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 et al., 2015). 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 and Ng, 2021; Maestri et al., 2006). Blood and liver are major sites of PFOS accumulation (Olsen et al., 2001c). Two studies measured PFOS levels in cerebrospinal fluid and serum (Wang et al., 2018; Harada et al., 2007) 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) 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. Another study of post-mortem tissues found varying PFOS levels in different tissues ranging from 1.0 ng/g in skeletal muscle to 13.6 ng/g in liver. PFOS was also detected in brain and basal ganglia, endocrine organs (pituitary, thyroid, pancreas), liver, kidney, and adipose tissue (Maestri et al., 2006). PFOS also accumulates in follicular fluid (Kang et al., 2020) and gonads (Maestri et al., 2006), 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 (Chang et al., 2017; Seacat et al., 2002). 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). 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 (Qiu et al., 2013) and mice (Bogdanska et al., 2011; Cui et al., 2009). In fact, one study in rats (Wang et al., 2015a) observed higher PFOS levels in the hippocampus than in serum measured on PND 1 in prenatally exposed rats. 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). However, some sex-dependent differences in PFOS levels were observed in rodents that varied by species, lifestage, and dose duration (Zhong et al., 2016; Curran et al., 2008; Thomford, 2002b).

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 (Chen et al., 2017a; Zhang et al., 2013c). Zhang et al. (2015b) 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) demonstrated that gestational age can affect PFOS concentrations in maternal serum and placentas, estimating a placental 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 et al., 2014; Porpora et al., 2013). The ratio of PFOS in cord serum relative to maternal serum ranged from 0.22 to 0.98 (see Appendix B, (U.S. EPA, 2024a)) and generally increased with gestational age (Li et al., 2020a). Li et al. (2020a) also showed a 6% increase in branched PFOS accumulation compared with linear PFOS isomers. Zhao et al. (2017) observed higher transplacental transfer efficiencies (TTEs) for 1m-, 4m-, 3 + 5m-, and m2-PFOS compared with n-PFOS. Together, these findings indicate that branched isomers of PFOS transfer more efficiently from maternal blood to cord blood compared with 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 (Brochot et al., 2019; Eryasa et al., 2019; Jusko et al., 2016).

Lower PFOS concentrations were measured in amniotic fluid compared with placenta and cord blood (Zhang et al., 2013c). 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) 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 et al., 2019). PFOS accumulated at higher levels in fetal tissues compared with 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 with the third trimester, and lowest in the lung in the second trimester compared with 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 (Gyllenhammar et al., 2018a; Cariou et al., 2015; Mogensen et al., 2015b; Mondal et al., 2014). Distribution is influenced by the chemical properties of PFAS including length, lipophilicity, and branching. In the Mondal study (Mondal et al., 2014), mean maternal serum PFOS concentrations were lower in breastfeeding mothers versus non-breastfeeding mothers. Conversely, breastfed infants had higher mean serum PFOS than infants who were never breastfed. Maternal serum concentrations decreased with each month of breastfeeding (Mogensen et al., 2015b; Mondal et al., 2014). Cariou et al. (2015) reported that PFOS levels in breastmilk were approximately 66-fold lower relative to maternal serum and the ratio between breastmilk and maternal serum PFOS was 0.38 ± 0.16. The authors noted that the transfer rates of PFAS from serum to breastmilk were lower compared with 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 (Ishida et al., 2017; Chen et al., 2012b; Zeng et al., 2011; Borg et al., 2010; Chang et al., 2009; Liu et al., 2009; Luebker et al., 2005a).

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 et al., 2010a). Gomis et al. (2017) used a V_d of 235 mL/kg by averaging V_d values estimated for both humans and animals. V_d values may be influenced by differences in distribution between males and females, between pregnant and 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) 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 with the peripheral compartment in females. Interestingly, another study found that for PFOS, a classical compartment model was not applicable (Iwabuchi et al., 2017). 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 5.6.2.

3.3.1.3 Metabolism

Consistent with other reports and reviews (ATSDR, 2021; Pizzurro et al., 2019; U.S. EPA, 2016b), the literature reviewed for this assessment do not provide evidence that PFOS is 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 Appendix B, (U.S. EPA, 2024a)).

3.3.1.4.1 Urinary and Fecal Excretion

Urinary excretion is considered the main route of PFOS excretion in humans. Zhang et al. (2015b) estimated a daily urinary excretion rate of 16% of the estimated total daily intake for PFOS for adults. Zhang et al. (2013d) 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) 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 with 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), 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) 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 et al., 2016). 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 with 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 Enterohepatic Resorption

Early evidence of enterohepatic resorption of PFOS was revealed by Johnson et al. (1984), 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 et al., 2010; Harada et al., 2007). Harada et al. (2007) estimated a biliary resorption rate of 0.97, which could contribute to the long half-life in humans. Genuis et al. (2010) 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 (Zhao et al., 2017; 2015) 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 et al., 2015). The opposite trend was seen for OATP-mediated uptake (Zhao et al., 2017). 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 yet unknown whether and to what extent these transporters function *in vivo*.

Studies describing renal resorption are discussed in Appendix B (U.S. EPA, 2024a).

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 and Qin, 2014). The ratio of branched:total PFOS isomers in cord blood was 0.27 and was higher in cord blood compared with maternal blood and placenta. These findings suggest branched PFOS isomers may transfer to the fetus more readily than linear forms. In another study in humans (Zhang et al., 2013c), 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 lifestage when excretion is altered, Zhang et al., (2015a) observed that the partitioning ratio of PFOS concentrations between urine and whole blood in pregnant women (0.0004) was 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).

Mamsen and colleagues (2017) measured placental samples and fetal organs in relation to maternal plasma levels of five PFAS in 39 Danish women (Mamsen et al., 2017). 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 with 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 (Lee et al., 2017; Thomsen et al., 2011; Tao et al., 2008) and it was shown that PFOS levels in breastmilk are affected by parity (Lee et al., 2017; Jusko et al., 2016). 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 et al., 2015). 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) 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 (Taylor et al., 2014; Knox et al., 2011). However, a re-analysis of these data (Ruark et al., 2017) 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) failed to find evidence of associations between menstrual cycle length and PFAS concentrations. Furthermore, Lorber et al. (2015) 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. 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) found that hair is a 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 with 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 Appendix B, (U.S. EPA, 2024a)). 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 et al., 2014) to up to 60.9 years for males occupationally exposed in a facility in China (Fu et al., 2016) (see Appendix B, (U.S. EPA, 2024a)). With one exception (Genuis et al., 2014), half-lives estimated for males are longer than those estimated for females and show an age-related increase (Zhang et al., 2013d). Also, linear isomers exhibit longer half-lives than branched isomers (Xu et al., 2020c; Zhang et al., 2013d). 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 et al., 2008).

Half-life estimates in humans rely on measured serum and/or urine concentrations. However, relatively few studies calculated PFOS half-lives along with measured intake and serum and urine PFOS concentrations (Xu et al., 2020c; Worley et al., 2017a; Fu et al., 2016; Zhang et al., 2013e) (see Appendix B, (U.S. EPA, 2024a)). PFOS half-life values among these four studies varied dramatically from 1.04 years in Xu et al. (2020c) to 60.9 years in Fu et al. (2016). These comparisons support principles suggested by the broader literature. First, sex related differences with males exhibiting much longer half-lives compared with 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. (2020c) suggest that linear PFOS molecules exhibit longer half-lives than branched forms, which may reflect differential affinities of linear versus branched forms for resorption transporters. Third, the relationships between blood and urine concentrations are not obvious, underscoring the role of non-urinary routes of excretion and the difficulty in measuring renal resorption. Finally, only two studies estimated PFOS intake in subjects (Xu et al., 2020c; Worley et al., 2017a). 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 et al., 2012; Seacat et al., 2002) 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 (Huang et al., 2019; Kim et al., 2016; Chang et al., 2012). 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) who used a model to describe historical changes in exposure in seafood and consumer products, Hu et al. (2019b) 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) who used measured concentrations 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) 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. (2017a) 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) 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. Because of 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, 2021a)) 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, 2016b). A similar approach was used in the recent toxicity assessment performed by CalEPA (CalEPA, 2021). Publications reporting applications of similar models include the work of Zhang et al. (2015b) 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) 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 PFOS HESD. In Huang et al. (2019), 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 et al., 2016).

Gomis et al. (2017) 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). However, because the scope of the Gomis et al. (2017) 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 et al., 2020). Verner et al. (2016) 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), 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 and allow for dose-dependent changes in excretion rate compared with the classic 1- or 2- compartment approaches have been published as tools to estimate internal doses for humans, monkeys, mice, and rats (Chou and Lin, 2019; Loccisano et al., 2013; Wambaugh et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011; Andersen et al., 2006). The underlying assumption for all the models is saturable resorption from the kidney filtrate, which consistently returns a portion of the excreted dose to the systemic circulation and prolongs both clearance from the body (e.g., extends half-life) and the time needed to reach steady state.

One of the earliest PK models (Andersen et al., 2006) was developed for PFOS using two dosing situations in cynomolgus monkeys. In the first, three male and three female monkeys received a single IV dose of potassium PFOS at 2 mg/kg (Noker and Gorman, 2003). 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 et al., 2002). 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 et al., 2004) and a cardiac output of 15 L/h-kg from the literature (Corley et al., 1990). 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 with PFOA, PFOS had a longer terminal half-life and more rapid approach to steady-state with repeated oral administration.

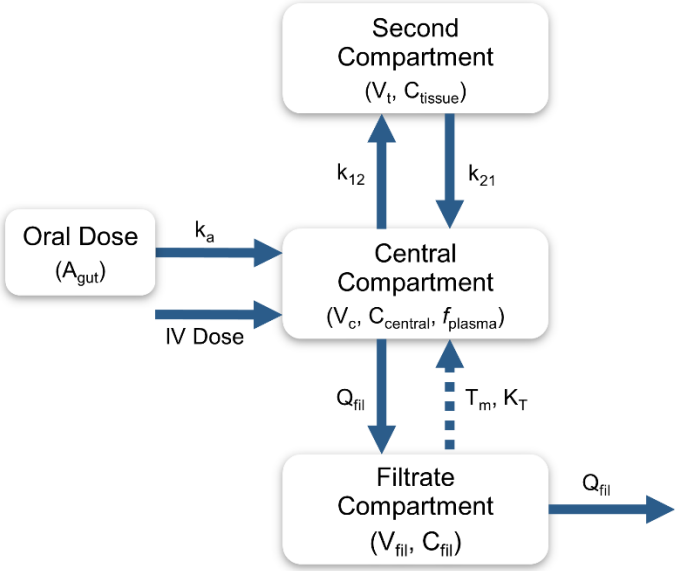


Figure 3-4. Schematic for a Physiologically Motivated Renal Resorption PK Model

Adapted from Wambaugh et al. (2013).

Building on the work of other researchers, Wambaugh et al. (2013) developed and published a PK model to support the development of an EPA RfD for PFOS (U.S. EPA, 2016b). 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)).

Wambaugh et al. (2013) placed bounds on the estimated values for some parameters of the Andersen et al. (2006) model to support the assumption that serum carries a significant portion of the total PFOS body load. The Andersen et al. (2006) 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) 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) model for each of the species evaluated.

The PK data that supported the Wambaugh et al. (2013) analysis were derived from two in vivo PFOS PK studies. The monkey PK data were derived from Seacat et al. (2002) and Chang et al. (2012). Data for the rats (male/females) and mice were both from Chang et al. (2012). The data were analyzed within a Bayesian framework using Markov Chain Monte Carlo sampler implemented as an R package developed by EPA to allow predictions across species, strains, and sexes and to identify serum levels associated with the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) external doses. Prior distributions for the parameters were chosen to be broad, log-normal distributions, allowing the fitted parameters to be positive and for the posterior distribution to be primarily informed by the data likelihood rather than by the priors.

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 with blood concentration.

The first PBPK model developed for PFOS 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 et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011). 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 et al., 2008). The work of Tan et al. (2008) was a development of the earlier work of Andersen et al. (2006) 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) 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) 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) 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. (2020b; 2020c) who applied a model of thyroid disease (Dzierlenga et al., 2019) to describe changes in PFOS renal clearance due to disease state.

In addition to this set of studies, Fabrega et al. (2014) updated the model of Loccisano et al. (2013) 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) 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 et al., 2007) 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 (Fàbrega et al., 2015).

Brochot et al. (2019) 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. (2017b) 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) developed a PFOS PBPK model for rat, mouse, monkey, and human. Using the model structure of Worley and Fisher (2015), parameters were determined using a hierarchical Bayesian framework to pool datasets across studies for each species. This model reflects saturable resorption in the proximal tubule cells of the kidney and fecal elimination through the bile. While the Bayesian approach is ideal for handling multiple datasets, the method for implementing the Bayesian inference raises questions about the final posterior parameter

distributions. Priors for the hierarchical model were determined using a least-squares fitting method on the most sensitive parameters as opposed to defining priors using information from previous studies and letting the data update those priors to determine the joint posterior distribution of the parameter space. In a subsequent study, Chou and Lin (2021) 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) with renal resorption, for the infant. Using this model, the authors developed human equivalent doses (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 Noncancer Health Effects Evidence Synthesis and Integration

3.4.1 Hepatic

EPA identified 24 epidemiological studies (30 publications)[5,6] and 25 animal toxicological studies that investigated the association between PFOS and hepatic effects. Of the epidemiological publications, 17 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 et al., 2005). Bilirubin and γ-glutamyltransferase (GGT) are also routinely used to evaluate potential hepatobiliary toxicity (Hall et al., 2012; EMEA, 2008; Boone et al., 2005). 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 7 epidemiological studies (8 publications)[6] from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and hepatic effects. Study quality evaluations for these eight studies are shown in Figure 3-5. Results from studies summarized in the 2016 PFOS HESD are described in Table 3-2 and below.

[5] Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu et al. (2018d); Gleason et al. (2015) all use NHANES data from overlapping years.
[6] Olsen (2003) is the peer-review paper of Olsen (2001a) and Olsen (2001b); however, data for PFOA and hepatic outcomes is reported in Olsen (2001a). Olsen (2001b) was considered overlapping and not evaluated because data in the technical report was completely described in Olsen (2003).

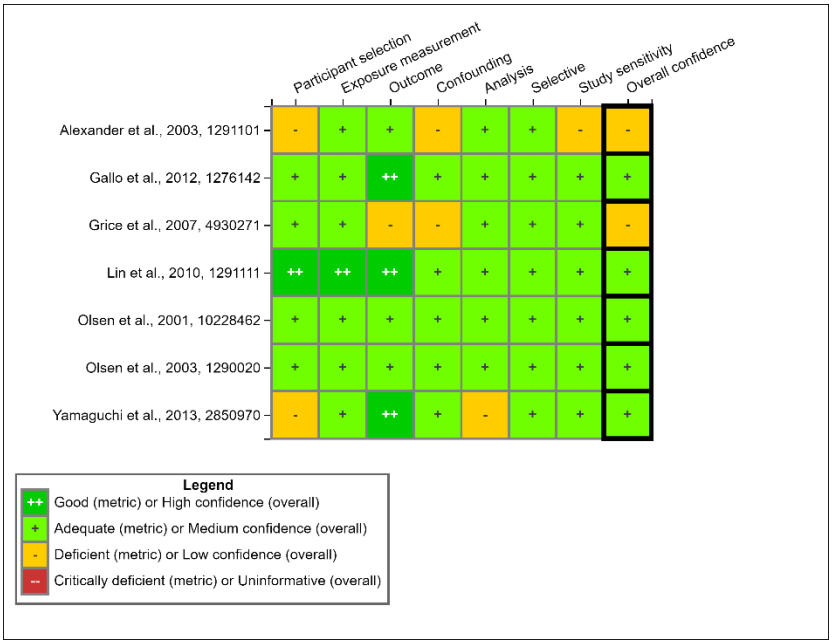


Figure 3-5. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Hepatic Effects Published Before 2016 (References in the 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

The 2016 PFOS HESD (U.S. EPA, 2016b) describes both cross-sectional and longitudinal studies that evaluated PFOS and liver enzymes in adults. Two available cross-sectional studies (Gallo et al., 2012; Lin et al., 2010) reported positive associations between PFOS exposure and ALT in adults of the general population (see Appendix D, (U.S. EPA, 2024a)). Lin et al. (2010) examined 2,216 adults in NHANES (1999–2000, and 2003–2004) and observed that higher serum concentrations of PFOS were associated with abnormal liver enzyme increases in the U.S. general population. With each increase in logPFOS, serum ALT and GGT concentrations (U/L) increased by 1.01 units (SE = 0.53) and 0.01 units (SE 0.03), respectively (Lin et al., 2010). When PFOA, PFHxS, and PFNA were simultaneously added in the fully adjusted regression models, one unit increase in serum logPFOS 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 et al., 2013) conducted in Japan reported a positive correlation with ALT in addition to factors influencing PFOS exposure.

Gallo et al. (2012) 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) 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 the Gallo et al. (2012) and Lin et al. (2010) studies observed a slight positive association between serum PFOS levels and increased serum ALT values (Figure 3-6). 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) study, the cross-sectional design and self-reported lifestyle characteristics are limitations of the study, and while both Lin et al. (2010) and Gallo et al. (2012) showed a trend, it was not large in magnitude.

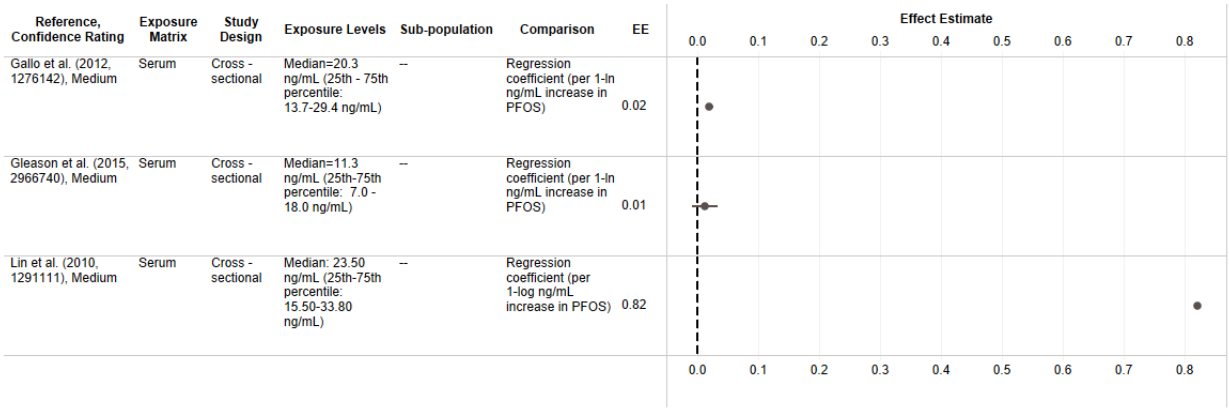


Figure 3-6. Overall ALT Levels from 2016 PFOS HESD Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

Several cross-sectional occupational studies in PFOS production workers reported mostly null or inconsistent findings with respect to biomarkers of liver disease. Exposure to PFOA was generally associated with increased ALT concentrations, but findings were inconsistent for some timepoints or in sex-stratified groups (Olsen et al., 2003; Olsen et al., 2001a). Null or inconsistent associations were also 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 et al., 2003). At the same plant, nonsignificant increases in noncancerous liver disease (including cirrhosis) were observed with cumulative exposure to PFOS (Grice et al., 2007).

Table 3-2. Associations Between Elevated Exposure to PFOS and Hepatic Outcomes From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Population	ALT[a]	AST[a]	GGT[a]	ALP[a]	Liver Disease[b]
Alexander, 2003, 1291101 *Low*	Cohort	Occupational	NA	NA	NA	NA	–

Reference, confidence	Study Design	Population	ALT[a]	AST[a]	GGT[a]	ALP[a]	Liver Disease[b]
Gallo, 2012, 1276142 *Medium*	Cross-sectional	Adults	↑↑	NA	–	NA	NA
Grice, 2007, 4930271 *Low*	Cohort	Occupational	NA	NA	NA	NA	↑
Lin, 2010, 1291111 *Medium*	Cross-sectional	Adults	↑↑	NA	–	NA	NA
Olsen, 2001, 10228462 *Medium*	Cohort	Occupational	↑	↑	–	–	NA
Olsen, 2003, 1290020 *Medium*	Cross-sectional	Occupational	↑	–	↑	↑	NA
Yamaguchi, 2013, 2850970 *Medium*	Cross-sectional	Adults and adolescents	↑↑	↑↑	↑↑	NA	NA

Notes: ALP = alkaline phosphatase; ALT = alanine transferase; AST = aspartate transaminase; GGT = gamma-glutamyl transferase; NA = no analysis was for this outcome was performed; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Jain et al., 2014, 2969807 was not included in the table due to their *uninformative* overall study confidence ratings.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

3.4.1.1.2 Study Quality Evaluation Results for the Updated Literature Review

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

Of these, 12 were classified as *medium* confidence, four as *low* confidence, and seven were considered *uninformative.* Of the informative studies, two cross-sectional studies (Nian et al., 2019; van den Dungen et al., 2017), multiple publications of data from NHANES (Omoike et al., 2020; Jain, 2019; Jain and Ducatman, 2019a, c; Liu et al., 2018d; Gleason et al., 2015), one prospective cohort in elderly adults (Salihovic et al., 2018), and one occupational cohort of fluorochemical plant workers (Olsen et al., 2012) examined liver enzymes in adults. In addition, two cross-sectional studies (Rantakokko et al., 2015 Liu, 2018, 4238396) examined functional liver endpoints in adults. In children and adolescents, four studies were available including one cohort study (Mora et al., 2018) and three cross-sectional studies (Jin et al., 2020; Attanasio, 2019; Khalil et al., 2018), with one examining function liver endpoints (Jin et al., 2020). All of the studies measured PFOS exposure using biomarkers in blood. The *uninformative* studies were excluded due to potential confounding (Abraham et al., 2020; Sinisalu et al., 2020; Predieri et al., 2015; Jiang et al., 2014), lack of information on participant selection (Sinisalu et al., 2021), or

[7] Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu et al. (2018d); Gleason et al. (2015) all use NHANES data from overlapping years.

use of PFAS as the dependent variable (in a publication with a more suitable analysis available (Jain, 2020a) or where the independent variable is a genetic variant and thus not affected by PFAS exposure (Fan et al., 2014)).

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

Interactive figure and additional study details available on HAWC.

[a] Multiple publications of the same data: Jain and Ducatman (2019a); Jain and Ducatman (2019c); Jain (2019); Jain (2020a); Omoike et al. (2020); Liu (2018d); Gleason et al. (2015) 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 Appendix D (U.S. EPA, 2024a). 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 (Nian et al., 2019; Salihovic et al., 2018) and multiple NHANES publications, including all the *medium* confidence studies. However, the positive associations in Jain et al. (2019) 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 ($p < 0.05$) was reported in Olsen et al. (2012) (see Appendix D, (U.S. EPA, 2024a)). 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), no association was observed. ALT findings from *low* confidence studies are not included in figures.

In children and adolescents, positive associations were observed in girls in the fourth quartile reported by Attanasio (2019) and in the *low* confidence study in obese children (Khalil et al., 2018). However, inverse associations were observed in Mora et al. (2018), 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 Appendix D (U.S. EPA, 2024a). In adults, statistically significant positive associations were observed in the one *medium* confidence study (Nian et al., 2019) and in NHANES studies. Van den Dungen et al. (2017) reported a nonsignificant positive association. No association was observed in Olsen et al. (2012). In children and adolescents, the *medium* confidence study (Attanasio, 2019) also observed a positive association in girls but not boys, while the *low* confidence study (Khalil et al., 2018) reported an inverse association, both not statistically significant. For the other liver enzymes (bilirubin, GGT), results were generally consistent with ALT and AST (Attanasio, 2019; Nian et al., 2019; van den Dungen et al., 2017) with the exception of inverse associations (not statistically significant) for GGT in Jain (2019) and bilirubin in Salihovic et al. (2018).

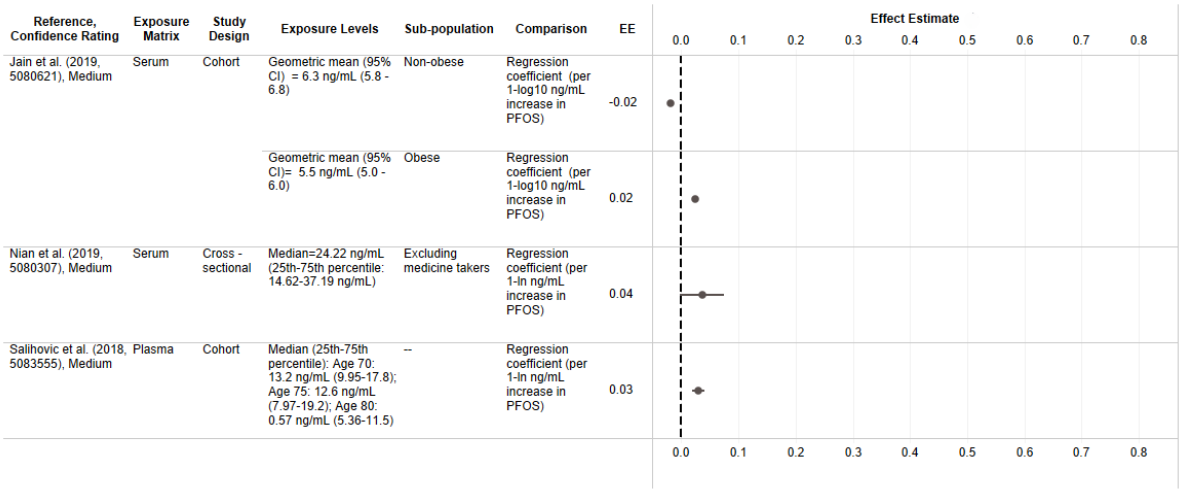


Figure 3-8. Overall ALT Levels from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

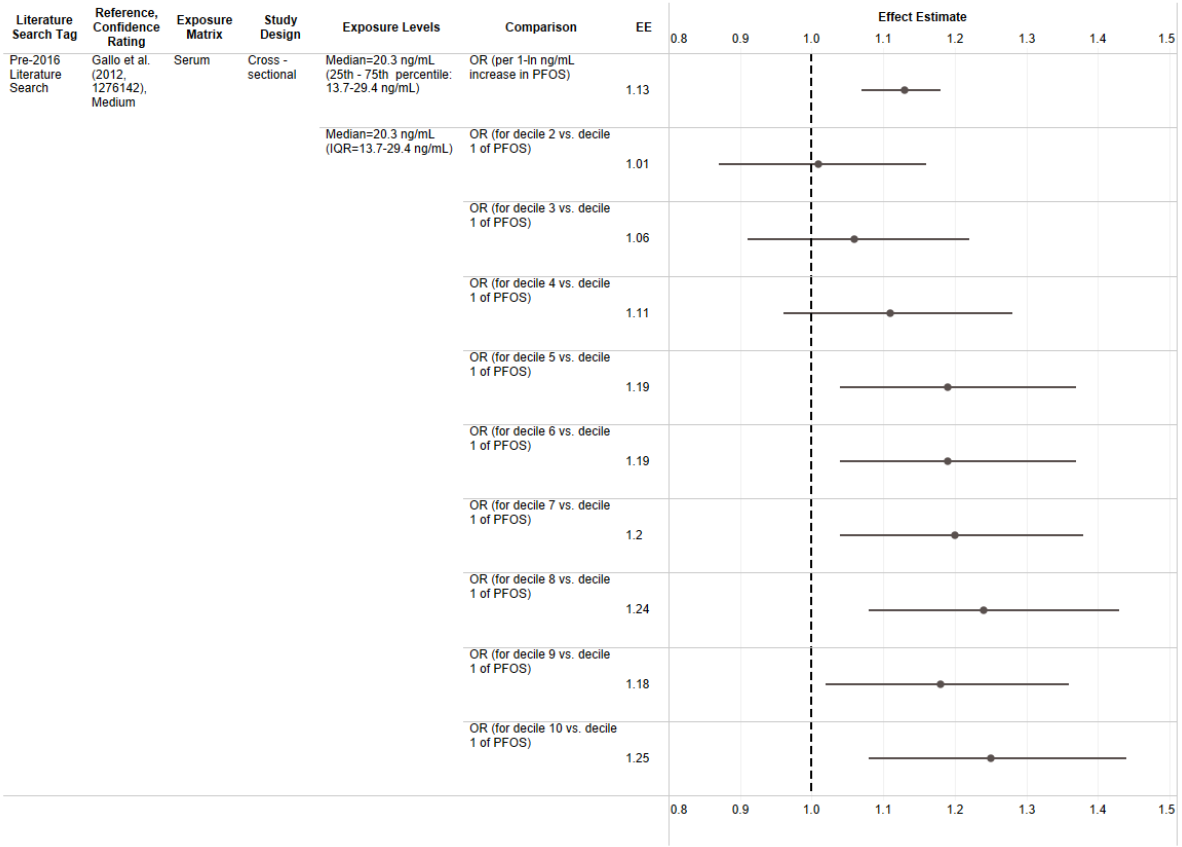


Figure 3-9. Odds of Elevated ALT Levels from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

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) reported that higher PFOS exposure levels were associated with reduced odds of lobular inflammation, whereas Jin et al. (2020) reported the opposite, with an OR of 2.9 for 2–4 foci versus. none, though the results in the latter study were non-monotonic and both were not statistically significant. Jin et al. (2020) additionally reported higher odds (not statistically significant) of non-alcoholic steatosis ($p < 0.05$), ballooning, fibrosis, and portal inflammation. Lastly, Liu et al. (2018b) examined hepatic fat mass and found no correlation with PFOS exposure.

In summary, across studies in the 2016 PFOS HESD (U.S. EPA, 2016b) 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 of the individual contaminants. This cannot be ruled out in this body of evidence given the attenuation of the association in Lin et al. (2010), 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 in epidemiological studies, there is residual uncertainty.

3.4.1.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 6 animal toxicological studies from the 2016 PFOS HESD (U.S. EPA, 2016b) and 19 animal toxicological 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.

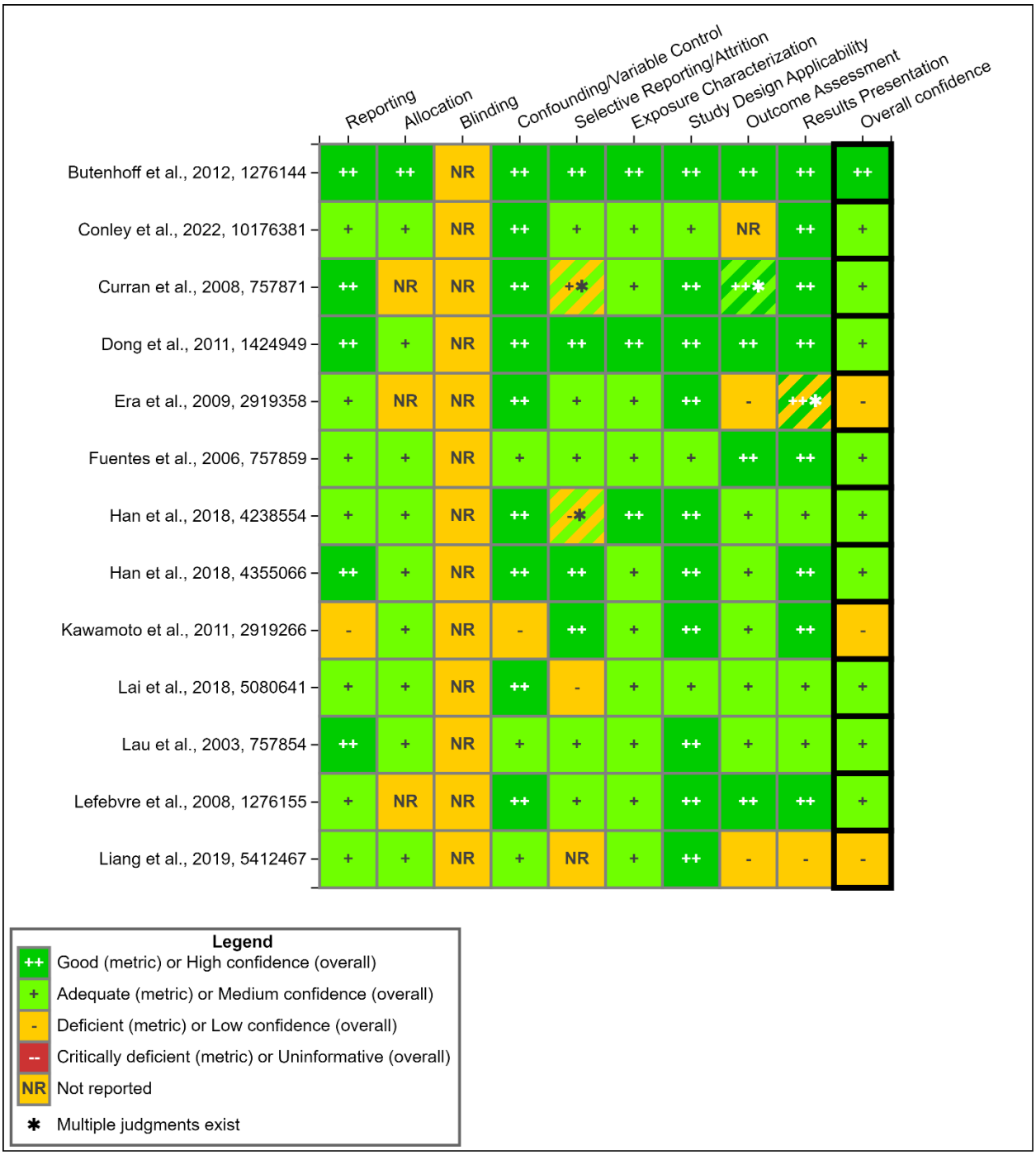


Figure 3-10. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Hepatic Effects[a,b]

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

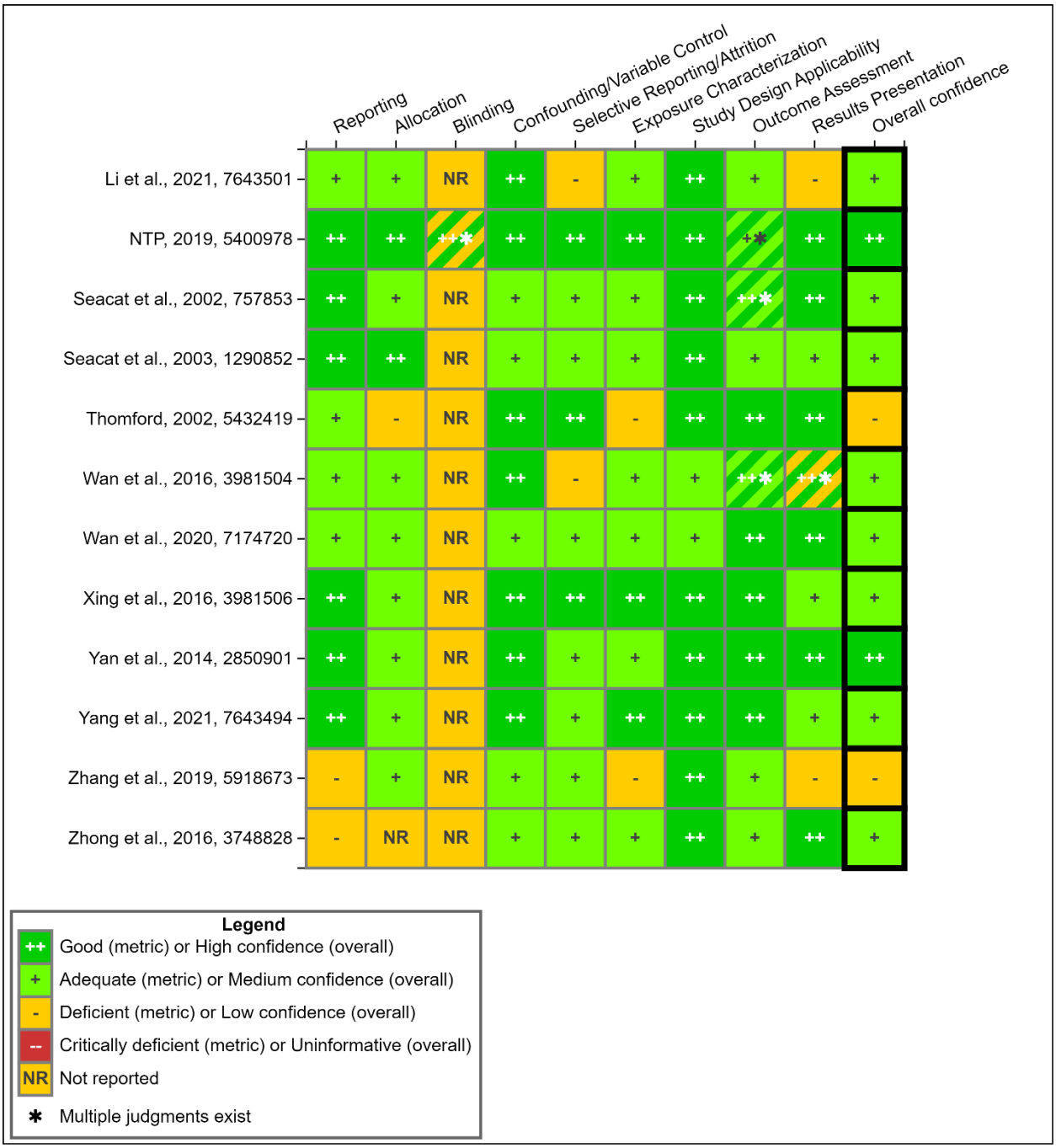


Figure 3-11. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Hepatic Effects (Continued) [a,b]

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

Hepatic effects were observed in male and female mice, rats, and monkeys after varying oral PFOS exposure durations and 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 (Yang et al., 2021; Lai et al., 2018; Xing et al., 2016; Zhong et al., 2016; Yan et al., 2014; Dong et al., 2011; Lau et al., 2003). In male BALB/c mice, significant increases in both relative and absolute liver weights were observed after a 28-day exposure to PFOS doses of 1.25 and 5 mg/kg/day (Yan et al., 2014). 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) or 2.5–10 mg/kg/day PFOS (Xing et al., 2016). In a 60-day study in male C57BL/6 mice, Dong et al. (2011) observed a dose-related increase in relative liver weights; at doses above 0.417 mg/kg/day PFOS, the increases were statistically significant compared with control. In a 7-week gavage study in female CD-1 mice, Lai et al. (2018) 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 increased liver weights in the dams following gestational PFOS exposure (Wan et al., 2020; Fuentes et al., 2006). Fuentes et al. (2006) 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) 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 et al., 2016; Lau et al., 2003). Zhong et al. (2016) 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) 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; Han et al., 2018b; Wan et al., 2016; Wan et al., 2012; Cui et al., 2009; Curran et al., 2008; Lau et al., 2003; Seacat et al., 2003). 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 et al., 2008). 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). In a 14-week feeding study, Seacat et al. (2003) 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 at 1.33 mg/kg/day.

In a developmental toxicity study, Lau et al. (2003) 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 2–21. 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 a relatively short 5-day exposure (GD 14–18) to PFOS concentrations of 0, 0.1, 0.3,1, 3, 10, or 30 mg/kg/day (Conley et al., 2022).

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 et al., 2002).

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)). Serum levels of 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, 2002a). Alterations in serum enzyme levels are generally considered to reach biological significance and indicate potential adversity at levels ≥ twofold compared with controls (i.e., ≥ 100% change relative to control response) (Hall et al., 2012; U.S. EPA, 2002a).

Two studies in male mice found statistically and biologically significant increases in serum enzymes indicative of hepatic or hepatobiliary damage after oral PFOS exposure (Figure 3-12) (Xing et al., 2016; Yan et al., 2014). Xing et al. (2016) 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) 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) and Xing et al. (2016) 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) 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) also reported a large increase in ALP (321% change relative to control) in the 5 mg/kg/day dose group. A statistically and biologically significant dose-dependent increase in GGT was observed by Xing et al. (2016), 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, 2002a).

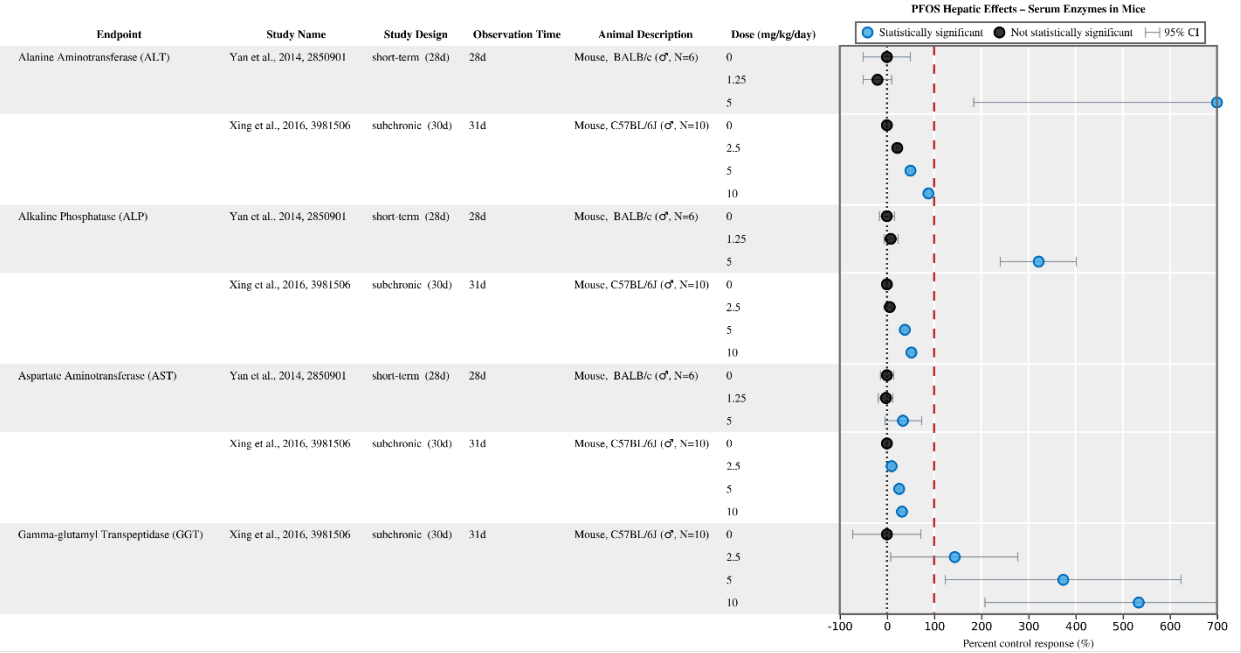


Figure 3-12. Percent Change in Serum Enzyme Levels Relative to Controls in Mice Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC here and here.
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) 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) (Conley et al., 2022; NTP, 2019; Han et al., 2018b; Han et al., 2018a; Wan et al., 2016; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003).

The NTP (2019), Han et al. (2018b), and Curran et al. (2008) 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) 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) 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 twofold compared with controls. Han et al. (2018b) 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) or not statistically significant between treated and control groups (Curran et al., 2008; Seacat et al., 2003). Butenhoff et al. (2012) 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) reported statistically significant increases in ALP in male rats after a 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) did not observe consistent effects of 28-day dietary consumption of PFOS on ALP levels at dose levels up to approximately 6.34 mg/kg/day in male rats.

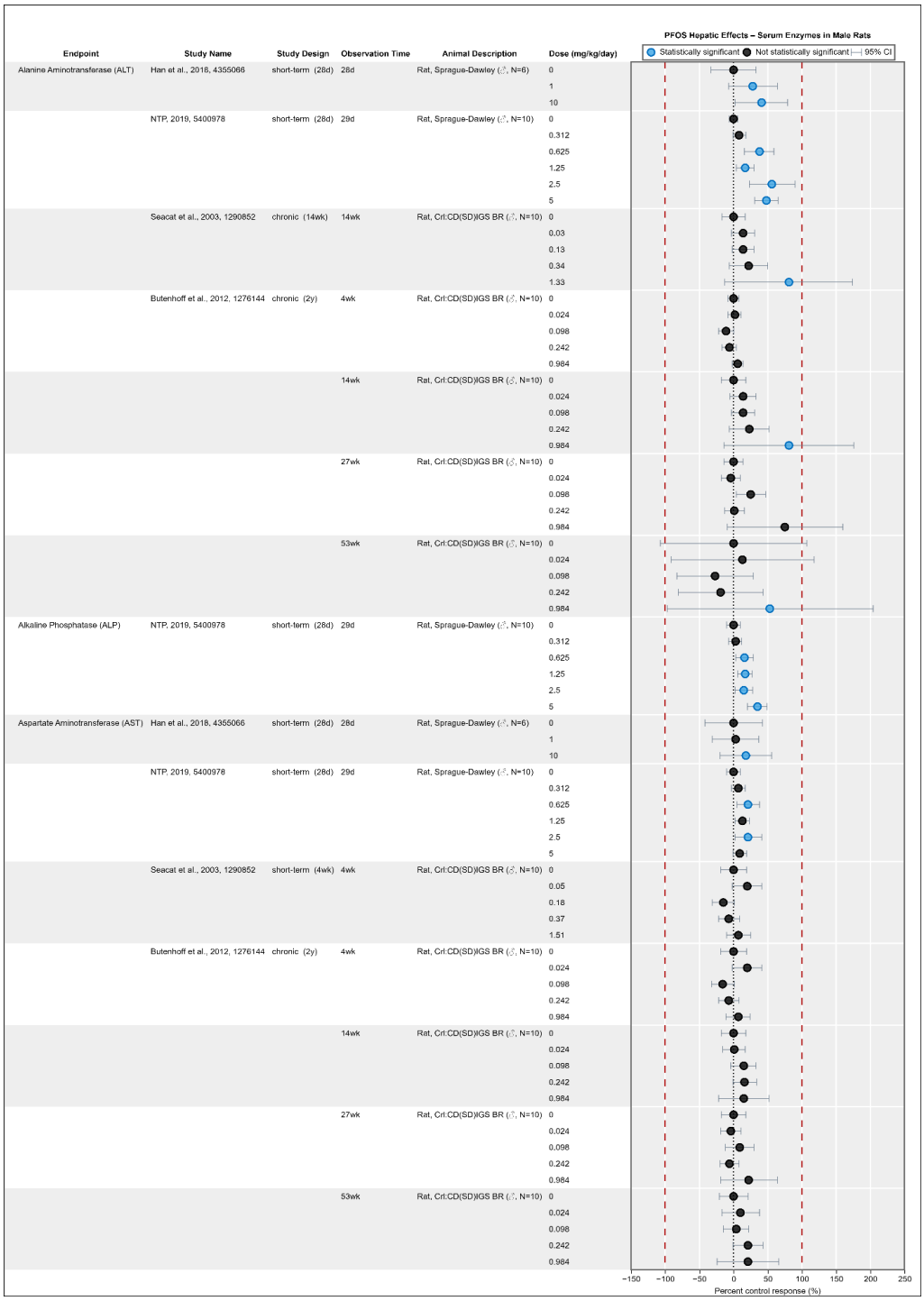


Figure 3-13. Percent Change in Serum Enzyme Levels Relative to Controls in Male Rats Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC here and here.
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. (2018a) and Wan et al. (2016) reported on the same data as Han et al. (2018b) 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; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003). In a 28-day study in female rats, NTP (2019) reported dose-dependent increases in ALT, though these increases reached only approximately 62% change with the highest dose tested (10 mg/kg/day). A 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). Similarly, Seacat et al. (2003) 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) 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 and Conley et al. (2022) did not observe effects on ALT levels in female Sprague-Dawley dams treated with up to 30 mg/kg/day PFOS from GD 14–18.

Both Curran et al. (2008) and Butenhoff et al. (2012) 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 with controls (NTP, 2019; Seacat et al., 2003) and the statistically significant decrease observed by Butenhoff et al. (2012) 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) observed no significant effect on AST in the serum of Sprague-Dawley dams exposed to PFOS concentrations between 0.1–30 mg/kg/day from GD 14–18.

NTP (2019) 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 et al., 2008).

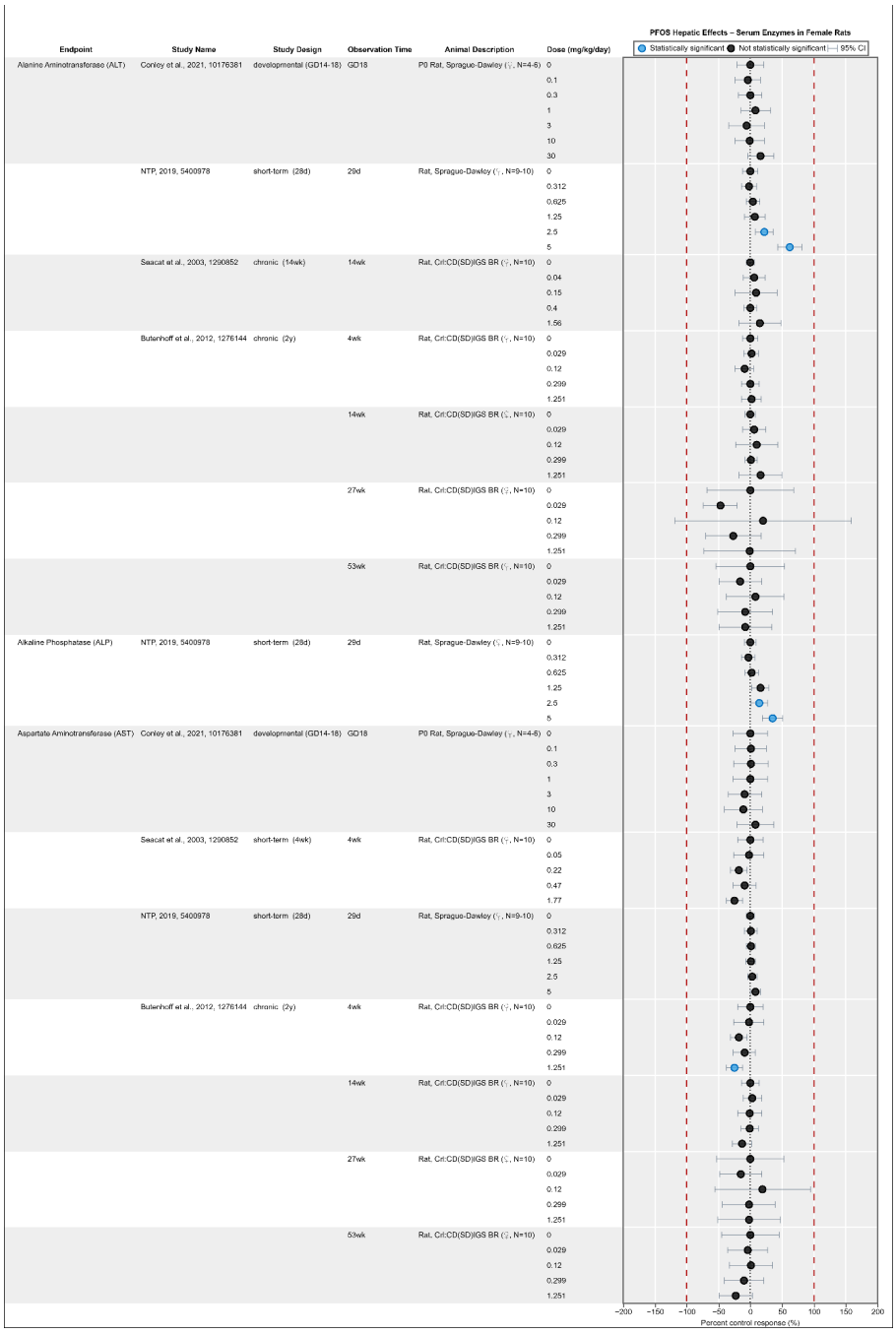


Figure 3-14. Percent Change in Serum Enzyme Levels Relative to Controls in Female Rats Following Exposure to PFOS[a,b]

Interactive figure and additional study details available on HAWC here and here.
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. (2018a) and Wan et al. (2016) reported on the same data as Han et al. (2018b) 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 et al., 2002).

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; Curran et al., 2008; Seacat et al., 2003). 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 et al., 2002). Six studies examined albumin levels, but only two studies found significant alterations due to PFOS treatment (Conley et al., 2022; NTP, 2019; Yan et al., 2014; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003). 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 et al., 2014). 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). 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). In monkeys, serum bile acids were significantly increased in males, but not in females, dosed with 0.75 mg/kg/day PFOS (Seacat et al., 2002).

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 (Li et al., 2021c; NTP, 2019; Han et al., 2018b; Han et al., 2018a; Wan et al., 2016; Xing et al., 2016; Wan et al., 2012; Cui et al., 2009; Curran et al., 2008) and in two chronic studies of male and female rats and monkeys (Butenhoff et al., 2012; Seacat et al., 2002). Only three of these studies provided quantitative incidence data (NTP, 2019; Butenhoff et al., 2012; Curran et al., 2008).

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) (Table 3-3). 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). 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; this lesion was not observed in females (Curran et al., 2008) (Table 3-4). 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 et al., 2008). In the chronic study in Sprague-Dawley rats (Butenhoff et al., 2012; Thomford, 2002b), 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 positive dose-response relationship was observed (Table 3-5).

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

	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 3-4. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by Curran et al. (2008)

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 3-5. Incidences of Nonneoplastic Lesions in Male and Female Sprague-Dawley Rats, as Reported by Thomford (2002b)

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

Males					
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) (peer-reviewed publication of data from a report by Thomford (2002b)) also observed a dose-dependent increase in cystic degeneration in male rats exposed to 0.024–0.984 mg/kg/day of PFOS (Table 3-5); 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 et al., 2012; Thomford, 2002b).

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 quantitative data 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 et al., 2016). Cytoplasmic vacuolation was also observed in one study of female mice exposed to 0.1 mg/kg/day PFOS for 60 days (Li et al., 2021c). 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 et al., 2018b; Han et al., 2018a; Wan et al., 2016); 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 et al., 2009). 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 et al., 2002).

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 (Han et al., 2018b; Han et al., 2018a; Wan et al., 2016; Xing et al., 2016; Cui et al., 2009). Livers of male C57BL/6J mice and Sprague-Dawley rats dosed with PFOS concentrations ranging from 2.5 to 20 mg/kg/day for approximately 4 weeks showed focal or flake-like necrosis, hepatocytic degeneration, and/or inflammatory cell infiltration (Xing et al., 2016; Cui et al., 2009). 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 et al., 2018b; Han et al., 2018a; Wan et al., 2016). 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 et al., 2021c). However, no quantification or statistical analyses were performed in 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, 2016b). 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 as organized by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-15.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Tot
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling				
Atherogenesis And Clot Formation	0	0	1	1
Big Data, Non-Targeted Analysis	9	0	6	15
Cell Growth, Differentiation, Proliferation, Or Viability	13	1	25	35
Cell Signaling Or Signal Transduction	13	1	15	25
Extracellular Matrix Or Molecules				
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	17	0	10	25
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
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	31	2	31	58

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

Interactive figure and additional study details available on HAWC.

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, 2016b). 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, 2016b). 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, 2016b).

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 (Behr et al., 2020b; Rosenmai et al., 2018; Wolf et al., 2014; Wolf et al., 2008; Takacs and Abbott, 2007). 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 et al., 2014) and a lowest observed effect concentration (LOEC) of 90 μM for PPARα activation in another study (Wolf et al., 2008). 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 and Abbott, 2007). 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 et al., 2008). 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 et al., 2020b). 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 et al., 2018) or 250 μM in COS-1 cells (Takacs and Abbott, 2007). In every study that compared the ability of PFOS to activate PPARα with that of PFOA, PFOS was a weaker PPARα activator (Behr et al., 2020b; Rosenmai et al., 2018; Wolf et al., 2014; Wolf et al., 2008; Takacs and Abbott, 2007).

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 (Behr et al., 2020b; Bagley et al., 2017; Zhang et al., 2014; Takacs and Abbott, 2007). One study showed that PFOS significantly activates human PPARγ by 1.5-fold at 10 μM and by threefold at 100 μM in a luciferase assay in HepG2 cells (Zhang et al., 2014). 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 et al., 2017). However, two other studies did not observe activation of PPARγ by PFOS (Behr et al., 2020b; Takacs and Abbott, 2007): PFOS did not activate human PPARγ or PPARδ in HEK29 cells at concentrations of up to 100 μM (Behr et al., 2020b), and neither human nor mouse PPARγ were activated by concentrations of up to 250 μM PFOS in COS-1 cells (Takacs

and Abbott, 2007). 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 and Abbott, 2007). 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 (Behr et al., 2020b; Bagley et al., 2017). 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 et al., 2017). 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

3.4.1.3.1.4 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, 2016b). Indeed, several studies in Sprague-Dawley rats have found evidence that PFOS may activate both PPARα and CAR/PXR in the liver (NTP, 2019; Dong et al., 2016; Elcombe et al., 2012b; Elcombe et al., 2012a; Chang et al., 2009; Martin et al., 2007). 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 et al., 2007). 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 et al., 2016). 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; Chang et al., 2009). 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 et al., 2009). 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). 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 et al., 2012b; Elcombe et al., 2012a). 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 et al., 2012b). 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 et al., 2012a). PPARα, CAR, and PXR activities (as measured by lauric acid 12-hydroxylation (CYP4A activity), pentoxyresorufin-O-depentylation (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 et al., 2012b), but was shown to be significantly increased at both concentrations after 28 days of exposure (Elcombe et al., 2012a). 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 et al., 2003).

Studies in various strains of wild-type (WT) mice also examined PPARα activation as a mechanism of PFOS-induced liver toxicity (Huck et al., 2018; Lai et al., 2017b; Wang et al., 2014; Wan et al., 2012; Bijland et al., 2011; Rosen et al., 2009). 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 (Lai et al., 2017b; Wang et al., 2014; Wan et al., 2012; Bijland et al., 2011; Rosen et al., 2009). 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 et al., 2012). 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 et al., 2018). 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 upregulated 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 et al., 2017; Rosen et al., 2010). The first study found that PPARα-regulated genes were altered in WT mice dosed with 10 mg/kg/day PFOS for 7 days (Rosen et al., 2010). 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 et al., 2010). In a connected study, the authors reanalyzed their data using different expression analysis software than the initial analysis (Rosen et al., 2017). 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 et al., 2017). 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 et al., 2015a), and *Pparα*-null mice (Oshida et al., 2015b). 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 et al., 2015a).

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. (Rosen et al., 2010) 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 et al., 2009b). 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), 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 et al., 2016). 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 et al., 2016). 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.

3.4.1.3.1.5 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 (Louisse et al., 2020; Song et al., 2016; Rosen et al., 2013; Bjork and Wallace, 2009). 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 et al., 2013), 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 and Wallace, 2009). 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 et al., 2016). However, transcripts associated with CAR/PXR activation were upregulated in human hepatocytes (Rosen et al., 2013). 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 et al., 2020; Song et al., 2016). Gene expression patterns in PFOS-exposed HepG2 cells were also consistent with activation of LXR (Louisse et al., 2020). 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 et al., 2020a).

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 (Behr et al., 2020a; Beggs et al., 2016). 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 et al., 2016). 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 et al., 2020a).

3.4.1.3.1.6 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 and Guo, 2019). 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 et al., 2017). 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 and Quiroga, 2016). 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 et al., 2011). 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, 2016b). 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 and Guo, 2019). 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 et al., 2020). 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 (NTP, 2019; Bagley et al., 2017).

In a study by Bagley et al. (2017), male and female rats were exposed to 0 or 100 ppm of PFOS in their diet for 3 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 3 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 ninefold in females (compared with twofold 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 with 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 et al., 2017).

NTP (2019) 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), Luebker (2005b) 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 (Liang et al., 2019; Huck et al., 2018; Lai et al., 2018; Xu et al., 2017). These changes often had potentially associated histopathological consequences, with steatosis and other lesions being observed in affected livers (Liang et al., 2019; Su et al., 2019; Huck et al., 2018).

In a 4-week study, decreased liver cholesterol was observed in male C57BL/6 mice dosed with 5 mg/kg/day PFOS (Xu et al., 2017); 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 et al., 2018). 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 et al., 2019). 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). 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) observed a decrease in hepatocyte nuclear factor alpha (HNF4α) protein, a master regulator of hepatic differentiation, in the livers of 10-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 et al., 2016).

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 (Liang et al., 2019; Su et al., 2019; Huck et al., 2018; Das et al., 2017; Rosen et al., 2017). Two of these studies, Das et al. (2017) and Rosen et al. (2017), 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) observed that a smaller subset of genes related to lipid homeostasis was activated in *Pparα*-null mice compared with WT mice. In addition, there were three-to-fourfold reductions in the genes related to lipid homeostasis that were expressed in PFOS-exposed *Pparα*-null mice compared with 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 twofold decrease in *Hnf4α*, a fourfold decrease in *Ppargc1a*, and a threefold 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) are concurrent with the findings in another study by the same authors (Rosen et al., 2017), which exposes WT and *Pparα*-null mice to 10 mg/kg/day PFOS for 7 days. PFOS exposure upregulated 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), 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 (Liang et al., 2019; Su et al., 2019; Huck et al., 2018). 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 et al., 2019). 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 et al., 2018). *Srebf1*, a regulator of hepatic lipogenesis, was significantly induced in PFOS-exposed mice in the HFD group compared with 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 datasets 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 (Lai et al., 2017b; Beggs et al., 2016).

Beggs et al. (2016) 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. (2017b) 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 (Khazaee et al., 2019; Cui et al., 2017; Cheng et al., 2016; Du et al., 2014). 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) 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 (Cui et al., 2017; Cheng et al., 2016). 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 with females (Cheng et al., 2016). In a 2-generation study, Cui et al. (2017) 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) 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 with 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) 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 10 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) 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) 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. (2020a) also exposed HepaRG cells to 0–100 μM PFOS for 24 or 48 hours. Similar to the results from Louisse et al. (2020), 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. (2020a) 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) 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 Appendix, (U.S. EPA, 2024a)), 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) 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 subfamily 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 (Song et al., 2016; Popovic et al., 2014). 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 et al., 2016).

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 et al., 2011). 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 et al., 2012a; Chang et al., 2009; Curran et al., 2008) and the remaining study using *Pparα*-null and WT mice (Rosen et al., 2010). In a gestational and lactational exposure study, Chang et al. (2009) reported increased *Cyp2b2* expression in dams and male pups (2.8-fold and 1.8-fold, respectively). Elcombe et al. (2012a) 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) and Rosen et al. (2010) 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 (NTP, 2019; Elcombe et al., 2012b). 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 et al., 2012b). 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 with the control group. However, there was a nonlinear 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) 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 et al., 2016b). 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 et al., 2016b), which are the genes responsible for OATPs and significant in the transport of xenobiotics (Popovic et al., 2014). Jantzen et al. (2016b) noted that in their previous study, PFOS exposure from 5 to 14 dpf resulted in significantly reduced slco2b1 expression in zebrafish at 5 dpf but significantly increased expression at 14 dpf (Jantzen et al., 2016a). 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 et al., 2013). 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 (Song et al., 2016; Shan et al., 2013) and HepaRG (Behr et al., 2020a; Franco et al., 2020b; Louisse et al., 2020). Franco et al. (2020b) 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 et al., 2020b).

Evidence varied for CYP3A4 induction, depending on the model and duration of exposure, as well as whether gene expression or enzyme activity was assessed (Behr et al., 2020a; Franco et al., 2020b; Louisse et al., 2020; Shan et al., 2013). Franco et al. (2020b) reported that after 24 hours, there were no clear trends in *CYP3A4* expression. However, after 48 hours, *CYP3A4* expression was significantly reduced (up to fivefold) across all concentrations (Franco et al., 2020b). Conversely, Behr et al. (2020a) and Louisse et al. (2020) 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) reported no significant changes in CYP3A4 enzyme activity following PFOS exposure (0, 100, 200, 300, and 400 μM) in HepG2 cells.

Franco et al. (2020b) 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 et al., 2020b).

Song et al. (2016) 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 with 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 (NTP, 2019; Elcombe et al., 2012b), zebrafish (Jantzen et al., 2016b), primary hepatocytes (Rosen et al., 2013), or hepatic cell lines (Behr et al., 2020a; Franco et al., 2020b; Louisse et al., 2020; Song et al., 2016; Shan et al., 2013). Jantzen et al. (2016b) reported significant reductions in the expression of OATPs (*slco1d1* and *slco2b1*). While the majority of studies reported upregulation of gene expression of CYP enzymes (Behr et al., 2020a; Franco et al., 2020b; Louisse et al., 2020; NTP, 2019; Song et al., 2016; Rosen et al., 2013; Elcombe et al., 2012b), direction and magnitude of change varied across doses and exposure times. Jantzen et al. (2016b) and Franco et al. (2020b) 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 (Xu et al., 2019b; Khansari et al., 2017) and in hepatic cell lines (Behr et al., 2020a; Franco et al., 2020b; Franco et al., 2020a; Louisse et al., 2020; Ojo et al., 2020; Rosenmai et al., 2018; Sheng et al., 2018; Bagley et al., 2017; Oh et al., 2017; Song et al., 2016; Wan et al., 2016; Cui et al., 2015b; Wielsøe et al., 2015; Huang et al., 2014; Shan et al., 2013; Florentin et al., 2011), 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 et al., 2019b) and by 64%, as determined by a trypan blue exclusion assay in rat primary hepatocytes exposed to 25 μM PFOS for 3 hours (Khansari et al., 2017). 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 et al., 2013).

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 et al., 2020b; Franco et al., 2020a), to 45–65 μM after 24 or 48 hours of exposure in HepG2 cells (Ojo et al., 2020; Wan et al., 2016), to 417 μM after 24 hours of exposure in HL-7702 cells (Sheng et al., 2018). However, two studies in HepG2 cells (Rosenmai et al., 2018) and HepaRG cells (Louisse et al., 2020) 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 (Han et al., 2018a; Eke et al., 2017; Wan et al., 2016; Elcombe et al., 2012b; Elcombe et al., 2012a) and two studies in male mice (Lv et al., 2018; Xing et al., 2016), 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 et al., 2012b; Elcombe et al., 2012a). In one of these studies, rats were exposed for 7 days and allowed to recover for 1, 28, 56, or 84 days (Elcombe et al., 2012b), while the other study exposed rats for 1, 7, or 28 days and collected liver directly after exposure (Elcombe et al., 2012a). 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 (Han et al., 2018a; Eke et al., 2017; Wan et al., 2016). Similarly, two short-term gavage studies in male mice showed an increase in liver apoptosis (Lv et al., 2018; Xing et al., 2016). 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 et al., 2016). 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 et al., 2018).

Several studies further examined the mechanisms by which PFOS exposure may lead to apoptosis in the liver (Xu et al., 2020b; Han et al., 2018a; Lv et al., 2018; Oh et al., 2017; Xing et al., 2016; Huang et al., 2014; Yao et al., 2014). 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 et al., 2018a). 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 et al., 2018; Xing et al., 2016). 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 et al., 2014).

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 et al., 2020b; Xu et al., 2019b; Oh et al., 2017; Song et al., 2016; Wan et al., 2016; Yao et al., 2016; Cui et al., 2015b; Huang et al., 2014). PFOS was shown to increase the percentage of apoptotic cells (Xu et al., 2019b; Oh et al., 2017; Yao et al., 2016; Cui et al., 2015b; Huang et al., 2014), to increase the expression of proteins and genes in apoptotic pathways (Song et al., 2016; Wan et al., 2016), or to increase CASP3 enzyme activity (Yao et al., 2016). Only one study in HL-7702 cells showed no change in the percentage of apoptotic cells (Cui et al., 2015a).

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 et al., 2020b). 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 et al., 2017). 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 et al., 2014). 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 et al., 2016; Yao et al., 2014).

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 et al., 2016). 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 et al., 2016).

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 (Louisse et al., 2020; Han et al., 2018b; Huck et al., 2018; Lai et al., 2017b; Beggs et al., 2016; Song et al., 2016; Cui et al., 2015b; Cui et al., 2015a; Elcombe et al., 2012b; Elcombe et al., 2012a; Thomford, 2002b). 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 et al., 2012b; Elcombe et al., 2012a). 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 et al., 2018b). 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, 2002b). 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 et al., 2016); 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 et al., 2018). 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 et al., 2017b).

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 et al., 2018b). 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 et al., 2015a). 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. (2015a) 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 et al., 2015b). 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 to 278 μM for 24 or 48 hours were associated with pathways involved in the regulation of cellular proliferation or the cell cycle (Louisse et al., 2020; Song et al., 2016).

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 et al., 2017b). Additionally, in two studies, PFOS has been shown to decrease the expression of HNF4α (Behr et al., 2020a; Beggs et al., 2016), a regulator of hepatic differentiation and quiescence that has been suggested as a mediator of steatosis following PFOS exposure (Armstrong and Guo, 2019). In one study by Beggs et al. (2016) (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 et al., 2013). 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 and Zhang, 2019). 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 et al., 2016). 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 et al., 2019), rat models (Han et al., 2018b; Han et al., 2018a), mouse models (Su et al., 2019; Lai et al., 2017b), and in vitro models (Han et al., 2018b; Song et al., 2016). Bassler et al. (2019) 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 et al., 2018b; Han et al., 2018a). In contrast to the C8 Panel study in humans (Bassler et al., 2019), 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. (2018b) 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. (2018b) 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. (2018b) 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. (2018b) 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 et al., 2019). 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 et al., 2017b). 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) 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 and Michalak, 2014). 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 (Han et al., 2018a; Wan et al., 2016). In hepatocytes extracted from dosed rats, Wan et al. (2016) 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. (2018a) 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 (Lv et al., 2018; Xing et al., 2016; Rosen et al., 2010; Liu et al., 2009). Rosen et al. (2010) 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) 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 et al., 2016). 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) subchronic exposure study also showed evidence of increased oxidative stress and decreased mechanisms of defense against oxidative stress following PFOS exposure (Lv et al., 2018). In an unspecified species of male mice, intragastric administration of 10 mg/kg/day PFOS for 3 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 with 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 et al., 2019). 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). 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 *Pppar*α-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 (Xu et al., 2020b; Xu et al., 2019b; Khansari et al., 2017; Rosen et al., 2013). Khansari et al. (2017) 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 with 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) 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 to 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. (2019b) 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. (2020b) 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 et al., 2016; Wielsøe et al., 2015; Shan et al., 2013; Florentin et al., 2011). Two studies reported increases in ROS levels following PFOS exposure (Wan et al., 2016; Wielsøe et al., 2015), while two studies did not observe statistical differences in ROS levels following 1- or 24-hour PFOS exposures up to 400 μM (Florentin et al., 2011) or following 3-hour PFOS exposures up to 400 μM (Shan et al., 2013). Wan et al. (2016) 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 with 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) concluded that *miR-155* plays a key role in the inhibition of the Nrf2 signaling pathway and can be upregulated with PFOS exposure.

Wielsoe et al. (2015) incubated HepG2 cells with up to 200 μM 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, 2016b) 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. Across studies in the 2016 PFOS HESD (U.S. EPA, 2016b) and this updated systematic review, there is generally consistent evidence of a positive association between exposure to PFOS and ALT. The positive associations with ALT are also supported by the recent meta-analysis of 25 studies in adolescents and adults (Costello et al., 2022). However, in several studies, the associations were not large in magnitude.

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), the only general population study that performed multi-pollutant modeling. Among the studies of ALT in adults, two presented correlations across PFAS (Nian et al., 2019; Salihovic et al., 2018); PFOA and PFOS were moderately correlated in both studies (r = 0.4–0.5). Jin et al. (2020), 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 entirely explain the observed associations.

Evidence for other liver enzymes and in children and adolescents is less consistent. Results for functional measures of liver toxicity from epidemiological studies, 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. Associations for functional hepatic outcomes such as liver disease were also less consistent than the associations between PFOS and ALT.

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 (Hall et al., 2012; EMEA, 2010; FDA, 2009; U.S. EPA, 2002a). 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, 2002a).

Multiple studies in mice and rats report increases in relative liver weights accompanied by statistically significant increases in serum enzymes, though the increases in serum enzymes were generally under twofold (100% change relative to control) as compared with controls (NTP, 2019; Han et al., 2018b; Xing et al., 2016; Yan et al., 2014; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003). However, across the animal toxicological database, 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 et al., 2012) 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 (Han et al., 2018b; Xing et al., 2016; Cui et al., 2009). A 28-day study in male and female rats also reported

dose-dependent increases in hepatocellular hypertrophy and cytoplasmic alterations (NTP, 2019). A second short-term study in rats (Curran et al., 2008) 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).

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 receptor 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 were 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 3-6). 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 3-6. Evidence Profile Table for PFOS Exposure and 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 12 *Medium* confidence studies 3 *Low* confidence studies	In adults, significant increases in ALT were observed in *medium* confidence studies (6/8). 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 occupational populations and children were mixed. However, significant increases in ALT were observed in one occupational study in men (1/2), and significant increases in AST and GGT were observed in female children (1/3).	• *Medium* confidence studies that reported an effect • *Consistent direction* of effect for ALT • *Coherence* of findings across biomarkers	• *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 histological changes in children, such as non-alcoholic steatosis. Minor uncertainties remain regarding mixed liver enzyme findings in children and limited availability of high-quality studies on liver disease.	*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 associations between PFOS exposure and other serum biomarkers of hepatic injury 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 support for the biological plausibility of the phenotypic effects observed
Liver disease or injury 3 *Medium* confidence studies 2 *Low* confidence studies	Findings for markers of liver inflammation were mixed in *medium* confidence studies (1/2). In adults, one study reported nonsignificant decreased odds of lobular inflammation (1/1). The only study in children reported significantly	• *Medium* confidence studies	• *Low* confidence studies • *Limited number* of studies examining the outcome • *Imprecision* of findings		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors That Increase Certainty**	**Factors That Decrease Certainty**	**Evidence Stream Judgment**	
	increased odds of non-alcoholic steatosis while associations with other histological markers of liver injury were generally positive but less precise. Both *low* confidence occupational studies reported nonsignificant increases in liver disease (2/2), but findings were generally imprecise.				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 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 status. No studies were conducted in children.	• *Medium* confidence studies that reported an effect • *Consistent direction* of effect for albumin	• *Low* confidence study • *Limited number* of studies examining the outcome		
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 Studies (Section 3.4.1.2)					
Liver histopathology 2 *High* confidence studies 5 *Medium* confidence studies	Histopathological alterations in the liver were reported in rodents or non-human primates exposed to PFOS for varying durations (6/7). Hepatocellular hypertrophy was most	• *High* and *medium* confidence studies • *Consistent* direction of effects across study design, sex, and species • *Dose-dependent* response	• No factors noted	⊕⊕⊕ *Robust* Evidence is based on 20 *high* or *medium* confidence animal toxicological studies indicating increased	

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**	
	consistently observed across sex, species, and duration of exposure and in a dose-responsive manner (5/7). 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).	• *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 groups (i.e., hypertrophy) or are considered severe (i.e., cell or necrosis and cystic degeneration)		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 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	
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	• *High* and *medium* confidence studies • *Consistent* direction of effects across study design, sex, and species	• *Limited number of studies* examining specific endpoints • *Inconsistent* direction of effects between sexes		

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**	
	exposure increased enzyme levels. Several studies found increased 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		tissue was an adverse, and not adaptive, response.	

Mechanistic Evidence and Supplemental Information (Section 3.4.1.3)		
Biological Events or Pathways	**Summary of Key Findings, Interpretation, and Limitations**	**Evidence Stream Judgment**
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:** • Conflicting results have been reported for activation of the PPARα signaling pathway in vitro between human and rodent cells.	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 pathways in the MOA for noncancer liver effects of PFOS, particularly CAR activation and decreased expression of HNF4α.

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**	
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 47 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, 29 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, one was classified as *high* confidence, nine as *medium* confidence, one as *low* confidence, and two 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).

There are 10 studies (11 publications[8]) from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and immune effects. Study quality evaluations for these 11 studies are shown in Figure 3-16. Results from studies summarized in the 2016 PFOS HESD are described in Table 3-7 and below.

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 (Granum et al., 2013; Grandjean et al., 2012). In one study of adults, no association was observed (Looker et al., 2014). Antibody responses for diphtheria and tetanus in children (n = 587) were examined at multiple timepoints in a study on a Faroese birth cohort (Grandjean et al., 2012). 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. Another study on Faroese children conducted a pilot investigation on the association between elevated PFOS exposure and autoantibodies to antigens indicating tissue damage, but the results were unclear (Osuna et al., 2014). Prenatal PFOS exposure was associated with diminished vaccine response in a different birth cohort study (Granum et al., 2013). Decreases in the anti-rubella antibody response were significantly associated with elevated prenatal PFOS concentrations among 3-year-old children. Stein et al. (Stein et al., 2016b) reported significant inverse associations between PFOS exposure and mumps and rubella

[8] Okada, 2012, 1332477 reports overlapping eczema results with Okada, 2014, 2850407.

antibody concentrations in seropositive adolescents (12–19 years old) from multiple NHANES cycles (1999–2000, 2003–2004), but no association was observed for measles. No association was observed for the only study (Looker et al., 2014) in adults, examining influenza vaccine responses in a high-exposure community (C8 Health Project).

Evidence based on studies of infectious disease in children from the 2016 PFOS HESD was limited. In the Danish National Birth Cohort (DNBC) study, Fei et al. (2010b) reported nonsignificant increases in risk of hospitalizations for infectious diseases in children 4 years and older, but no association was observed at younger ages. In sex-stratified analyses the risk for hospitalization for infectious disease was significantly increased in girls (IRR = 1.18, 95% CI: 1.03, 1.36), while findings for boys were null. No association was observed for gastroenteritis or common cold in children from the Norwegian Mother and Child Cohort study (MoBa) (Granum et al., 2013).

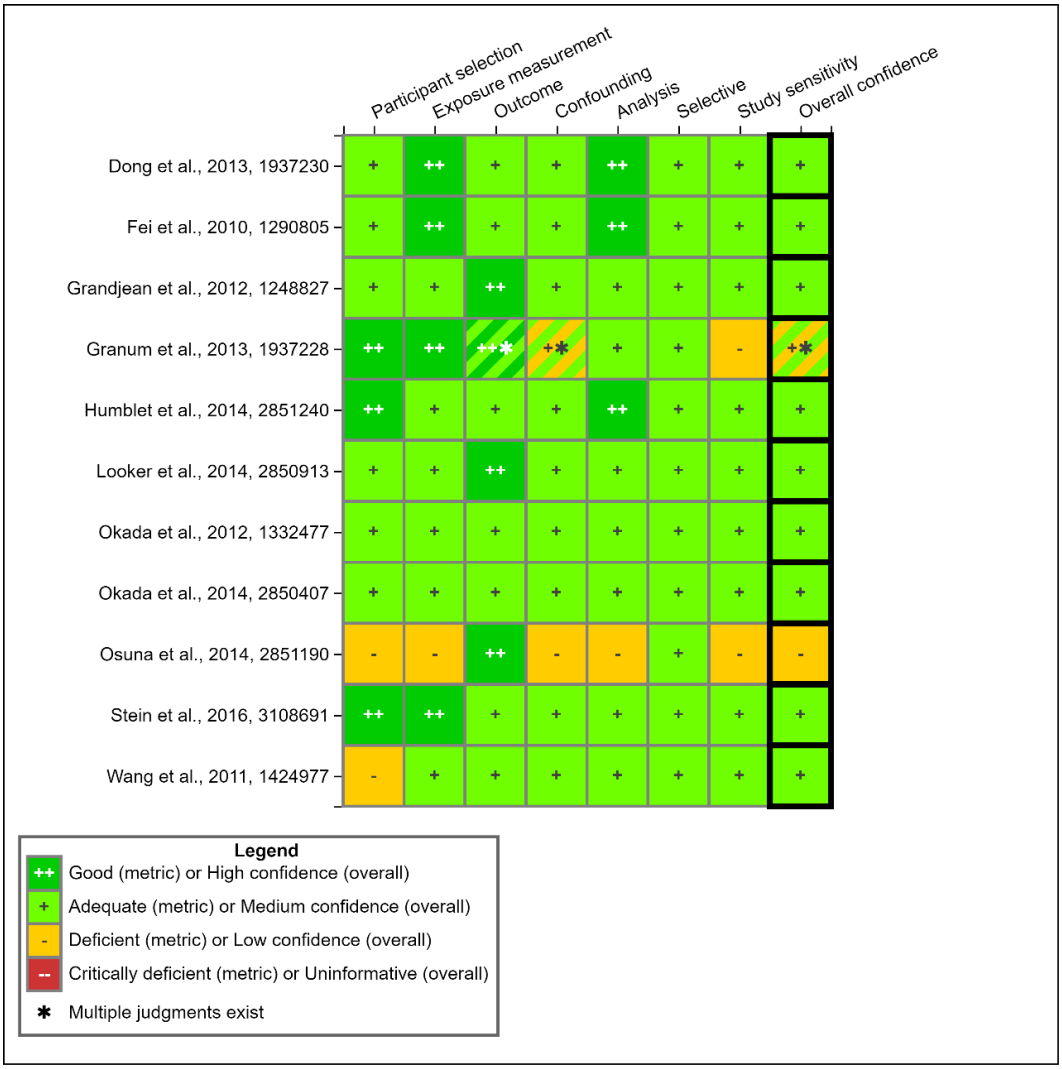


Figure 3-16. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Immune Effects Published Before 2016 (References in 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

Table 3-7. Associations Between Elevated Exposure to PFOS and Immune Outcomes From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Population	Tetanus Ab[a]	Diphtheria Ab[a]	Rubella Ab[a]	Influenza Ab[a]	Infectious Disease[b]	Asthma[b]	Eczema[b]	Autoimmune Disease[b]
Dong, 2013, 1937230 *Medium*	Case-control	Children	NA	NA	NA	NA	NA	↑↑	NA	NA
Fei, 2010, 1290805 *Medium*	Cohort	Children	NA	NA	NA	NA	↑	NA	NA	NA
Grandjean, 2012, 1248827 *Medium*	Cohort	Children	↓↓	↓↓	NA	NA	NA	NA	NA	NA
Granum, 2013, 1937228 *Mixed*	Cohort	Children	–	NA	↓↓	NA	–	NA	NA	NA
Humblet, 2014, 2851240 *Medium*	Cross-sectional	Adolescents	NA	NA	NA	NA	NA	–	NA	NA
Looker, 2014, 2850913 *Medium*	Cohort	Children	NA	NA	NA	–	NA	NA	NA	NA
Stein, 2016, 3108691 *Medium*	Cross-sectional	Children	NA	NA	↓↓	NA	NA	↑	NA	NA
Okada, 2014, 2850407 *Medium*	Cohort	Children	NA	NA	NA	NA	NA	NA	–	NA
Wang, 2011, 1424977 *Medium*	Cohort	Children	NA	NA	NA	NA	NA	NA	↑	NA

Notes: Ab = antibody; NA = no analysis was for this outcome was performed; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Osuna, 2014, 2851190 analyzed autoantibody response to indicators of tissue damage and was not included in the table.

Okada, 2012, 1332477 reports overlapping eczema results with Okada, 2014, 2850407, which was considered the most updated data.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

Granum, 2013, 1937228 was rated *medium* confidence for antibody response, common cold, and gastroenteritis, and *low* confidence for all other outcomes.

There are 28 new studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) 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 et al., 2012) was updated during this period, and the update was included in the systematic review (Grandjean et al., 2017a).

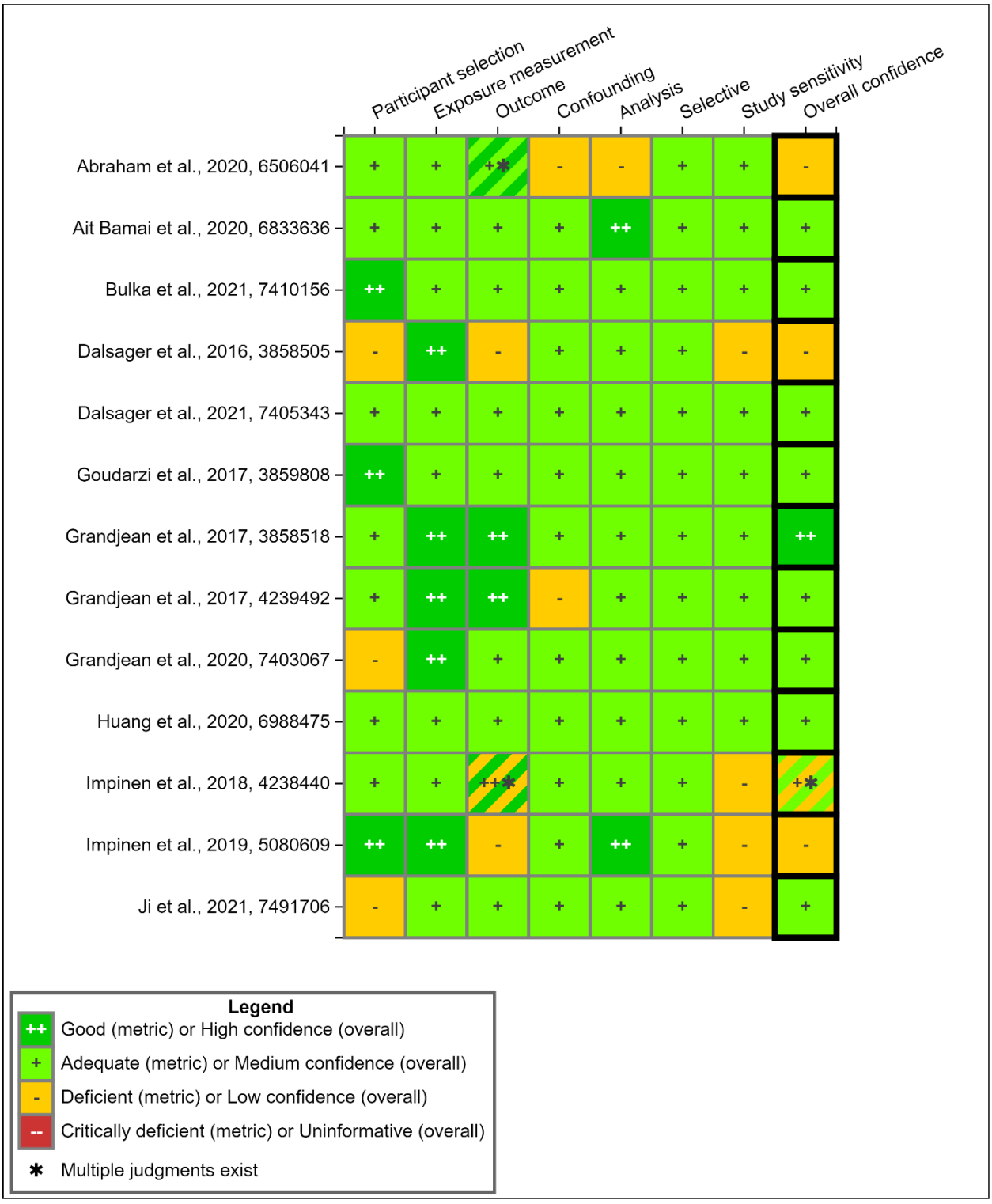


Figure 3-17. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Immunosuppression Effects

Interactive figure and additional study details available on HAWC.

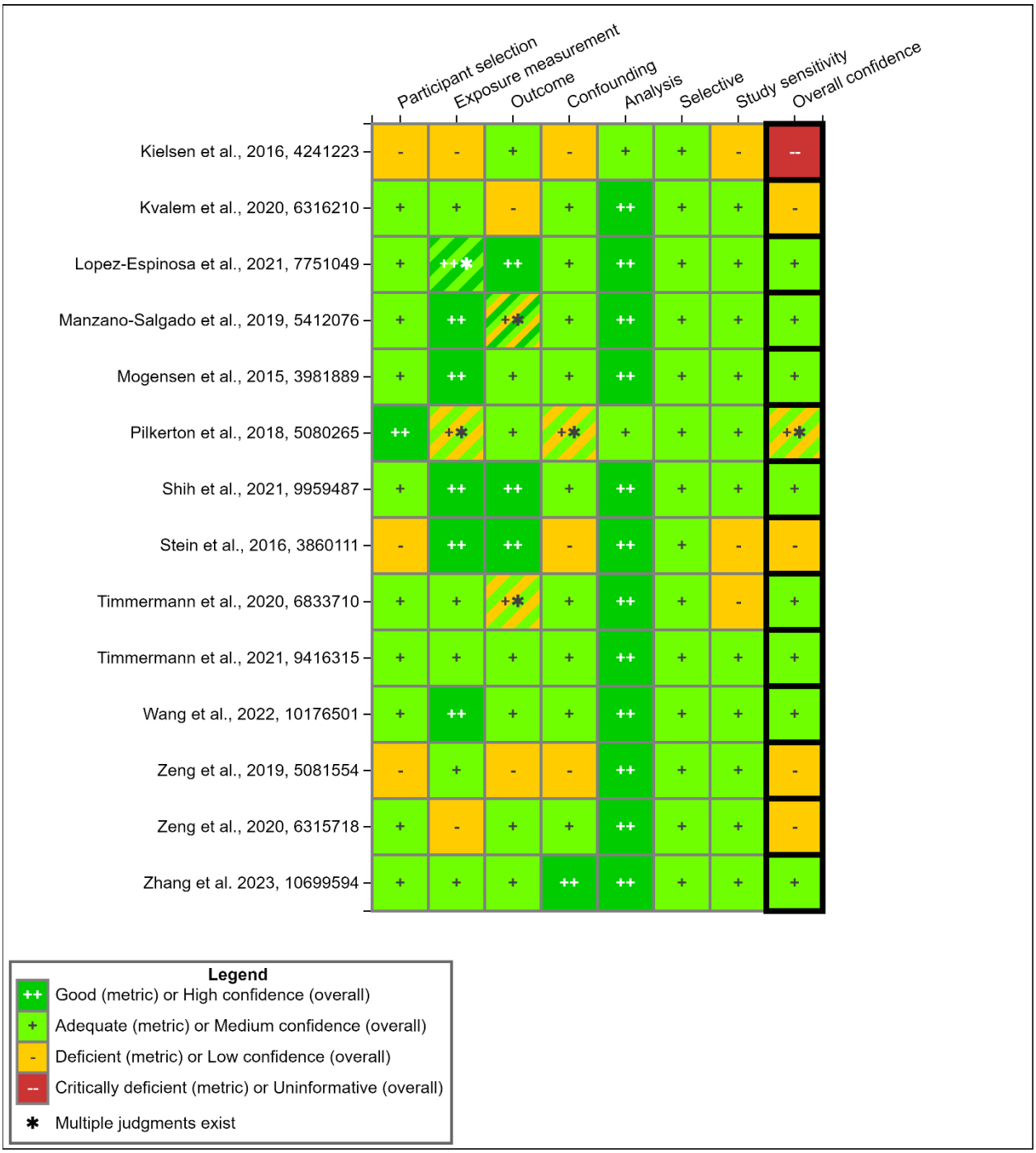


Figure 3-18. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Immunosuppression Effects (Continued)

Interactive figure and additional study details available on HAWC.

3.4.2.1.1.1 Vaccine Response

Thirteen studies (14 publications[9,10]) studied the relationship between antibody response to vaccination and PFOS exposure. Six of these studies investigated antibody response to vaccination in children (Zhang et al., 2023; Timmermann et al., 2021; Abraham et al., 2020; Timmermann et al., 2020; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a). In adults, two studies investigated antibody response to diphtheria and tetanus (Shih et al., 2021; Kielsen et al., 2016), one study investigated hepatitis A and B vaccine response (Shih et al., 2021), one study investigated adult flu vaccine response (Stein et al., 2016a), one study measured rubella antibodies in both adolescents (aged 12 and older) and adults (Pilkerton et al., 2018), and one study measured rubella, measles, and mumps antibodies in adolescents (Zhang et al., 2023). In addition, one study (Zeng et al., 2019b) measured natural antibody exposure to hand, foot, and mouth disease (HFMD), and one study (Zeng et al., 2020) measured hepatitis B antibodies in adults. Overall, one study was *high* confidence (Grandjean et al., 2017a), six studies were *medium* confidence (Zhang et al., 2023; Shih et al., 2021; Timmermann et al., 2021; Timmermann et al., 2020; Grandjean et al., 2017b; Mogensen et al., 2015a), four were *low* confidence (Abraham et al., 2020; Zeng et al., 2020; Zeng et al., 2019b; Stein et al., 2016a), one was *mixed* (*medium/low* confidence) (Pilkerton et al., 2018), and one was *uninformative* (Kielsen et al., 2016).

Of the studies that measured antibody response to vaccination in children and adolescents, four studies were cohorts (Timmermann et al., 2020; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a), and four were cross-sectional (Zhang et al., 2023; Timmermann et al., 2021; Abraham et al., 2020; Pilkerton et al., 2018) (maternal serum was available for a subset of participants in Timmermann et al. (2021)). These included multiple prospective birth cohorts in the Faroe Islands, one with enrollment in 1997–2000 and subsequent follow-up to age 13 (Grandjean et al., 2017a) and one with enrollment in 2007–2009 and follow-up to age 5 (Grandjean et al., 2017b) (one additional cohort in the Faroe Islands examined outcomes in adults with enrollment in 1986–1987 and follow-up to age 28 (Shih et al., 2021)). Five of these studies measured antibody response to tetanus vaccination (Timmermann et al., 2021; Abraham et al., 2020; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a); the same studies also measured antibody response to diphtheria vaccination. In addition, two studies measured antibody response to measles vaccination (Zhang et al., 2023; Timmermann et al., 2020), two studies measured antibody response to rubella vaccination (Zhang et al., 2023; Pilkerton et al., 2018), one study measured antibody response to mumps vaccination (Zhang et al., 2023), and one study to *Haemophilus influenzae* type b (Hib) vaccination (Abraham et al., 2020).

The results for this set of studies in children are shown in Table 3-8 and Appendix D (U.S. EPA, 2024a). The Faroe Islands studies (Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a) 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

[9] Multiple publications of the same study: the study populations are the same in Grandjean et al. (2017a) and Mogensen et al. (2015a).
[10] Zhang (2023) analyzes NHANES cycles 2003–2004 and 2009–2010 partially overlapping with Pilkerton (2018) and Stein (2016b) which both analyze cycles 1999–2000 and 2003–2004.

13 years (Table 3-8). 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. Results from all *medium* and *high* confidence studies on tetanus and diphtheria antibody response in children are provided in Figure 3-19 and Figure 3-20.

Reference, Confidence Rating	Exposure Levels	Comparison	Sub-population	Exposure Age	Outcome Age	EE
Grandjean et al. (2017a, 3858518), High	PFOS at 7 years median (25th-75th percentile)=15.3 ng/mL (12.4-19.0 ng/mL)	percent change (per doubling of PFOS)	--	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)	--	Age 13	Age 13	22.2
Grandjean et al. (2012, 1248827), Medium	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)	--	Age 5	Age 7	-23.8
			Adj for Age 5 Ab	Age 5	Age 7	-11.4
			Pre-booster	Age 5	Age 5	-11.9
			Post-booster	Age 5	Age 5	-28.5
	Maternal PFOS: Geometric mean=27.3 ng/mL (25th-75th percentile=23.2-33.1 ng/mL)	Percent difference (per doubling in maternal PFOS)	--	Prenatal	Age 7	35.3
			Adj for Age 5 Ab	Prenatal	Age 7	33.1
					Age 5	-2.3
			Pre-booster	Prenatal	Age 5	-10.1
Grandjean et al. (2017b, 4239492), Medium	--	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, 3981889), Medium	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, 9416315), Medium	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

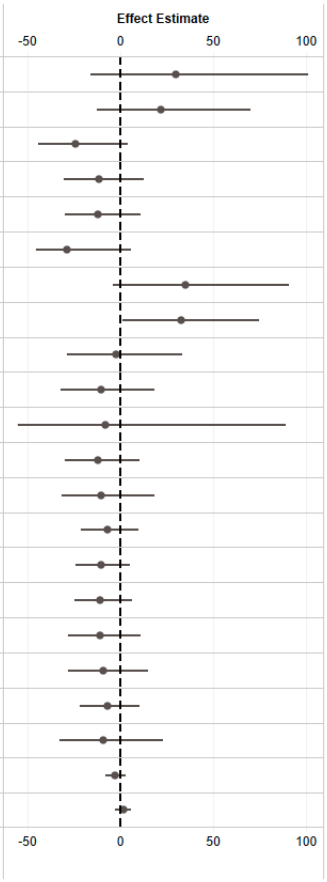
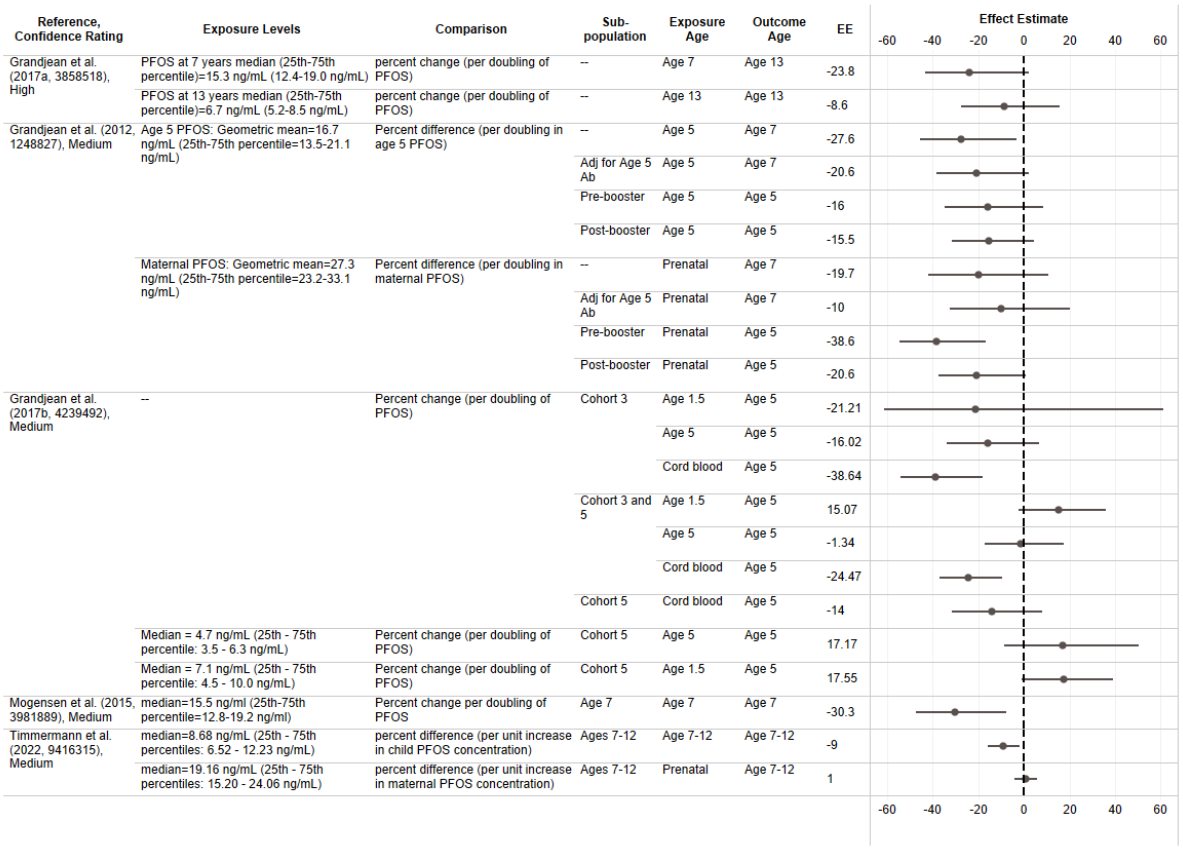


Figure 3-19. Overall Tetanus Antibody Levels in Children from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
Grandjean et al. (2012) was reviewed as a part of the 2016 PFOS HESD.



Reference, Confidence Rating	Exposure Levels	Comparison	Sub-population	Exposure Age	Outcome Age	EE
Grandjean et al. (2017a, 3858518), High	PFOS at 7 years median (25th-75th percentile)=15.3 ng/mL (12.4-19.0 ng/mL)	percent change (per doubling of PFOS)	--	Age 7	Age 13	-23.8
	PFOS at 13 years median (25th-75th percentile)=6.7 ng/mL (5.2-8.5 ng/mL)	percent change (per doubling of PFOS)	--	Age 13	Age 13	-8.6
Grandjean et al. (2012, 1248827), Medium	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)	--	Age 5	Age 7	-27.6
			Adj for Age 5 Ab	Age 5	Age 7	-20.6
			Pre-booster	Age 5	Age 5	-16
			Post-booster	Age 5	Age 5	-15.5
	Maternal PFOS: Geometric mean=27.3 ng/mL (25th-75th percentile=23.2-33.1 ng/mL)	Percent difference (per doubling in maternal PFOS)	--	Prenatal	Age 7	-19.7
			Adj for Age 5 Ab	Prenatal	Age 7	-10
			Pre-booster	Prenatal	Age 5	-38.6
			Post-booster	Prenatal	Age 5	-20.6
Grandjean et al. (2017b, 4239492), Medium	--	Percent change (per doubling of PFOS)	Cohort 3	Age 1.5	Age 5	-21.21
				Age 5	Age 5	-16.02
				Cord blood	Age 5	-38.64
			Cohort 3 and 5	Age 1.5	Age 5	15.07
				Age 5	Age 5	-1.34
				Cord blood	Age 5	-24.47
			Cohort 5	Cord blood	Age 5	-14
	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	17.17
	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	17.55
Mogensen et al. (2015, 3981889), Medium	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	-30.3
Timmermann et al. (2022, 9416315), Medium	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	-9
	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	1

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

Interactive figure and additional study details available on HAWC.
Grandjean et al. (2012) was reviewed as a part of the 2016 PFOS HESD.

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-Jørgensen and Grandjean, 2018) where PFOS 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 does not show that confounding across PFAS is likely to completely explain the observed effects.

Table 3-8. 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, logPFOS)[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, logPFOS)[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, logPFOS)[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, logPFOS)[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 (25th–75th percentile) unless otherwise noted.
[b] Grandjean et al. (2012); *medium* confidence.
[c] Budtz-Jørgensen and Grandjean (2018); *medium* confidence.
[d] Grandjean et al. (2017b); *medium* confidence.
[e] Grandjean and Budtz-Jørgensen (2013); *medium* confidence.
[f] Mogensen et al. (2015a); *medium* confidence.
[g] Grandjean et al. (2017a); *medium* confidence.

The cross-sectional study of these antibodies in Greenlandic children (Timmermann et al., 2021) 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) 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).

Two *medium* cross-sectional studies of adolescents examined associations between elevated levels of PFOS and vaccine response (Zhang et al., 2023; Pilkerton et al., 2018). Inverse associations were observed in cross-sectional analyses in adolescents from NHANES (2003–2004; 2009–2010) for rubella, mumps, and measles (Zhang et al., 2023), including a significant reduction in the antibody response to rubella per 2.7-fold increase in serum PFOS. No association was observed for rubella vaccine response in the other cross-sectional study of adolescents (Pilkerton et al., 2018), however, an overlapping study (Stein et al., 2016b) on adolescents from the same NHANES cycles (i.e., 1999–2000 and 2003–2004) reported a significant inverse association for rubella antibody response in seropositive adolescents.

Lastly, the *low* confidence cross-sectional study at age one, Abraham et al. (2020), 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, two were cohorts (Shih et al., 2021; Stein et al., 2016a), and one was a cross-sectional analysis (Pilkerton et al., 2018). Shih et al. (2021) 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. (2016a) utilized a convenience sampling to recruit participants, had low seroconversion rates, and was at high risk of residual confounding, so was *low* confidence. The study of the adult population in Pilkerton et al. (2018) was considered *low* confidence as the analysis suffered from potential exposure misclassification due to concurrent exposure and outcome measurements, considering the amount of time since rubella vaccination in childhood. This was less of a concern for the study of adolescent participants, which was rated as *medium* confidence for adolescence antibody response to vaccinations. Shih et al. (2021) 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 et al., 2019b) and hepatitis B infection (Zeng et al., 2020). This birth cohort in China (Zeng et al., 2019b) measured antibody levels in infants at birth

and age 3 months, which represent passive immunity from maternal antibodies. This study (Zeng et al., 2019b) 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 protective 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) 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) 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 for 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, 10 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 (Wang et al., 2022; Dalsager et al., 2021; Ait Bamai et al., 2020; Huang et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Manzano-Salgado et al., 2019; Goudarzi et al., 2017; Dalsager et al., 2016), one was a case-control study nested in a cohort (Impinen et al., 2018), and one was a cross-sectional study (Abraham et al., 2020). Five studies measured PFOS concentrations from mothers during pregnancy (Ait Bamai et al., 2020; Impinen et al., 2019; Manzano-Salgado et al., 2019; Goudarzi et al., 2017; Dalsager et al., 2016). Impinen et al. (2018) measured PFOS concentrations from cord blood at delivery. Two studies measured PFOS concentrations in children's serum at age 1 year (Abraham et al., 2020) and at age 10 years (Kvalem et al., 2020).

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

[11] Multiple publications of the same study: both Dalsager et al. (2016) and Dalsager et al. (2021) 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.

al., 2020; Manzano-Salgado et al., 2019; Goudarzi et al., 2017) and four were *low* confidence (Kvalem et al., 2020; Impinen et al., 2019; Impinen et al., 2018; Dalsager et al., 2016).

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 Appendix D (U.S. EPA, 2024a). The most commonly examined type of infections was respiratory, including pneumonia/bronchitis, upper and lower respiratory tract, throat infections, and common colds. Dalsager et al. (2021), 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 et al., 2019) and 7-year-old children (Ait Bamai et al., 2020); however, two other *medium* confidence studies did not report an increase in infections (Wang et al., 2022; Abraham et al., 2020). Huang et al. (2020) 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 (Dalsager et al., 2021; Kvalem et al., 2020; Impinen et al., 2018), while another *medium* confidence study reported no association (Manzano-Salgado et al., 2019). There were also non-statistically significant positive associations seen for PFOS in relation to chickenpox (Ait Bamai et al., 2020), common cold (Wang et al., 2022), and cough (Dalsager et al., 2016), but statistically significant inverse associations were observed for RSV (Ait Bamai et al., 2020) and common cold (Impinen et al., 2018). Outside of respiratory infections, two *medium* confidence studies examined total infectious diseases. Dalsager et al. (2021) reported higher rates of hospitalization for any infections with higher PFOS exposure (not statistically significant), while (Goudarzi et al., 2017) 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, (Bulka et al., 2021; Ji et al., 2021; Grandjean et al., 2020). Results from these studies are available in Appendix D (U.S. EPA, 2024a). All three studies were *medium* confidence. Ji et al. (2021) 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) reported no association between PFOS exposure and increased COVID-19 severity. Bulka et al. (2021) used NHANES data from 1999 to 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). 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 et al., 2013) 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 et al., 2014). No association was observed for eczema in a Hokkaido birth cohort study (Okada et al., 2014).

There are 23 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) 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 3-21.

Figure 3-21. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Immune Hypersensitivity Effects

Interactive figure and additional study details available on HAWC.

Thirteen studies (15 publications)[12] examined asthma (or asthma symptoms) and PFOS exposure. Ten of these studies were cohorts (Kvalem et al., 2020; Averina et al., 2019; Beck et al., 2019; Gaylord et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Workman et al., 2019; Zeng et al., 2019a; Timmermann et al., 2017; Smit et al., 2015); three studies (five publications) were case-control investigations (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016), including one nested case-control, (Impinen et al., 2018); and one was a cross-sectional analysis (Jackson-Browne et al., 2020). Seven studies measured the prevalence of "current" asthma for at least one time point (Kvalem et al., 2020; Averina et al., 2019; Beck et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Zeng et al., 2019a; Impinen et al., 2018). Eight studies measured "ever" asthma for at least one time point (Jackson-Browne et al., 2020; Averina et al., 2019; Gaylord et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Impinen et al., 2018; Timmermann et al., 2017; Smit et al., 2015). Incident or recurrent wheeze was examined in one study (Workman et al., 2019). Overall, nine studies were rated *medium* confidence, and six studies were *low* confidence for asthma (Figure 3-21). Timmermann et al. (2017) was *low* confidence for asthma because the questionnaire used to ascertain status was not validated. Averina et al. (2019) was considered *low* confidence because results were not provided quantitatively. Studies from the Genetic and Biomarkers study for Childhood Asthma (GBCA) (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016) 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 Appendix D, (U.S. EPA, 2024a)). Several studies observed positive associations with ORs greater than 1.2 between PFOS concentration levels and increased "current" or "ever" asthma (Jackson-Browne et al., 2020; Averina et al., 2019; Beck et al., 2019; Zeng et al., 2019a; Impinen et al., 2018; Timmermann et al., 2017), but often only within population subgroups. Averina et al. (2019) 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) 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) 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. (2019a) observed a positive association in boys and an inverse association in girls (both $p > 0.05$). Impinen et al. (2018) reported higher odds of ever asthma. The *low* confidence study, Timmermann et al. (2017), observed positive associations ($p > 0.05$) between increased asthma odds and elevated PFOS concentrations in small subset of children aged 5 and 13 who did not receive their measles, mumps, and rubella (MMR) vaccination before age 5. However, in children of the same ages who had received their MMR vaccination before age 5, no association was observed. *Low* confidence studies from the GBCA study (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016) observed elevated PFOS levels ($p = 0.002$) in children with asthma compared with those without (Zhou et al., 2017b), and the odds of current asthma was

[12] Three publications (Zhou et al., 2017c; Zhou et al., 2017b; Zhu et al., 2016) reported on the same cohort (Genetic and Biomarker study for Childhood Asthma) and outcome and are considered one study.

also found to be elevated among boys and girls with increasing PFOS exposure (Zhu et al., 2016). One other study (Impinen et al., 2019) observed a small positive association (OR: 1.1) with current asthma in boys only. Two studies reported nonsignificant inverse associations with asthma (Manzano-Salgado et al., 2019; Smit et al., 2015), and in one study, all results were nonsignificant (Gaylord et al., 2019). One *low* confidence study did not observe a significant effect for recurrent wheeze (Workman et al., 2019).

In addition to the studies of asthma in children, one *medium* confidence study (Xu et al., 2020a) 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 versus 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 (Ait Bamai et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Timmermann et al., 2017; Goudarzi et al., 2016), one study was a case-control analysis (Impinen et al., 2018), and one study was a cross-sectional study using data from NHANES 2005–2006 and 2007–2010 (Buser and Scinicariello, 2016). All studies were considered *medium* confidence for allergy outcomes. Results for these outcomes are presented in Appendix D (U.S. EPA, 2024a).

Three studies conducted skin prick tests on participants to determine allergy sensitization at age 10 years (Kvalem et al., 2020; Impinen et al., 2018), at age 13 years (Timmermann et al., 2017), and at age 16 years (Kvalem et al., 2020). 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) 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) 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) reported an inverse association ($p > 0.05$). Five studies measured symptoms of "current" or "ever" allergic rhinitis or rhinoconjunctivitis (Ait Bamai et al., 2020; Kvalem et al., 2020; Impinen et al., 2018; Timmermann et al., 2017; Goudarzi et al., 2016), and one study measured symptoms at 16 years old (Kvalem et al., 2020). 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) 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) 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 (Manzano-Salgado et al., 2019; Wen et al., 2019a; Wen et al., 2019b; Chen et al., 2018; Timmermann et al., 2017; Goudarzi et al., 2016), and one was a case-control analysis (Impinen et al., 2018). Four studies

measured PFOS concentrations in cord blood at delivery (Wen et al., 2019a; Wen et al., 2019b; Chen et al., 2018; Impinen et al., 2018), three studies measured PFOS concentrations in pregnancy (Manzano-Salgado et al., 2019; Timmermann et al., 2017; Goudarzi et al., 2016), and one study measured child blood at age 5 and 13 years (Timmermann et al., 2017). All the studies were considered *medium* confidence for eczema. Results are presented in Appendix D (U.S. EPA, 2024a).

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

One *medium* confidence nested case-control study examined chronic spontaneous urticaria (Shen et al., 2022). 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). 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, 2016b) that investigated the association between PFOS and autoimmune disease effects. Study quality evaluations for these 4 studies are shown in Figure 3-22.

Four case-control studies examined PFOS exposure and autoimmune diseases (Figure 3-22). Two studies examined MS (Ammitzbøll et al., 2019) and ulcerative colitis (Steenland et al., 2018b) in adults, and two studies examined celiac disease in children (Sinisalu et al., 2020) and young adults (Gaylord et al., 2020). PFOS was measured in blood components (i.e., blood, plasma, or serum) for all studies (see Appendix D, (U.S. EPA, 2024a)). One study was *medium* confidence (Gaylord et al., 2020) with minimal deficiencies, and three studies were considered *low* confidence (Sinisalu et al., 2020; Ammitzbøll et al., 2019; Steenland et al., 2018b). Information on participant selection, particularly control selection, was not reported in Ammitzbøll et al. (2019). 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. (2018b) examined exposure concentrations 1 to 2 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.

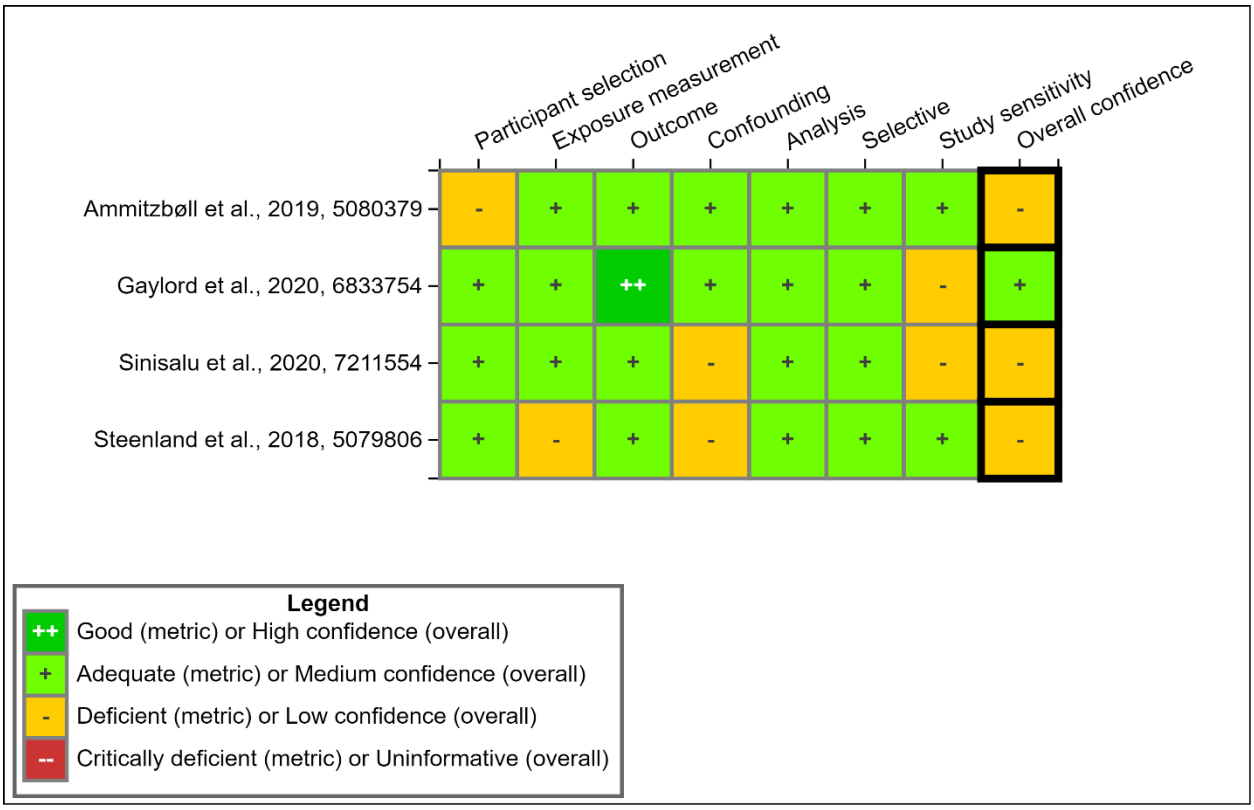


Figure 3-22. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Autoimmune Effects

Interactive figure and additional study details available on HAWC.

Ammitzbøll et al. (2019) observed lower PFOS concentrations among healthy controls compared with those with MS. Serum PFOS concentrations were 17% lower (95% CI: −27%, −6%; p = 0.004) in healthy controls compared with 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 with 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 et al., 2020). 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 with healthy controls (Steenland et al., 2018b).

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 et al., 2020), 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 et al., 2018b) or lower than in MS cases (Ammitzbøll et al., 2019).

3.4.2.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 3 studies from the 2016 PFOS HESD (U.S. EPA, 2016b) 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 3-23.

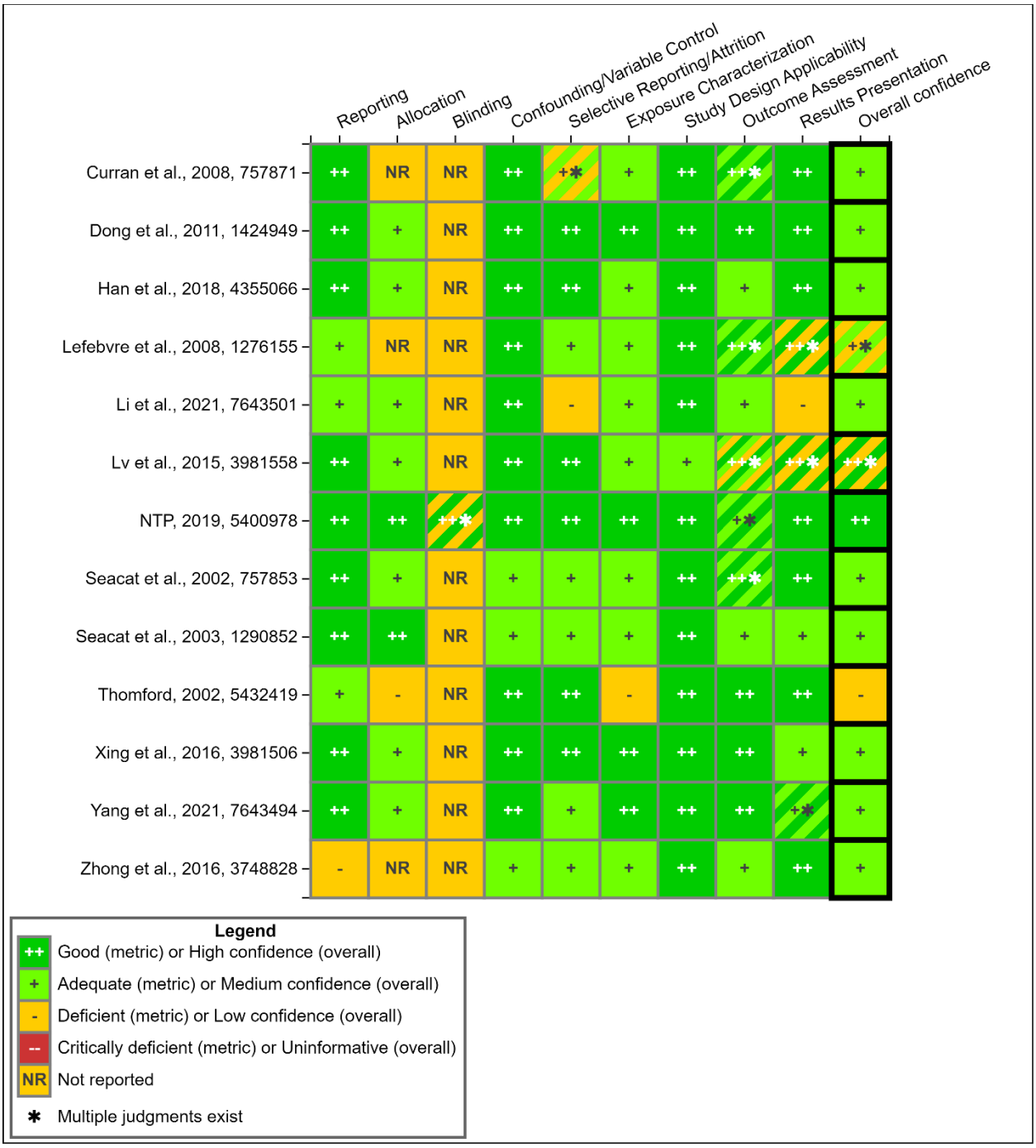


Figure 3-23. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Immune Effects[a]

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

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) 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) 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 et al., 2009), fed chow with 0.001, 0.005, or 0.02% PFOS (equivalent to ~40 mg/kg/day) for 10 days (Qazi et al., 2009b), 0.008–2.083 mg/kg/day PFOS for 60 days (Dong et al., 2009), or administered 0.008–0.833 mg/kg/day PFOS for 60 days via gavage (Dong et al., 2011). 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), 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 et al., 2016).

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 4 weeks. No significant effects were observed in lower dose groups, at the 8-week time point, or in females (Zhong et al., 2016).

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 et al., 2008), male C57BL/6 mice exposed to 0.25 or 2.5 mg/kg/day PFOS for 28 days (Yang et al., 2021), and male C57BL/6 (H-2^b) mice administered 0.005% PFOS in the diet for 10 days (Qazi et al., 2010). Similarly, relative

spleen weight in male BALB/c mice was not affected at the end of a 3-week exposure to 2.5–5 mg/kg/day PFOS (Lv et al., 2015). Although Qazi et al. (2010), 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) and Lefebvre et al. (2008) 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 3-24). Reductions in absolute thymus weight were also observed in females of the highest dose in Lefebvre et al. (2008). 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) (Figure 3-24).

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 et al., 2009), 0.02% PFOS for 10 days in diet (Qazi et al., 2009b), or 0.417–2.083 mg/kg/day PFOS for 60 days (Dong et al., 2009). A follow-up from the latter study (Dong et al., 2009) by Dong et al. (2011) 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) 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 1–17 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 et al., 2016) (Figure 3-24).

In contrast to the several studies that reported reductions in thymus weight, Qazi et al. (2010) and Peden-Adams et al. (2008) did not observe any changes in thymus weight. Qazi et al. (2010) exposed male C57BL/6 (H-2^b) mice to 0.005% PFOS in the diet for 10 days, while Peden-Adams et al. (2008) 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) and NTP (2019) 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).

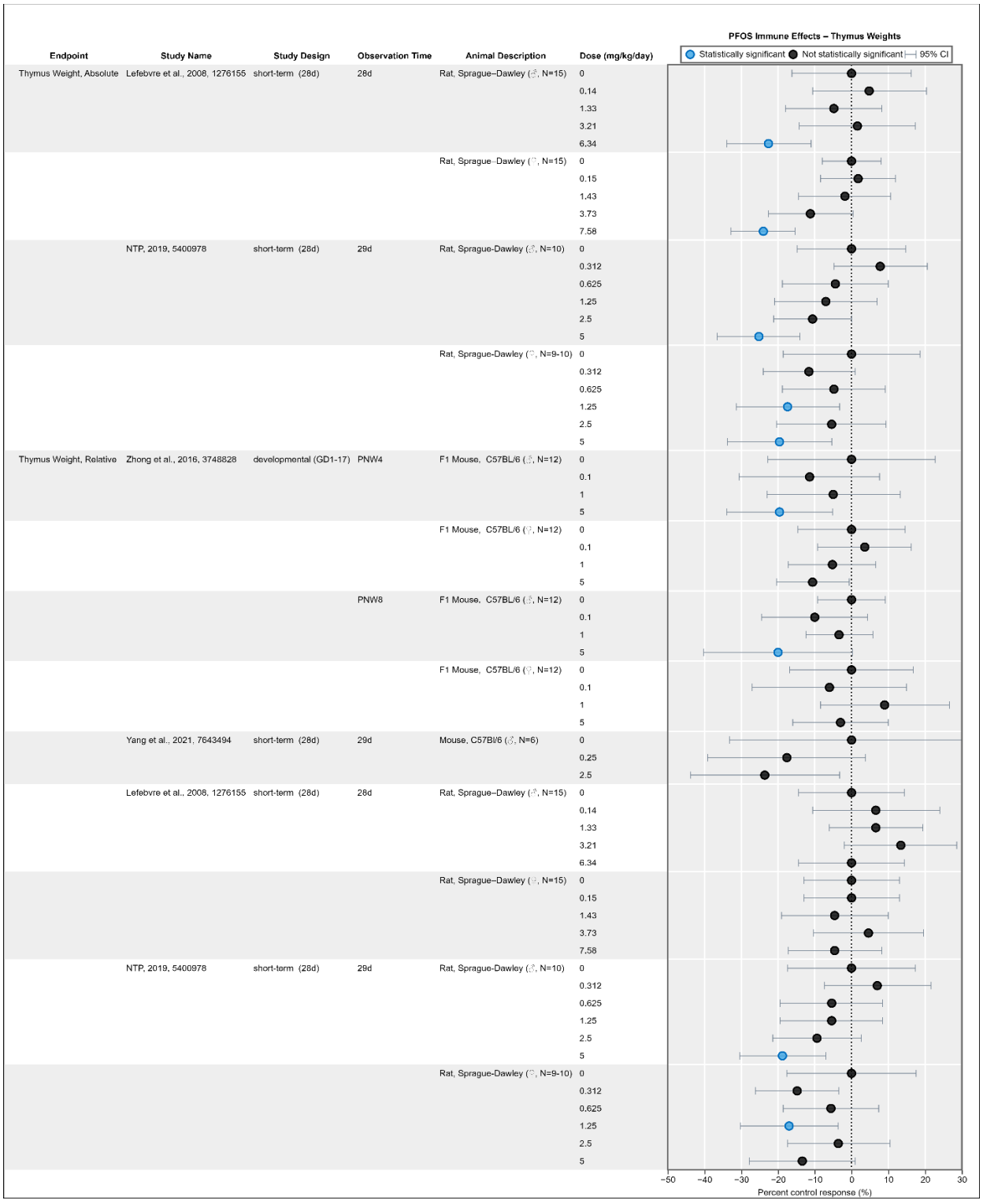


Figure 3-24. Percent Change in Thymus Weights Relative to Controls in Rodents Following Exposure to PFOS

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 3-25). In general, short-term and subchronic studies have observed histopathology such as extramedullary hematopoiesis (NTP, 2019), bone marrow hypocellularity (NTP, 2019), and other aberrations in the immune organs (Lv et al., 2015; Qazi et al., 2009b).

One study included in the 2016 PFOS HESD (U.S. EPA, 2016b) by Qazi et al. (2009b) 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 (NTP, 2019; Qazi et al., 2009b). 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), and this finding was complemented by a chronic non-human primate study by Seacat et al. (2002), 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 nonneoplastic lesions were observed following exposure to 2.5, 5, or 10 mg/kg/day PFOS for 3 weeks, though quantitative results were not reported (Lv et al., 2015). However, the authors (Lv et al., 2015) 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.

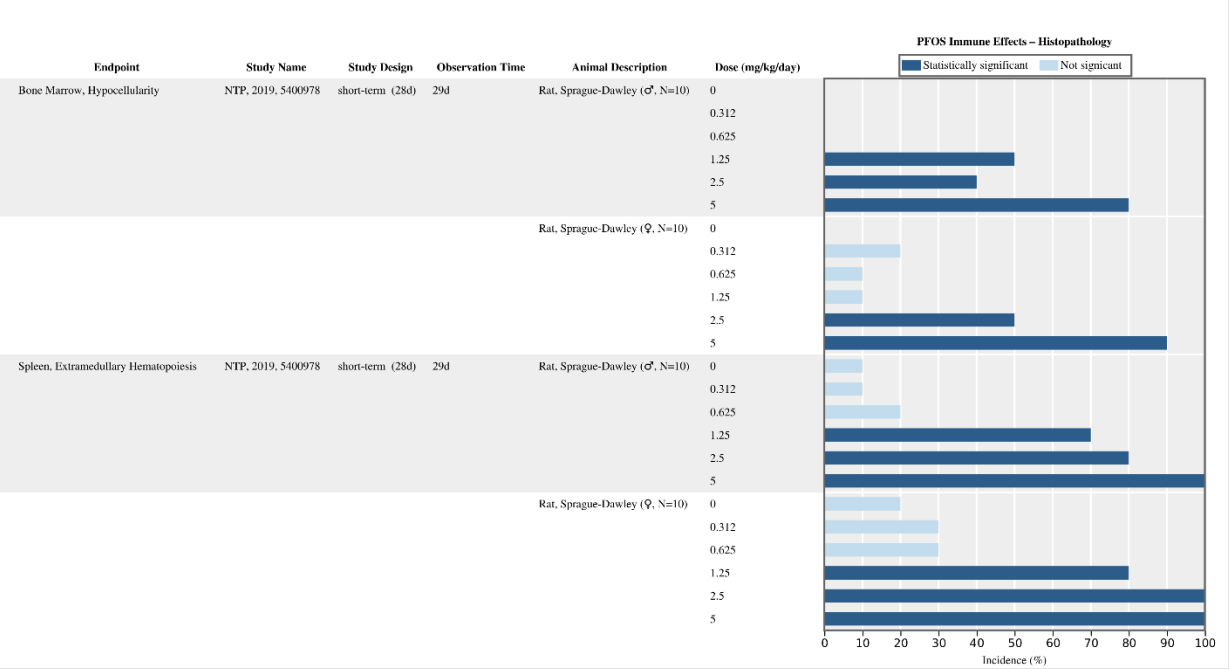


Figure 3-25. Incidences of Immune Cell Histopathology in Rodents Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

Xing et al. (2016) 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 (2021c) 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).

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. (2009a) 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 et al., 2009a). In a study by Seacat et al. (2003), 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 et al., 2003). 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 et al., 2008). 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) 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 et al., 2002) 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 et al., 2002). In contrast, reduced numbers of neutrophils were observed in male rats, but not females, in an NTP (2019) study. In that report, NTP also reported that male rats, and not females, exhibited significantly reduced WBC counts (NTP, 2019).

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 et al., 2008). 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 et al., 2009). 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 et al., 2009). Similarly, Zhong et al. (2016) 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) 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 (Zhong et al., 2016; Keil et al., 2008) 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 et al., 2008). 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 3-9).

Table 3-9. Associations Between PFOS Exposure and Natural Killer Cell Activity in Mice

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
Peden-Adams et al. (2008)	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)	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)	7 days	0, 5, 20, 40	M	↓ (20–40 mg/kg/day)
Zhong et al. (2016)	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)	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 3-26). 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 et al., 2008). 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 et al., 2009b), 20 or 40 mg/kg/day PFOS for 7 days (Zheng et al., 2009), and 0.417–2.083 mg/kg/day for 60 days (Dong et al., 2009). Female mice were not evaluated in these studies.

Altered splenic cellular composition was observed in a study by Lv et al. (2015) where male BALB/c mice were exposed to 0, 2.5, 5, or 10 mg/kg/day PFOS for 3 weeks (Lv et al., 2015), 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 nonsignificant 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 et al., 2015).

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

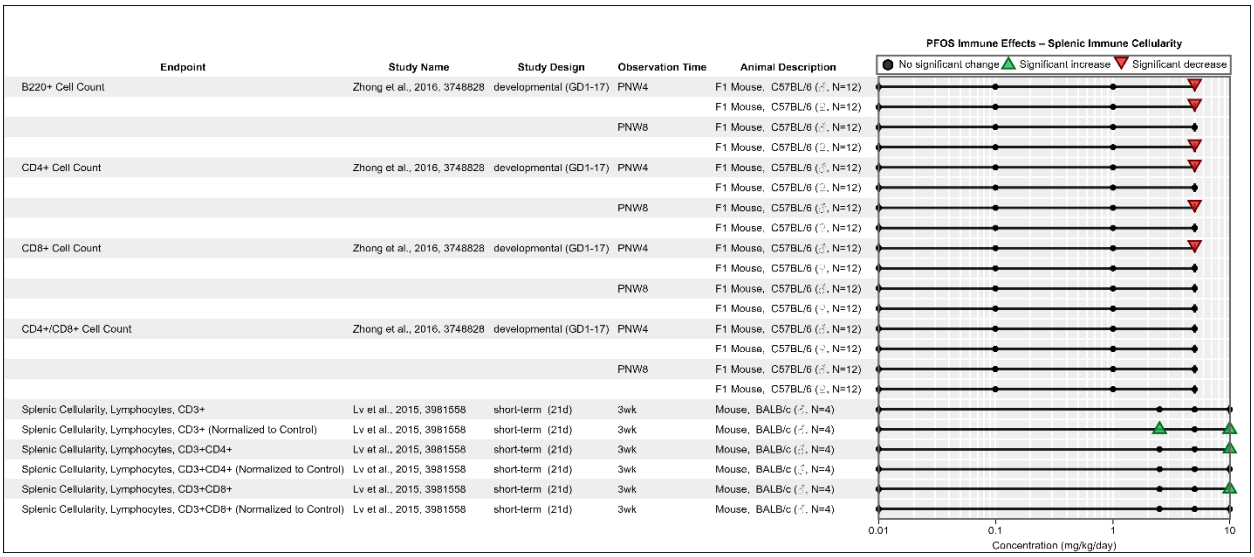


Figure 3-26. Splenocyte Cellularity in Rodents Following Exposure to PFOS (Logarithmic Scale)[a]

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 with 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 3-27). 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 et al., 2008). In contrast, significantly decreased thymocyte populations were observed in male C57BL/6 mice exposed to 0.02% PFOS for 10 days (Qazi et al., 2009b), 20 or 40 mg/kg/day PFOS for 7 days (Zheng et al., 2009), and 0.417–2.083 mg/kg/day for 60 days (Dong et al., 2009). 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 1–17, and immune cell populations were quantified in male and female pups at 4 and 8 weeks after birth. Decreased thymic lymphocyte subpopulations ($CD4^+$, 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 et al., 2016). 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), 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 1–17 (Keil et al., 2008).

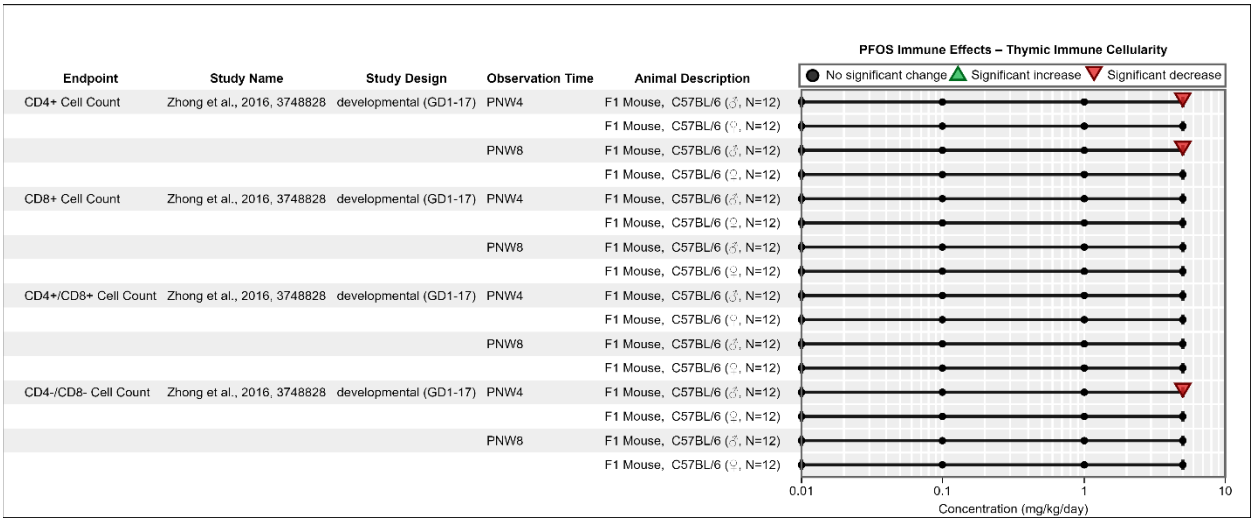


Figure 3-27. Thymocyte Cellularity in Rodents Following Exposure to PFOS (Logarithmic Scale)

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 (Yang et al., 2021; Lee et al., 2018a) 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) 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 et al., 2008). Similarly, Dong et al. (2009) 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 et al., 2011). 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 et al., 2011). 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) in male and female rats sensitized with the T-dependent antigen, keyhole limpet hemocyanin (KLH), during a 28-day exposure to 0.14–7.58 mg/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 with control, and there were no changes in footpad swelling. Zheng et al. (2009) 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 3-10).

Table 3-10. Associations Between PFOS Exposure and Immune Response in Mice

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
Peden-Adams et al. (2008)[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)[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)[a]	60 days	0, 0.008, 0.083, 0.417, 0.833, 2.083	M	↓ 0.083–2.083
Dong et al. (2011)[a]	60 days	0, 0.008, 0.0167, 0.083, 0.417, 0.833	M	↓ 0.083–0.833
Zheng et al. (2009)[a]	7 days	0, 5, 20, 40	M	↓ 5–40 mg/kg/day
Zhong et al. (2016)[a]	GD 1–17 4-week assessment	0, 0.1, 1, 5	M	↓ 1–5 mg/kg/day
			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)[a]	GD 1–17	0, 0.1, 1, 5	M	↓

Reference	Exposure Length	Dose (mg/kg/day)	Sex	Change
	8-week assessment			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) and Zhong et al. (2016), each exposed pregnant female C57BL/6 mice to 0.1–5 mg/kg/day PFOS from GD 1–17 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. Keil et al. (2008) reported that the primary IgM response to SRBC was significantly suppressed by 53% at 8-weeks in males from the 5 mg/kg/day exposure group. In females, the primary IgM response was not altered (Keil et al., 2008). Similarly, Zhong et al. (2016) 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). Two 28-day studies (NTP, 2019; Curran et al., 2008) 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). These findings are complemented by a study by Curran et al. (2008), 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 et al., 2008) 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. (2018a) and Yang et al. (2021) 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). 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 with 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, 2016b). 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 by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-28.

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 3-28. Summary of Mechanistic Studies of PFOS and Immune Effects

Interactive figure and additional study details available on HAWC.

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

Since the 2016 PFOS 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 et al., 2017) and alterations in gene expression related to immune and inflammation responses in

human cord blood (Pennings et al., 2016) 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) 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) 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) 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 et al., 2016), 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) 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) 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 et al., 2019). 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) 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 4 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. (2020c) 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) and Pennings et al. (2016), 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 et al., 2016), glycosylation of immunoglobulins in humans (Liu et al., 2020c), and lymphocyte toxicity in vitro (Zarei et al., 2018). Zhong et al. (2016) 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 et al., 2020c). Altered IgG glycosylation patterns are associated with disease states and immune functions including cancer immunosurveillance and anti-inflammatory reactions (Cobb, 2020). 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) 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 et al., 2018b) or lower than in multiple sclerosis (MS) cases (Ammitzbøll et al., 2019). 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 et al., 2020; Fu et al., 2020). Suo et al. (2017) 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 with 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) 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) 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 nonsignificant 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, 2016a; Corsini et al., 2012; Corsini et al., 2011).

Lee et al. (2018a) 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 upregulation 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 3-9 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 et al., 2009). 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 et al., 2014). 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) 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) 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 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) 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 (Ishigame et al., 2009; Takatori et al., 2009; Zheng et al., 2008). 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 with 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 Appendix, (U.S. EPA, 2024a)) 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) 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). 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, 2016b), 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 et al., 2009a). 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 3-11. Pro-inflammatory cytokines were elevated in PFOS-exposed rodents and in human and animal cells in culture. In both studies evaluating human subjects (Mitro et al., 2020; Bassler et al., 2019), 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 3-11. 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)	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)	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. (2020c)	Human lymphocytes	IL-1	culture supernatant protein (ELISA)	↑
		IL-6	culture supernatant protein (ELISA)	↑
Sørli et al. (2020)	Human bronchial epithelial cell line	IL-1α	culture supernatant protein (ELISA)	↑
		IL-1β	culture supernatant protein (ELISA)	↑
Liao et al. (2013)	Human umbilical vein endothelial cells (HUVECs)	IL-6	cellular mRNA (qRT-PCR)	↑
		IL-1β	cellular mRNA (qRT-PCR)	↑
Han et al. (2018b)	Sprague-Dawley male rats	IL-6	serum protein (ELISA)	↑
		TNF-α	serum protein (ELISA)	↑
Su et al. (2019)	ICR male mice	IL-6	serum protein (ELISA)	↑
		TNF-α	serum protein (ELISA)	↑
Han et al. (2018b)	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)	↑

Study	Species or Cell Type	Cytokine or Inflammatory Marker	Matrix and Measurement	Direction of Change Following PFOS Exposure
Zhu et al. (2015)	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. (2018b) 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) 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 et al., 2020). Li et al. (2020c) 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) 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) 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 et al., 2015) via similar pathways observed in primary rat hepatocytes and Kupffer cells exposed to 100 μM PFOS (Han et al., 2018b). Zhu et al. (2015) 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. (2019b) 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 et al., 2005; Yeung et al., 2004). 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) 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 non-alcoholic fatty liver disease (NAFLD), PFAAs are associated with immunotoxic suppressive effects on innate immunity and inflammation.

Mitro et al. (2020) 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, 2016b), 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, 2016b) linked PFOS-mediated PPARγ activation to decreased spleen and thymus weight and reduced spleen and thymus cellularity (NTP, 2016b; Yang et al., 2002). Recent studies such as Zhong et al. (2016) 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 et al., 2015), and reduced numbers of myeloid, pro/pre-B, immature B, and early mature B cells in bone marrow (Qazi et al., 2012).

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 et al., 2016; Zhu et al., 2016), glycosylation of immunoglobulins in humans (Liu et al., 2020c), and lymphocyte toxicity in vitro (Zarei et al., 2018). Effects of PFOS exposure on allergy (Lee et al., 2018a) included release of histamine and β hexosaminidase associated with upregulation 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 et al., 2017). Additional insights were provided by transcriptomic and lipidomic studies (Li et al., 2020c; Pennings et al., 2016; Lv et al., 2015). 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 et al., 2016). 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 et al., 2015).

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 (Berntsen et al., 2018; Rainieri et al., 2017). 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) 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 with control mice.

Very little information is available regarding whether PFOS impacts intrinsic cellular defenses. One recent study, Sørli et al. (2020), 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 et al., 2018b; Zhu et al., 2015). 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 et al., 2020c). 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, and a change in antibody levels of approximately 11% per 2.7-fold increase of PFOS exposure was observed in adolescents from NHANES. 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 in these populations.

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 subgroups. 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" versus "current" asthma, and no studies beyond the Dong et al. (2013) described in the 2016 PFOS HESD 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 PFOS HESD, 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 3-12). 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. Given 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 lifestages represents a major source of uncertainty in the immunotoxicity database of PFOS.

Table 3-12. Evidence Profile Table for PFOS Exposure and 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 20 *Medium* confidence studies 8 *Low* confidence studies 2 *Mixed*[a] confidence studies	Studies conducted in the Faroe Islands examined antibody levels among children at various timepoints compared with 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. Similarly, antibody levels against rubella (2/2) were significantly decreased in *medium* confidence studies of children. Findings in the four studies examining adults were less consistent than children. Infectious disease was examined in 14 studies of children. Studies examining infections of the respiratory system observed some positive associations (5/12), although many findings from other studies were	• *High* and *medium* confidence studies that reported effects • *Consistent direction* of effect • *Coherence* of findings across antibody response and increased infectious disease	• *Imprecision* of findings	⊕⊕⊙ *Moderate* Evidence for immune effects is based on decreases in childhood antibody responses to pathogens such as diphtheria and tetanus, and some effect for rubella. 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, 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 under 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**	
	not precise. Findings for infectious disease in adults were mixed, with two studies reporting inconsistent results for COVID-19 infections.				*Human relevance and other inferences:* Given 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 lifestages represents a major source of uncertainty in the immunotoxicity database of PFOS.
Immune hypersensitivity 1 *High* confidence study 20 *Medium* confidence studies 4 *Low* confidence studies 3 *Mixed*[a] confidence studies	Examination of immune hypersensitivity includes outcomes such as asthma, allergies, and eczema. Increased odds of asthma were reported in multiple *medium* confidence studies (7/12), 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. Nine studies examined allergies, rhinitis, or rhinoconjunctivitis. Some positive associations (3/9) were observed, although this varied by outcome timing and were at times inconsistent. Ten studies examined eczema, and	• *High* and *medium* confidence studies • *Consistent direction* of effect for asthma across *medium* 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**	
	results were generally mixed.				
Autoimmune disease 1 *Medium* confidence study 3 *Low* confidence studies	Lower exposure levels were observed in healthy controls compared with 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 with healthy controls.	• *No factors* identified	• *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 hematopoiesis were	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	and 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).			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	• *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**	
	monkeys. 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 study 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		
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	• *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**	
	decreases in absolute spleen (2/2) (males only) and thymus 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	• *High* and *medium* confidence studies	• *Limited number* of studies examining specific outcomes • *Inconsistent direction* of effects across 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**	
	rats, and one short-term study found no changes in IgE (1/1) in male mice				

Mechanistic Evidence and Supplemental Information (Section 3.4.2.3)		
Biological events or pathways	**Summary of Key Findings, Interpretation, and Limitations**	**Evidence Stream Judgment**
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:**	

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**	
	• Only a single study; no studies directly evaluated the mechanism by which PFOS could promote autoimmunity.				
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: HFMD = hand, foot, and mouth disease; 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 13 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, 2 and were 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 et al., 2012). Studied health effects include ischemic heart diseases (IHD), coronary artery disease (CAD), coronary heart disease (CHD), hypertension, cerebrovascular disease, atherosclerosis (plaque buildup 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. Cardiovascular outcomes were synthesized separately by population (i.e., adults, children, occupational populations), and definitions of certain conditions may vary by age. For example, high blood pressure and/or hypertension is generally defined as SBP ≥ 140 mmHg and DBP ≥ 90 mmHg in adults and SBP ≥ 130 mmHg and DBP ≥ 80 mmHg in children and adolescents, although consistent blood pressure measurements in youth can be challenging (Falkner et al., 2023).

The 2016 PFOS HESD (U.S. EPA, 2016b) 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, 2016b) that investigated the association between PFOS and cardiovascular effects. Study quality evaluations for these 2 studies are shown in Figure 3-29. Results from studies summarized in the 2016 PFOS HESD are described in Table 3-13 and below.

The developmental section in the 2016 PFOS HESD describes results from Geiger et al. (2014b) 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 et al., 2003) 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.

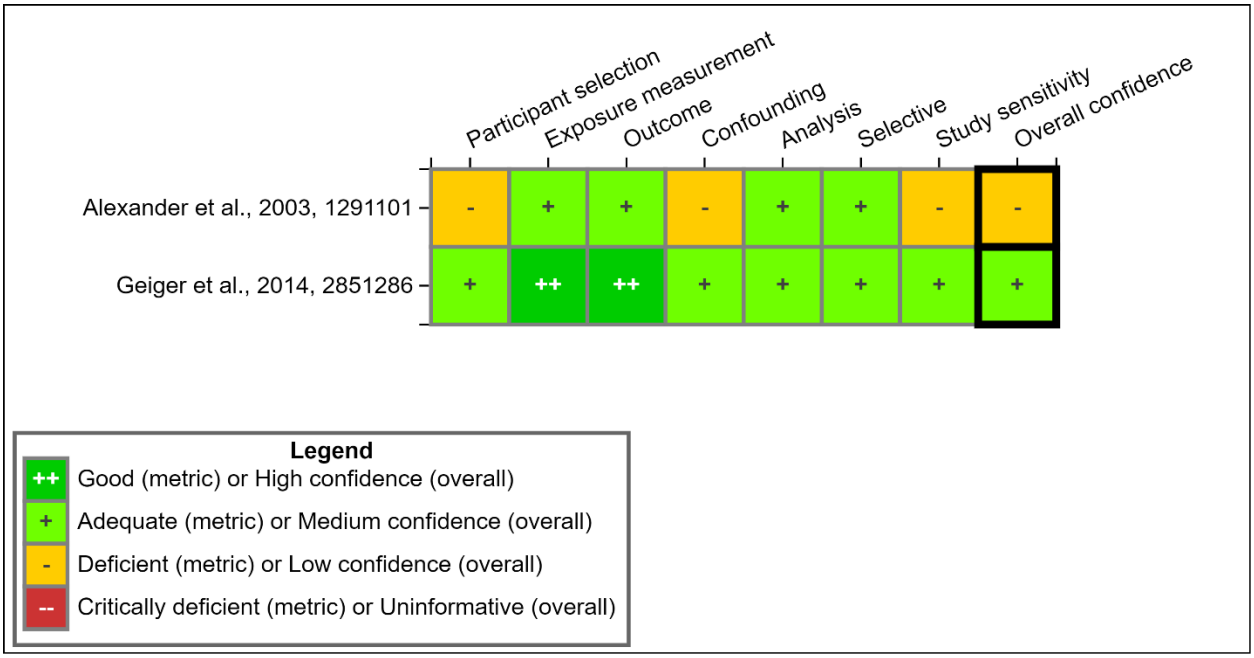


Figure 3-29. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Cardiovascular Effects Published Before 2016 (References in the 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

Table 3-13. Associations Between Elevated Exposure to PFOS and Cardiovascular Outcomes From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Population	Hypertension[b]	Heart Disease Mortality[b]	Cerebrovascular Disease Mortality[b]
Alexander, 2003, 1291101 *Low*	Cohort	Occupational	NA	↓	↓
Geiger, 2014, 2851286 *Medium*	Cross-sectional	Children	–	NA	NA

Notes: NA = no analysis was for this outcome was performed; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

Since publication of the 2016 PFOS HESD (U.S. EPA, 2016b), 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 the synthesis on female reproductive effects (see Appendix D, (U.S. EPA, 2024a)). All studies were conducted on the general population with six (Ye et al., 2021; Yu et al., 2021; Hutcheson et al., 2020; Mi et al., 2020; Honda-Kohmo et al., 2019; Bao et al., 2017) conducted in a high-exposure community in China (i.e., C8 Health Project and "Isomers of C8 Health Project" populations), and three studies (Canova et al., 2021; Zare Jeddi et al., 2021; Pitter et al., 2020) were conducted in a high-exposure community in Italy (i.e., Vento Region). Different study designs were also used including three controlled trial studies (Osorio-Yáñez et al., 2021; Cardenas et al., 2019; Liu et al., 2018b), 11 cohort studies (Li et al., 2021b; Papadopoulou et al., 2021; Lin et al., 2020c; Mitro et al., 2020; Donat-Vargas et al., 2019; Warembourg et al., 2019; Fry and Power, 2017; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017), one case-control study (Mattsson et al., 2015), and 33 cross-sectional studies (Koskela et al., 2022; Averina et al., 2021; Canova et al., 2021; Ye et al., 2021; Yu et al., 2021; Zare Jeddi et al., 2021; Hutcheson et al., 2020; Jain and Ducatman, 2020; Jain, 2020a, b; Khalil et al., 2020; Leary et al., 2020; Liao et al., 2020; Lin et al., 2020d; Mi et al., 2020; Pitter et al., 2020; Chen et al., 2019; Christensen et al., 2019; Graber et al., 2019; Honda-Kohmo et al., 2019; Ma et al., 2019; Huang et al., 2018; Khalil et al., 2018; Liu et al., 2018d; Mobacke et al., 2018; Yang et al., 2018; Bao et al., 2017; Koshy et al., 2017; Lind et al., 2017b; Christensen et al., 2016; Lin et al., 2016; Lin et al., 2013). The three controlled trial studies (Osorio-Yáñez et al., 2021; Cardenas et al., 2019; Liu et al., 2018b) were not controlled trials of PFAS exposures, but rather health interventions: prevention of type 2 diabetes in Diabetes Prevention Program and Outcomes Study (DPPOS) (Osorio-Yáñez et al., 2021; Cardenas et al., 2019) and weight loss in the Prevention of Obesity Using Novel Dietary Strategies Lost (POUNDS-Lost) Study (Liu et al., 2018b). Thus, these studies could be interpreted as cohort studies for evaluating cardiovascular risk purposes.

The available studies were conducted in different study populations with the majority of studies conducted in the United States (Koskela et al., 2022; Li et al., 2021b; Osorio-Yáñez et al., 2021; Hutcheson et al., 2020; Jain and Ducatman, 2020; Jain, 2020a, b; Khalil et al., 2020; Leary et al., 2020; Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Mitro et al., 2020; Cardenas et al., 2019; Christensen et al., 2019; Graber et al., 2019; Honda-Kohmo et al., 2019; Ma et al., 2019; Huang et al., 2018; Khalil et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Fry and Power, 2017; Koshy et al., 2017; Christensen et al., 2016). The remaining studies were conducted in China (Ye et al., 2021; Yu et al., 2021; Yang et al., 2018; Bao et al., 2017), Taiwan (Lin et al., 2016; Lin et al., 2013), Spain (Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017), Croatia (Chen et al., 2019), Sweden (Donat-Vargas et al., 2019; Mobacke et al., 2018; Lind et al., 2017b; Mattsson et al., 2015), Denmark (Jensen et al., 2020), Italy (Canova et al., 2021; Ye et al., 2021; Zare Jeddi et al., 2021; Pitter et al., 2020), Norway (Averina et al., 2021), and two studies conducted in several European countries (Papadopoulou et al., 2021; Warembourg et al., 2019). All the studies measured PFOS in blood components (i.e., serum or plasma) with three studies measuring levels in maternal serum (Li et al., 2021b; Papadopoulou et al., 2021; Warembourg et al., 2019), and four studies measuring levels in maternal plasma (Papadopoulou et al., 2021; Mitro et al., 2020; Warembourg et al., 2019; Manzano-Salgado et al., 2017b).

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, 2016b) that investigated the association between PFOS and cardiovascular effects. Study quality evaluations for these 45 studies are shown in Figure 3-30 and Figure 3-31.

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, 2020a, b; Leary et al., 2020; Seo et al., 2018). 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 (Girardi and Merler, 2019; Graber et al., 2019; Khalil et al., 2018; Christensen et al., 2016). 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, 2020a, b). 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 et al., 2020). Studies considered *uninformative* were not considered further.

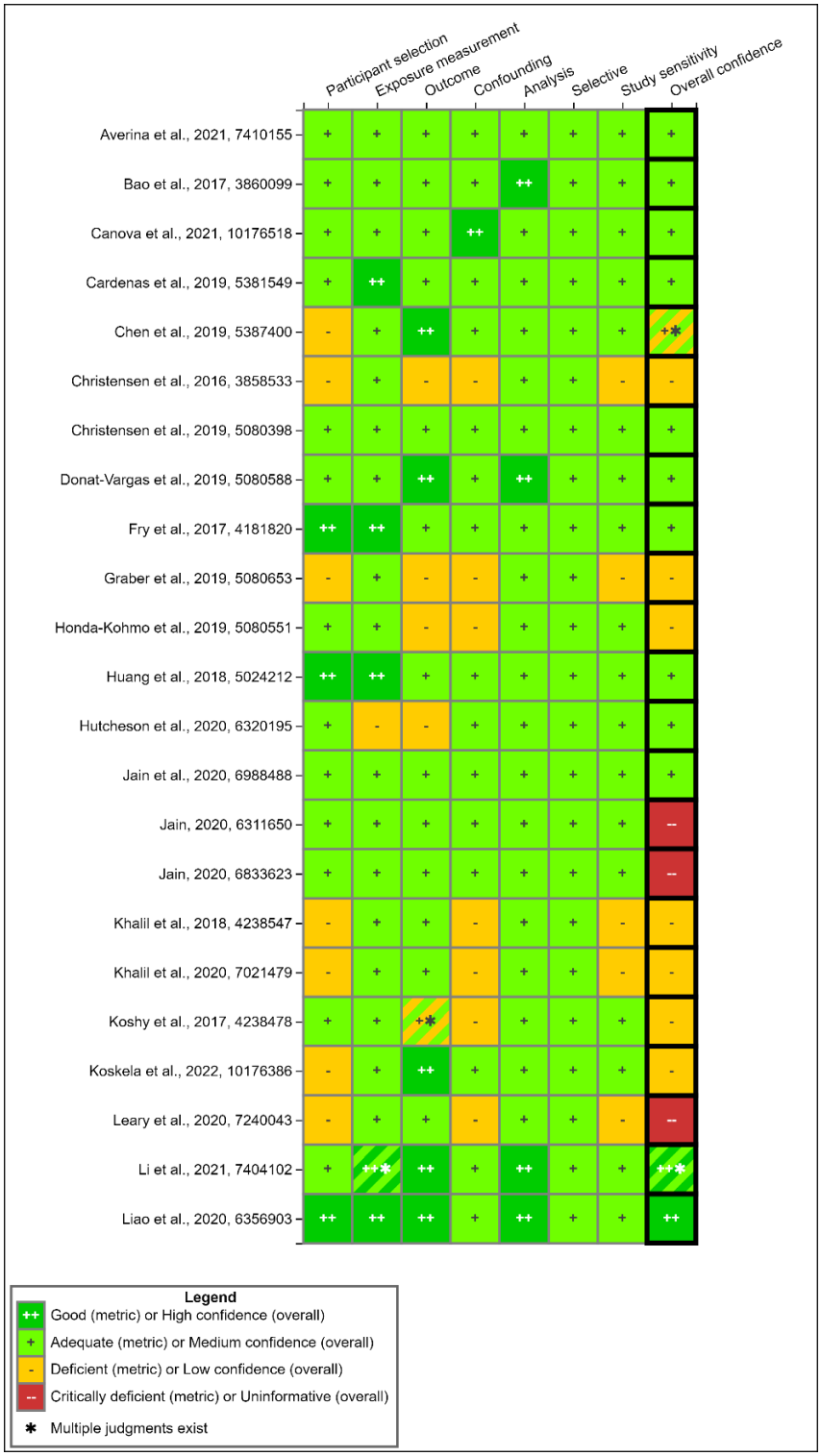


Figure 3-30. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

Figure 3-31. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure 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 et al., 2019), 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 et al., 2021). Results from the remaining *medium* confidence studies were essentially null (see Appendix D, (U.S. EPA, 2024a)). Among 2,251 NHANES (2003–2012) adolescents (mean age 15.5 years) Ma et al. (2019) observed a positive association with DBP, which was 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 et al., 2021). In quartile analyses, the association was positive for the second to fourth quartiles compared with 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. (2017b) 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) observed that PFOS measured in maternal blood during the pre-natal period, and in plasma during the postnatal period were not associated with blood pressure in single-pollutant models. Results from an overlapping study (Papadopoulou et al., 2021) on the same cohort were consistent with Warembourg et al. (2019)

Two *low* confidence studies did not observe associations between serum PFOS and blood pressure in children or adolescents (Khalil et al., 2018; Lin et al., 2013).

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 et al., 2013) and 848 (Lin et al., 2016) 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 et al., 2017). 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 Appendix D, (U.S. EPA, 2024a)). A total of 16 studies examined PFOS in association with SBP, DBP, hypertension, and elevated blood pressure (Ye et al., 2021; Yu et al., 2021; Zare Jeddi et al., 2021; Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Mitro et al., 2020; Pitter et al., 2020; Chen et al., 2019; Christensen et al., 2019; Donat-Vargas et al., 2019; Liu et al., 2018d; Liu et al., 2018b; Yang et al., 2018; Bao et al., 2017; Christensen et al., 2016).

Of the eight studies that examined blood pressure as a continuous measure, five observed statistically significant positive associations (Liao et al., 2020; Mi et al., 2020; Mitro et al., 2020; Liu et al., 2018b; Bao et al., 2017). 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 et al., 2020). Using a generalized additive model and restricted cubic splines, a nonlinear (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 et al., 2020) conducted in 761 women that examined associations between PFOS concentrations measured during pregnancy and blood pressure assessed at 3 years postpartum 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 et al., 2020; Bao et al., 2017) also reported positive associations for blood pressure. In adults with very high PFOS levels (median 24.22 ng/mL), Bao et al. (2017) 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) 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 nonsignificant overall or among women. Another high-exposure community study (Pitter et al., 2020) 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 et al., 2021) 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. (2020c) 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 toward 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. (2018b) 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 et al., 2019; Yang et al., 2018).

Of the eight studies that examined risk of elevated blood pressure (hypertension), two reported statistically significant associations (Mi et al., 2020; Bao et al., 2017). 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) and Bao et al. (2017), which had overlapping data on high exposed Isomers of C8 Health Project participants, reported significant associations. Bao et al. (2017) 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 with linear isomers (n-PFOS). Mi et al. (2020) 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 et al., 2020) reported in a fully adjusted analysis that the OR among adults exposed to PFOS levels in the highest tertile compared with 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 nonsignificant among males and females in stratified analysis. No association was observed for elevated blood pressure in two *medium* confidence studies (Christensen et al., 2019; Liu et al., 2018d) and for hypertension in one *medium* (Lin et al., 2020c) and one *low* confidence study (Christensen et al., 2016). One *medium* confidence study (Donat-Vargas et al., 2019) 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 et al., 2021; Yu et al., 2021), both of which examined participants of the Isomers of C8 Health Project (overlapping with Mi et al. (2020) and Bao et al. (2017)). Yu et al. (2021) 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 et al. (2021) reported a nonsignificant 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 et al. (2019) 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 nonsignificant associations, and one study reporting negative associations. A *high* confidence study (Mattsson et al., 2015), a *medium* confidence NHANES study (Huang et al., 2018), and a *low* confidence study (Christensen et al., 2016) reported positive nonsignificant associations with CHD. A *low* confidence study from the C8 Health Project (Honda-Kohmo et al., 2019) 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 et al., 2018) reported significantly higher odds of heart attack for the third quartile (OR: 1.56; 95% CI: 1.01, 2.43) compared with 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 et al., 2020) 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 et al. (2019) 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 et al., 2018) and carotid atherosclerosis (Lind et al., 2017b) in participants 70 years and older, with mixed results. Mobacke et al. (2018) 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. (2017b) 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 et al., 2021) 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 with a mild score (< 11) was increased with respect to PFOS exposure, and the odds of a severe score (> 400) compared with a mild score were significantly increased. Koskela et al. (2022), a *low* confidence study, examined abdominal aortic calcification among participants aged 40 years and older in NHANES (2013–2014) and 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 postpartum women (Mitro et al., 2020; Matilla-Santander et al., 2017).

Mortality due to heart/cerebrovascular diseases was examined in one *medium* confidence study (Fry and Power, 2017). 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 et al., 2020c) 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 et al., 2020; Bao et al., 2017) 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. Evidence for changes in serum lipids was synthesized by population (i.e., children, pregnant women, adults, occupational populations), and there may be differences in the interpretation of an effect depending on age. For example, while elevated levels of TC, LDL, and triglycerides are associated with increased cardiovascular risks in adults, serum lipid changes in children are age-dependent and fluctuate during puberty (Daniels et al., 2008).

There are 15 studies (17 publications)[13] from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and serum lipid effects. Study quality evaluations for these 15 studies are shown in Figure 3-32. Results from studies summarized in the 2016 PFOS HESD are described in Table 3-14 and below.

In the 2016 PFOS HESD (U.S. EPA, 2016b), 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 (Geiger et al., 2014a; Eriksen et al., 2013; Frisbee et al., 2010; Nelson et al., 2010; Steenland et al., 2009). Steenland (Steenland

[13] Olsen (2003) is the peer-review paper of Olsen (2001a) and Olsen (2001b).

et al., 2009) 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 et al., 2010) 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 et al., 2013) conducted among Danish adults (50–65 years old). A positive association between PFOS and hypercholesterolemia also was observed in two separate cohorts (C8 Health Project and Canadian Health Measures Survey) (Fisher et al., 2013; Steenland et al., 2009). Cross-sectional occupational studies (Olsen et al., 2003; Olsen et al., 2001a) 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.

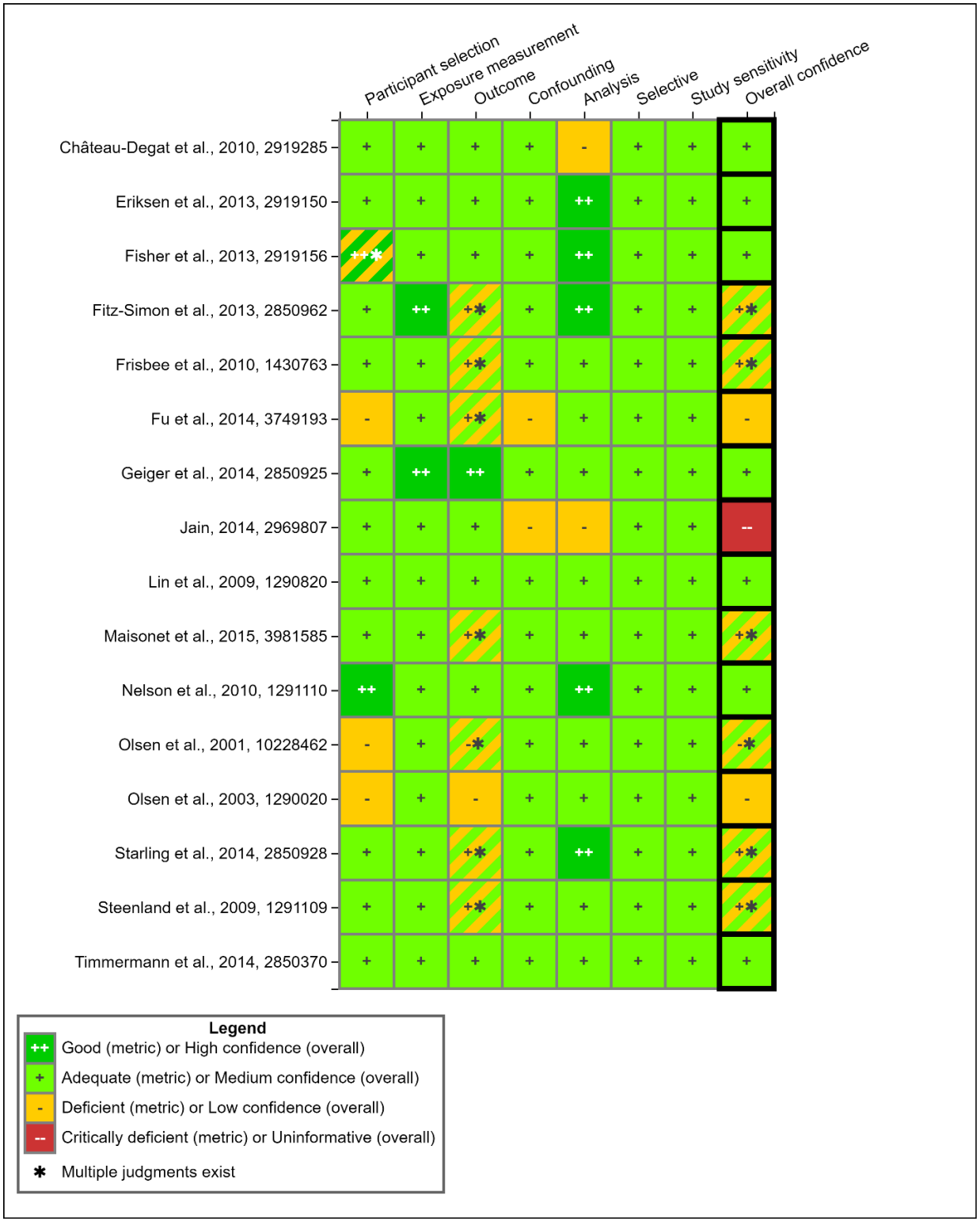


Figure 3-32. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Serum Lipids Published Before 2016 (References in the 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

Table 3-14. Associations Between Elevated Exposure to PFOS and Serum Lipids From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Population	TC[a]	HDL[a]	LDL[a]	TG[a]
Chateau-Degat, 2010, 2919285 *Medium*	Cross-sectional	Adults	↑	↑↑	↓	↓
Eriksen, 2013, 2919150 *Medium*	Cross-sectional	Adults	↑↑	NA	NA	NA
Fisher, 2013, 2919156 *Medium*	Cross-sectional	Adults	–	–	–	–
Fitz-Simon, 2013, 2850962 *Mixed*[b]	Cohort	Adults	↑	↓	↑	–
Frisbee, 2010, 1430763 *Mixed*[b]	Cross-sectional	Children	↑↑	–	↑↑	↑
Fu, 2014, 3749193 *Low*	Cross-sectional	Adults and children	↑	↓	↑	↑
Geiger, 2014, 2850925 *Medium*	Cross-sectional	Adolescents	↑↑	–	↑↑	↓
Lin, 2009, 1290820 *Medium*	Cross-sectional	Adults	NA	↑↑	NA	–
Maisonet, 2015, 3981585 *Mixed*[b]	Cohort	Children	–	–	–	↓
Nelson, 2010, 1291110 *Medium*	Cross-sectional	Adults	↑↑	↑	↑	NA
Olsen, 2001, 10228462 *Mixed*[b]	Cohort	Adults	↑	↓	NA	↑
Olsen, 2003, 1290020 *Low*	Cohort	Occupational	–	NA	NA	–

Reference, confidence	Study Design	Population	TC[a]	HDL[a]	LDL[a]	TG[a]
Starling, 2014, 2850928 *Mixed*[b]	Cohort	Children	↑↑	↑↑	↑	–
Steenland, 2009, 1291109 *Mixed*[b]	Cross-sectional	Occupational	↑	↑	↑	↑
Timmerman, 2014, 2850370 *Medium*	Cohort	Children	NA	NA	NA	↑

Notes: HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein; NA = no analysis was for this outcome was performed; TC = total cholesterol; TG = triglycerides; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Jain et al., 2014, 2969807 was not included in the table due to their *uninformative* overall study confidence ratings.

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] *Mixed* confidence studies were rated *medium* confidence for TC and HDL and *low* confidence for LDL and TG due to non-fasted blood samples.

Since publication of the 2016 PFOS HESD (U.S. EPA, 2016b), 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 10 studies (Blomberg et al., 2021; Li et al., 2021b; Liu et al., 2020b; Sinisalu et al., 2020; Tian et al., 2020; Donat-Vargas et al., 2019; Lin et al., 2019; Liu et al., 2018b; Domazet et al., 2016; Olsen et al., 2012), all studies were cross-sectional. Some cohort studies provided additional cross-sectional analyses (Blomberg et al., 2021; Li et al., 2021b; Sinisalu et al., 2020). 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 methods in Appendix A (U.S. EPA, 2024a) and Section 2.1.3. 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), 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). 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 = 3.4 years) (Li et al., 2018b), current blood concentrations are expected to correlate well with past exposures. Furthermore, although reverse causation due to hypothyroidism (Dzierlenga et al., 2020b) or enterohepatic cycling of bile acids (Fragki et al., 2021) 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, 2016b) that investigated the association between PFOS and serum lipid effects. Study quality evaluations for these 65 studies are shown in Figure 3-33, Figure 3-34, and Figure 3-35.

Consistent with 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 lipid outcomes, and 7 studies were rated *uninformative* for all lipid outcomes (Sinisalu et al., 2021; Abraham et al., 2020; Leary et al., 2020; Huang et al., 2018; Seo et al., 2018; Predieri et al., 2015). Notably, nine studies (Blomberg et al., 2021; Canova et al., 2021; Dalla Zuanna et al.,

[14] Dong et al. (2019) counted as two studies, one in adolescents and one in adults.

2021; Canova et al., 2020; Tian et al., 2020; Yang et al., 2020b; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017; Zeng et al., 2015) 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 (Cong et al., 2021; Kobayashi et al., 2021; Liu et al., 2021; Ye et al., 2021; Yu et al., 2021; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020a; Chen et al., 2019; Graber et al., 2019; He et al., 2018; Khalil et al., 2018; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; van den Dungen et al., 2017; Christensen et al., 2016; Rotander et al., 2015; Lin et al., 2013; Wang et al., 2012), outcome measures (Kobayashi et al., 2021; Graber et al., 2019; Yang et al., 2018; Koshy et al., 2017; Christensen et al., 2016; Kishi et al., 2015; Rotander et al., 2015), confounding (Liu et al., 2021; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020a; Sinisalu et al., 2020; Graber et al., 2019; Khalil et al., 2018; Yang et al., 2018; Koshy et al., 2017; van den Dungen et al., 2017; Christensen et al., 2016; Lin et al., 2013; Olsen et al., 2012; Wang et al., 2012), analysis (He et al., 2018; Liu et al., 2018b; Sun et al., 2018), sensitivity (Khalil et al., 2020; Sinisalu et al., 2020; Graber et al., 2019; Khalil et al., 2018; van den Dungen et al., 2017; Christensen et al., 2016; Rotander et al., 2015; Olsen et al., 2012; Wang et al., 2012), or selective reporting (Dong et al., 2019) (adolescent portion only).

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 (Cong et al., 2021; Liu et al., 2021; Ye et al., 2021; Yu et al., 2021; Li et al., 2020d; Lin et al., 2020a; Chen et al., 2019; He et al., 2018; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; van den Dungen et al., 2017; Wang et al., 2012), potential for self-selection (Li et al., 2020d; Graber et al., 2019; van den Dungen et al., 2017; Christensen et al., 2016; Rotander et al., 2015), highly unequal recruitment efforts in sampling frames with potentially different joint distributions of PFOS and lipids (Lin et al., 2013), and missing key information on the recruitment process (Khalil et al., 2020; Khalil et al., 2018; Yang et al., 2018). Another common reason for *low* confidence was a serious risk for residual confounding by SES (Li et al., 2020d; Lin et al., 2020a; Sinisalu et al., 2020; Graber et al., 2019; Khalil et al., 2018; Yang et al., 2018; Koshy et al., 2017; van den Dungen et al., 2017; Christensen et al., 2016; Lin et al., 2013; Olsen et al., 2012; Wang et al., 2012). 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 (Abraham et al., 2020; Huang et al., 2018; Seo et al., 2018), convenience sampling (Sinisalu et al., 2021), 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 et al., 2015). Small sample size (n = 45) and missing details on exposure measurements were the primary concerns of the remaining *uninformative* study (Leary et al., 2020). 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.

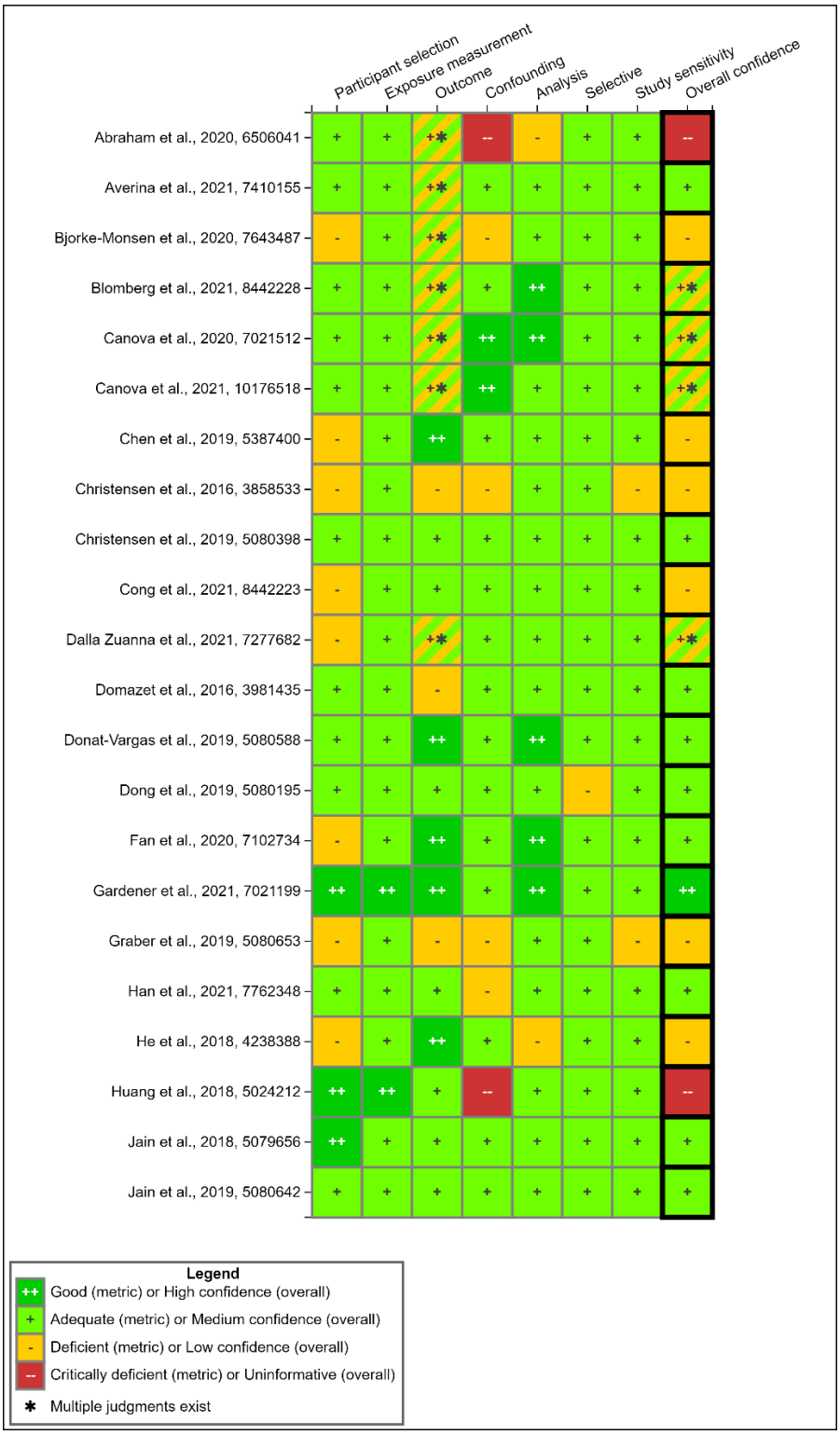


Figure 3-33. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids

Interactive figure and additional study details available on HAWC.

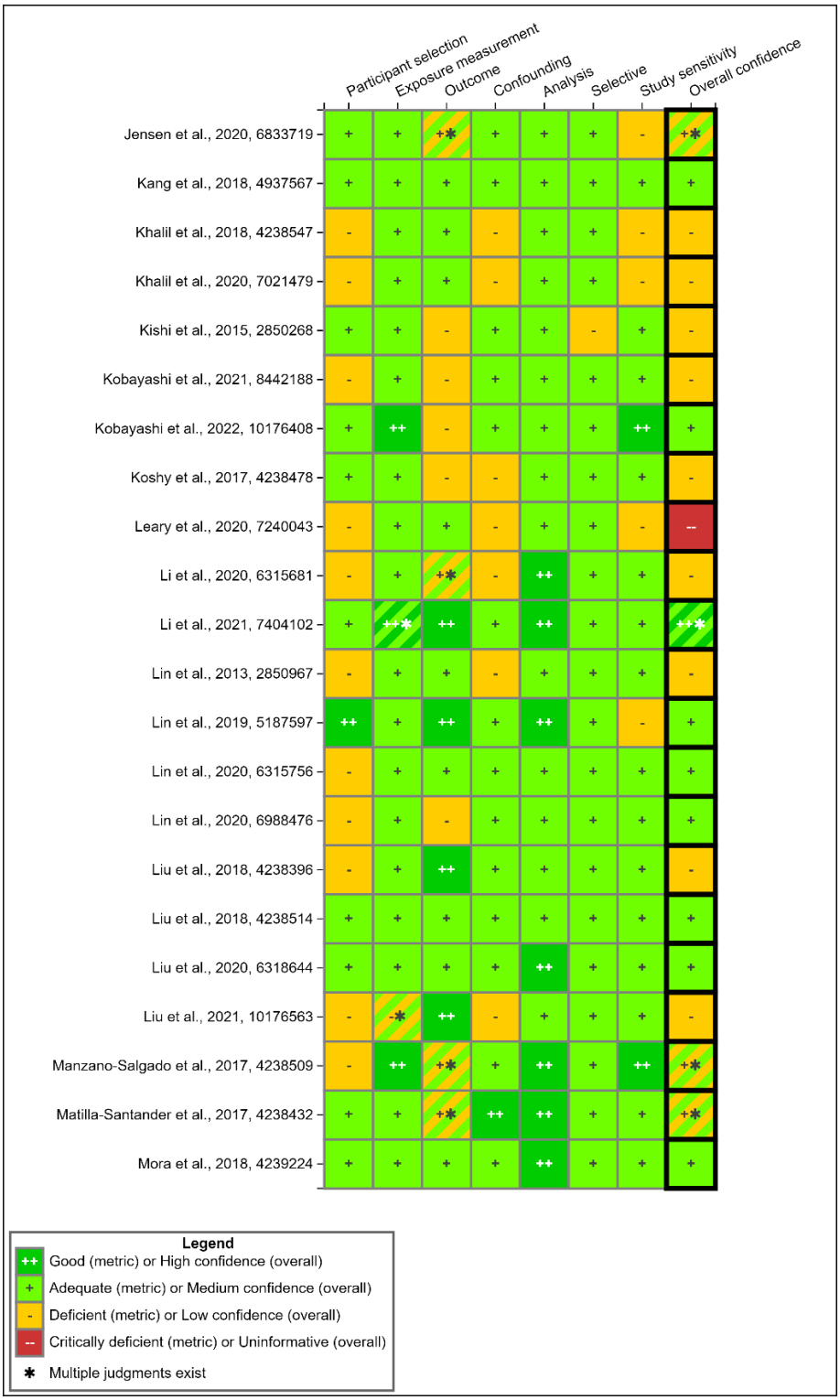


Figure 3-34. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

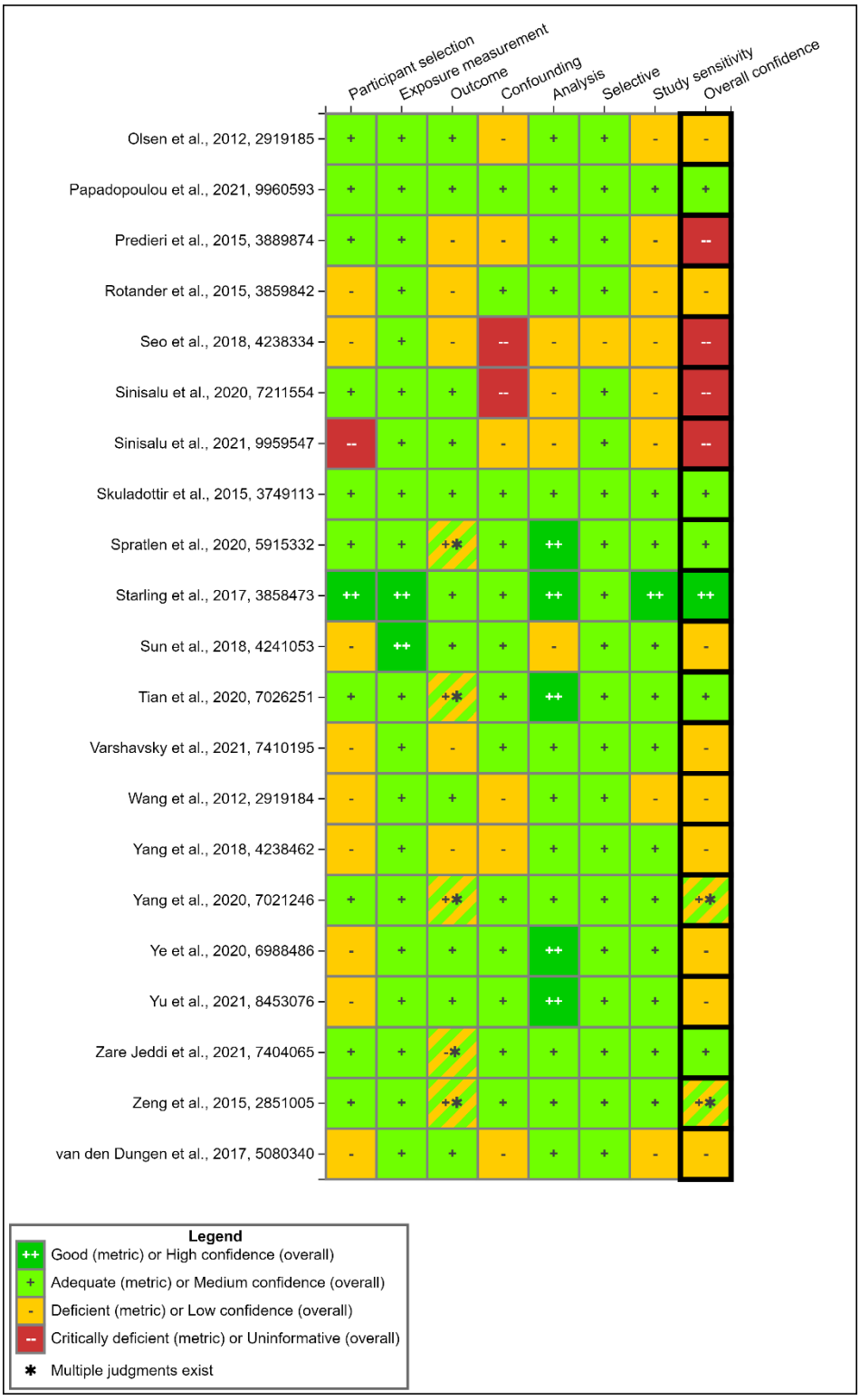


Figure 3-35. Summary of Study Evaluation for Epidemiology Studies of PFOS and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

3.4.3.1.2.3 Findings From Children

Results for the studies that examined TC in children are presented in Appendix D (U.S. EPA, 2024a). 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 (Jensen et al., 2020; Spratlen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b), one examined exposure and TC at multiple timepoints throughout childhood (Blomberg et al., 2021), and 10 examined the association between childhood PFOS exposure and concurrent TC (Averina et al., 2021; Canova et al., 2021; Tian et al., 2020; Dong et al., 2019; Jain and Ducatman, 2018; Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). Higher PFOS was significantly associated with higher TC in all children in five *medium* confidence studies (Averina et al., 2021; Blomberg et al., 2021; Canova et al., 2021; Jain and Ducatman, 2018; Zeng et al., 2015). 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 with adolescents. Significant positive associations were observed in 9-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 et al., 2021). Comparisons of PFOS and TC measured at other timepoints were less consistent. Positive associations were also found in four other *medium* confidence studies (Jensen et al., 2020; Spratlen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b), but the associations were small and statistically not significant except for girls in mid-childhood (Mora et al., 2018). In contrast, one *medium* confidence study (Tian et al., 2020) 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 (Khalil et al., 2018; Koshy et al., 2017). 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 et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b), one examined prenatal and childhood exposure (Papadopoulou et al., 2021) and nine examined childhood exposure (Averina et al., 2021; Canova et al., 2021; Tian et al., 2020; Dong et al., 2019; Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). 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 et al., 2021). None of the associations were statistically significant in the remaining *medium* confidence studies (see Appendix D, (U.S. EPA, 2024a)) (Jensen et al., 2020; Kang et al., 2018; Mora et al., 2018). Most *low* confidence studies found a positive association between PFOS and LDL (Canova et al., 2021; Khalil et al., 2018; Koshy et al., 2017; Manzano-Salgado et al., 2017b; Zeng et al., 2015), including statistically significant findings in three studies (Canova et al., 2021; Khalil et al., 2018; Koshy et al., 2017). However, residual confounding by SES (Khalil et al., 2018; Koshy et al., 2017) and the use of non-fasting samples (Canova et al., 2021; Manzano-Salgado et al., 2017b; Zeng et al., 2015) were concerns in these studies. Overall, increases in LDL with increasing PFOS were observed in children, but the magnitudes were small.

One *high* confidence, 11 *medium* confidence, and 3 *low* confidence studies examined the association between PFOS and HDL in children. Of these, three examined prenatal exposure (Jensen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b), one examined prenatal and postnatal exposure (Papadopoulou et al., 2021), two examined exposure and HDL at multiple timepoints throughout childhood (Blomberg et al., 2021; Li et al., 2021b), and six examined childhood exposure (Averina et al., 2021; Canova et al., 2021; Tian et al., 2020; Dong et al., 2019; Jain and Ducatman, 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). The only *high* confidence study (Li et al., 2021b) 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 (Canova et al., 2021; Mora et al., 2018). The positive association observed in Canova et al. (2021) was consistent when examining adolescent participants. In Faroese children (Blomberg et al., 2021), 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 and Ducatman, 2018), inverse (HDL at 18 months in Jensen et al. (2020); Papadopoulou et al. (2021), prenatal PFOS; Manzano-Salgado et al. (2017b); Zeng et al. (2015); Tian et al. (2020)), or close to zero (HDL at 3 months in Jensen et al. (2020); Papadopoulou et al. (2021), 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 et al., 2018; Koshy et al., 2017). 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 (Jensen et al., 2020; Spratlen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and six examined childhood exposure (Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Domazet et al., 2016; Zeng et al., 2015). Higher mid-childhood PFOS exposure was significantly associated with lower triglycerides in one *medium* confidence study (Mora et al., 2018). The other *medium* confidence studies reported positive (Spratlen et al., 2020; Kang et al., 2018), inverse (triglycerides at 3 months in Jensen et al. (2020); PFOS exposure at age 9 years in Domazet et al. (2016)), or close to zero associations (triglycerides at 18 months in Jensen et al. (2020); PFOS exposure at age 15 years in Domazet et al. (2016)); none of these associations were statistically significant. Of note, in Jensen et al. (2020) and Domazet et al. (2016), the direction of association changed depending on the timing of outcome or exposure assessment. One *medium* confidence study (Kobayashi et al., 2022) and one *low* confidence study (Kobayashi et al., 2021) 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 (Sinisalu et al., 2020; Khalil et al.,

2018; Koshy et al., 2017; Manzano-Salgado et al., 2017b; Zeng et al., 2015). The use of non-fasting samples and residual confounding by SES may have biased these results upward. 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

One *high* confidence study (Gardener et al., 2021) and four *medium* confidence studies examined the association between PFOS and TC in pregnant women and four reported positive associations between PFOS and TC (see Appendix D, (U.S. EPA, 2024a)) (Dalla Zuanna et al., 2021; Matilla-Santander et al., 2017; Skuladottir et al., 2015). A significant positive trend across quartiles of PFOS exposure was observed for TC in a cohort study of pregnant women from the United States (Gardener et al., 2021). Skuladottir et al. (2015) 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 et al., 2021) 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 et al., 2020b). No association was found in the single *low* confidence study (Varshavsky et al., 2021) 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 et al., 2021; Yang et al., 2020b) 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 et al., 2021), 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 nonsignificant, association between PFOS and HDL (see Appendix D, (U.S. EPA, 2024a)) (Starling et al., 2017). No association between PFOS and HDL was observed in a Chinese study of pregnant women (Yang et al., 2020b).

One *high* confidence, one *medium* confidence and three *low* confidence studies examined the association between PFOS and triglycerides in pregnant women. A significant positive trend across quartiles of PFOS exposure was observed for triglycerides in a cohort study of pregnant women from the United States (Gardener et al., 2021). The *medium* confidence study reported no association between PFOS and triglycerides (see Appendix D, (U.S. EPA, 2024a)) (Starling et al., 2017). Two *low* confidence studies reported statistically significant, inverse associations between PFOS and triglycerides (Matilla-Santander et al., 2017; Kishi et al., 2015) while the remaining study (Yang et al., 2020b) reported a nonsignificant 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. The inverse associations observed in the *low* confidence studies provides support for an inverse association

between PFOS and triglycerides. These inverse associations are inconsistent with the finding in the only *medium* confidence study. In summary, the available evidence suggests an inverse association between PFOS and triglycerides in pregnant women. However, additional *high* or *medium* confidence evidence is needed to confirm this association.

Kishi et al. (2015) additionally examined the association between PFOS and select fatty acids in serum. Except for stearic acid and eicosapentaenoic acid, PFOS was inversely associated with serum fatty acids; most of these associations were statistically significant (Kishi et al., 2015). 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 12 *low* confidence studies examined PFOS and TC or hypercholesterolemia in adults. All studies examined the cross-sectional association (Cong et al., 2021; Han et al., 2021; Liu et al., 2021; Bjorke-Monsen et al., 2020; Canova et al., 2020; Fan et al., 2020; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020d; Liu et al., 2020b; Chen et al., 2019; Donat-Vargas et al., 2019; Dong et al., 2019; Graber et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Sun et al., 2018; Christensen et al., 2016; Wang et al., 2012); two studies additionally examined the association between baseline PFOS and changes in TC or incident hypercholesterolemia (Liu et al., 2020b; Lin et al., 2019).

Of the 10 *medium* confidence studies, nine reported positive associations (Figure 3-36, Figure 3-37, Figure 3-38, and Figure 3-39). 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) reported statistically positive associations with TC. Canova et al. (2020) also reported a concentration-response curve for risk of high TC when PFOS was categorized in quartiles or deciles, with a higher slope at higher PFOS concentrations (Figure 3-40). Another high-exposure community study (Lin et al., 2020d) 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 (Fan et al., 2020; Dong et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d) (see Appendix D, (U.S. EPA, 2024a)). The association was statistically significant when data from all cycles were pooled in analyses (Dong et al., 2019). A cross-sectional analysis (Han et al., 2021) 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 et al., 2020b) and the DPPOS (Lin et al., 2019), 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 et al. (2019) 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 et al., 2019). All associations in Donat-Vargas et al. (2019) were small and few were statistically significant. It is noteworthy that all participants in Lin et al. (2019) were prediabetic, approximately half of all participants in Han et al. (2021) were diabetic, all participants in Liu et al. (2020b) were obese and enrolled in a weight loss trial, and all participants in Donat-Vargas et al. (2019) were free of diabetes for at least 10 years of follow-up. It is unclear whether 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 10 of 12 studies (Cong et al., 2021; Liu et al., 2021; Bjorke-Monsen et al., 2020; Li et al., 2020d; Chen et al., 2019; Graber et al., 2019; He et al., 2018; Liu et al., 2018b; Sun et al., 2018; Christensen et al., 2016). However, oversampling of persons with potentially high PFOS exposure and health problems was a concern in three of these studies (Li et al., 2020d; Graber et al., 2019; Christensen et al., 2016). Medication status and potential residual confounding by SES was a concern in three other studies (Cong et al., 2021; Liu et al., 2021; Bjorke-Monsen et al., 2020). Further, He et al. (2018) used similar data as the four *medium* NHANES studies and thus added little unique information. Considering *medium* and *low* confidence studies together, small increases in TC with increased PFOS were observed, though less consistently.

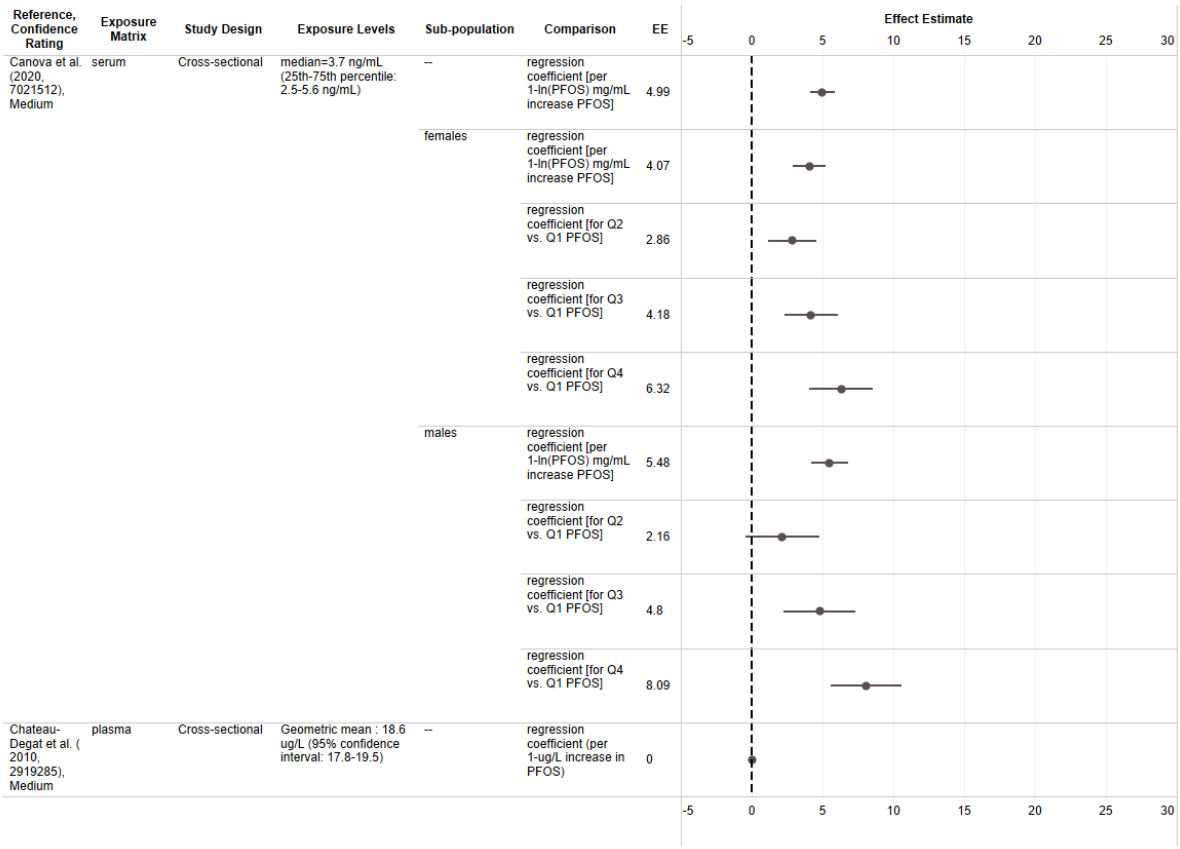


Figure 3-36. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

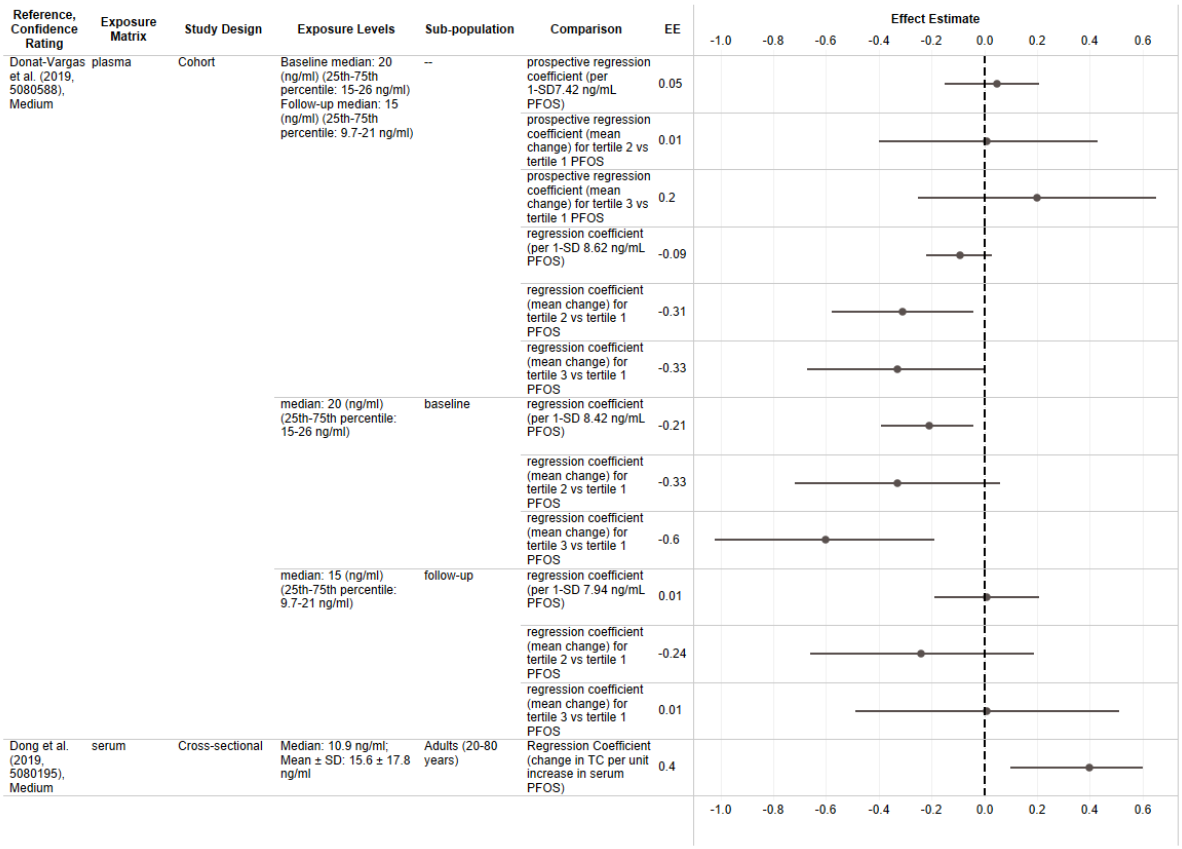


Figure 3-37. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

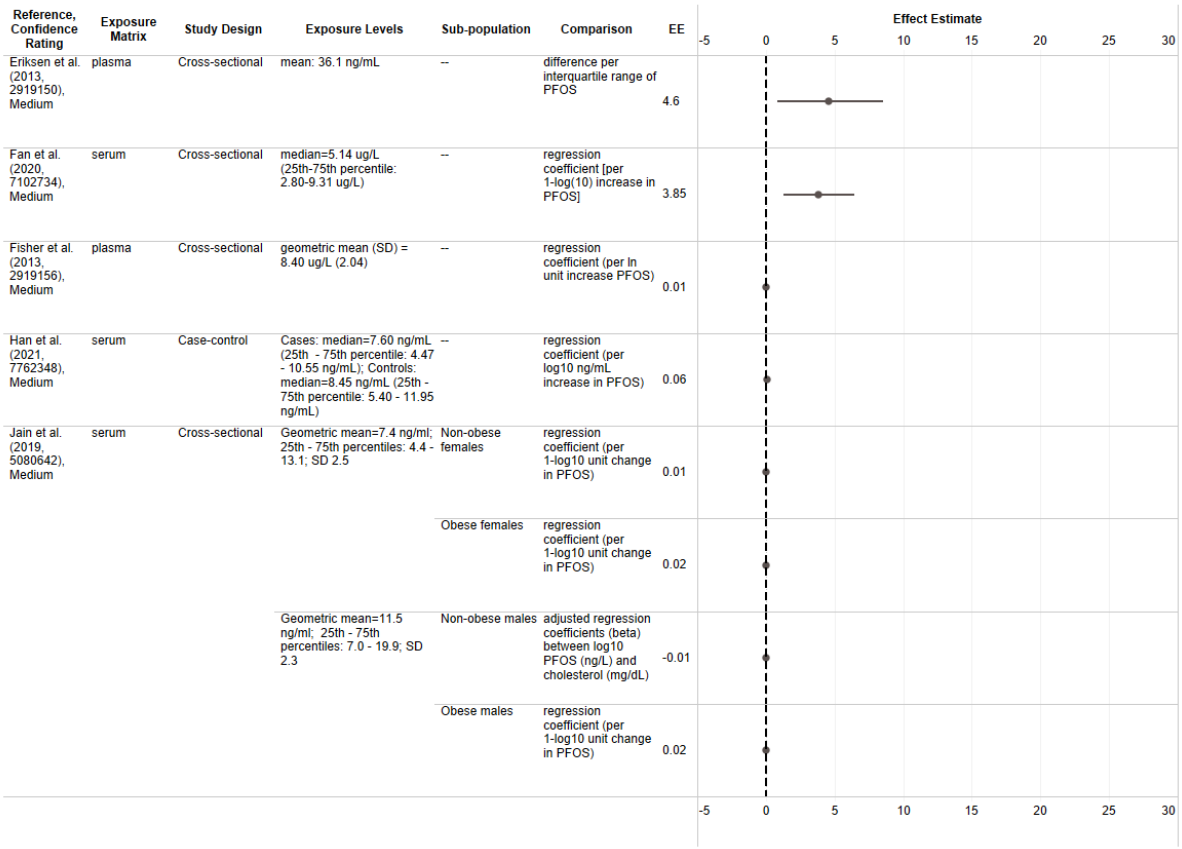


Figure 3-38. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

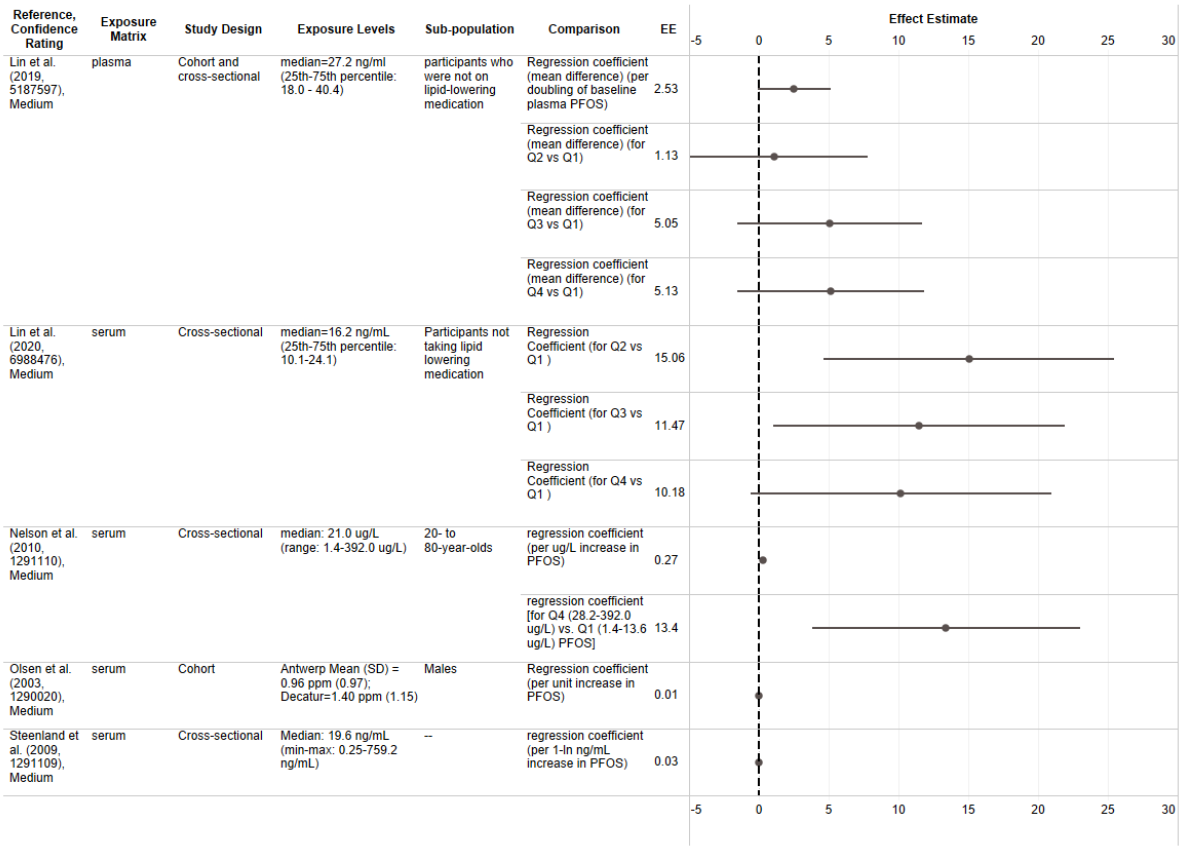


Figure 3-39. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

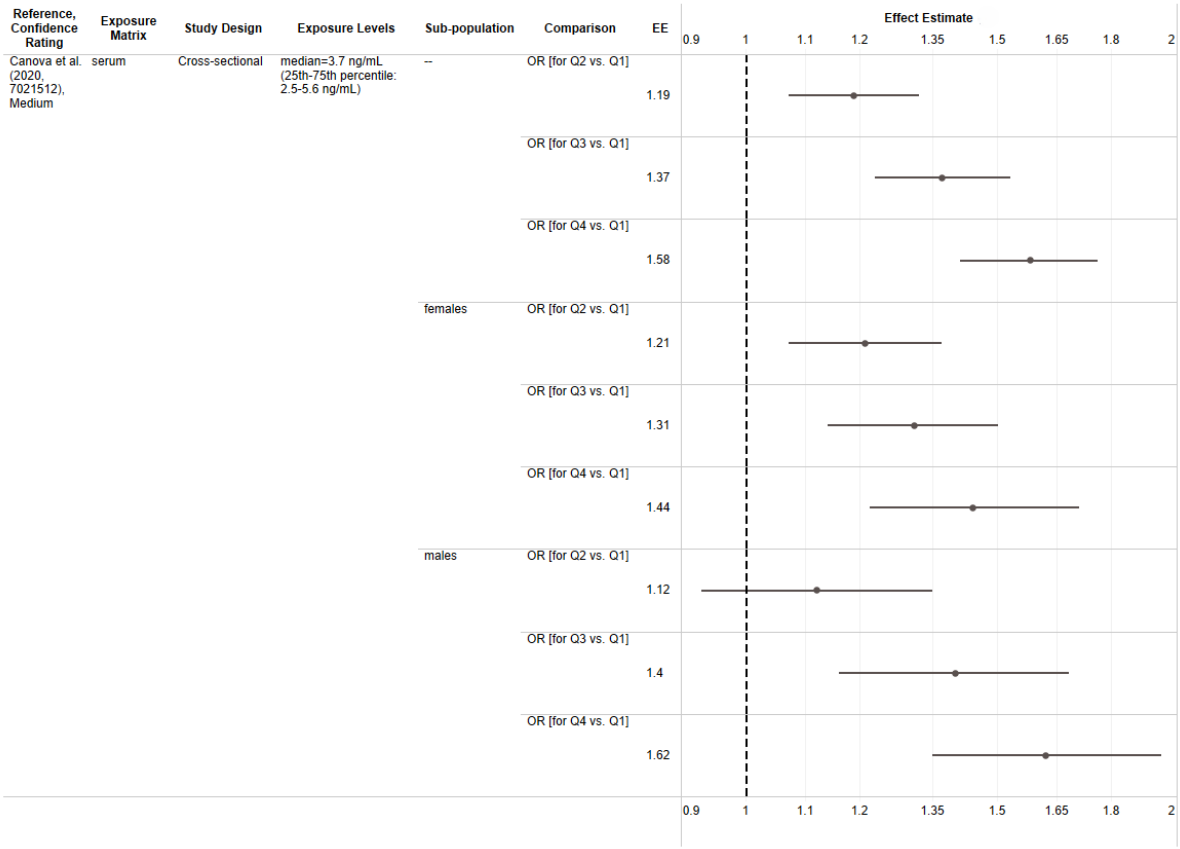


Figure 3-40. Odds of High Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

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 et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d), but the association was statistically significant in obese women only (Jain and Ducatman, 2019b) (see Appendix D, (U.S. EPA, 2024a)). The association was inverse, but not statistically significant, in non-obese persons (Jain and Ducatman, 2019b). A cross-sectional analysis (Han et al., 2021) 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 whether 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 et al., 2019). This study additionally reported that PFOS was significantly associated with higher VLDL and non-HDL (Lin et al., 2019), which are cholesterol species related to LDL and known to increase cardiovascular risks. Liu et al. (2020b) 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 and Ducatman, 2020) that reported a significantly positive association among non-diabetic, non-lipid-lowering medication users. Consistent with these findings, 9 of the 10 *low* confidence studies reported positive associations between PFOS and LDL (Cong et al., 2021; Bjorke-Monsen et al., 2020; Canova et al., 2020; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020a; He et al., 2018; Liu et al., 2018b; Lin et al., 2013). However, residual confounding by SES (Cong et al., 2021; Bjorke-Monsen et al., 2020; Lin et al., 2020a; Lin et al., 2013) and oversampling of persons with potentially high PFOS exposure and health problems (Li et al., 2020d) were major concerns in these studies. In addition, He et al. (2018) 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 13 *low* confidence studies examined PFOS and HDL or clinically defined low HDL in adults. All studies examined the cross-sectional association (Cong et al., 2021; Han et al., 2021; Ye et al., 2021; Zare Jeddi et al., 2021; Bjorke-Monsen et al., 2020; Canova et al., 2020; Fan et al., 2020; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020d; Lin et al., 2020a; Liu et al., 2020b; Chen et al., 2019; Christensen et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Yang et al., 2018; van den Dungen et al., 2017; Wang et al., 2012) including Dong et al. (2019) in the adult portion of the study. Two studies additionally examined the association between baseline PFOS and changes in HDL (Liu et al., 2020b; Liu et al., 2018b). 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) reported statistically positive associations with HDL. Canova et al. (2020) also reported a concentration-response curve when PFOS was categorized in deciles. An overlapping study (Zare Jeddi et al., 2021) in the same community was consistent with Canova et al. (2020), 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 et al., 2019) (see Appendix D, (U.S. EPA, 2024a)). The POUNDS-Lost study (Liu et al., 2020b), most cycles of NHANES 2003–2014 (Dong et al., 2019), a study conducted in a Taiwanese high-exposure community (Lin et al., 2020d), and a cross-sectional analysis (Han et al., 2021) 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 5 of 13 studies (Li et al., 2020d; Lin et al., 2020a; He et al., 2018; Liu et al., 2018b; Yang et al., 2018) (association with concurrent HDL). Of note, in Lin et al. (2020a), the positive association was limited to linear PFOS only; the association between branched PFOS and HDL was inverse and statistically significant (Lin et al., 2020a). 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 13 *low* confidence studies examined the association between PFOS and TG or hypertriglyceridemia. All studies examined the cross-sectional association (Cong et

al., 2021; Han et al., 2021; Ye et al., 2021; Zare Jeddi et al., 2021; Canova et al., 2020; Fan et al., 2020; Khalil et al., 2020; Li et al., 2020d; Lin et al., 2020a; Liu et al., 2020b; Chen et al., 2019; Christensen et al., 2019; Donat-Vargas et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; Lin et al., 2013; Wang et al., 2012); three studies additionally examined the association between baseline PFOS and changes in TG or incident hypertriglyceridemia (Liu et al., 2020b; Lin et al., 2019; Liu et al., 2018b). Higher PFOS was significantly associated with higher levels of TG in the DPPOS (Lin et al., 2019) (see Appendix D, (U.S. EPA, 2024a)). 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 et al., 2019). In contrast, PFOS was not associated with triglycerides or changes in triglycerides in the POUNDS-Lost study (Liu et al., 2020b), a cross-sectional analysis (Han et al., 2021) of type 2 diabetes cases and healthy controls, and a high-exposure community study in Italian young adults (aged 20–39 years) (Zare Jeddi et al., 2021). Furthermore, PFOS was inversely associated with TG in the three studies using overlapping NHANES data (Christensen et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d) and in Donat-Vargas et al. (2019). 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 et al., 2019). In *low* confidence studies, five reported inverse associations (Li et al., 2020d; Lin et al., 2020a; He et al., 2018; Liu et al., 2018b; Lin et al., 2013), six reported essentially null associations (Cong et al., 2021; Ye et al., 2021; Canova et al., 2020; Khalil et al., 2020; Chen et al., 2019; Sun et al., 2018), one reported a positive association (Yang et al., 2018), and one qualitatively stated the association was not statistically significant (Wang et al., 2012). Altogether, the association between PFOS and TG was inconsistent.

In summary, in the general adult population, the available evidence 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). 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 (Rotander et al., 2015; Wang et al., 2012); one investigated the association between baseline PFOS and changes in TC over the course of a fluorochemical plant demolition project (Olsen et al., 2012). PFOS was positively associated with TC in Rotander et al. (2015), but the association was not statistically significant. The other cross-sectional study simply reported no significant association (Wang et al., 2012). Olsen et al. (2012) reported an inverse or positive association between changes in PFOS and changes in TC,

depending on whether the outcome was log transformed (Olsen et al., 2012). 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. On the basis of 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 et al. (2015), but this association was not statistically significant. The other cross-sectional study simply stated that no significant association was found (Wang et al., 2012). 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 et al., 2012). 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 (Rotander et al., 2015; Olsen et al., 2012; Wang et al., 2012). PFOS was positively associated with HDL in Rotander et al. (2015), but this association was not statistically significant. The other cross-sectional study simply stated that no significant association was found (Wang et al., 2012). In Olsen et al. (2012), changes in PFOS over the demolition project was positively associated with changes in HDL (Olsen et al., 2012). Together, the occupational studies suggest a positive association between PFOS and HDL in workers, although these findings were limited by potentially unmeasured confounding (Rotander et al., 2015; Olsen et al., 2012) and self-selection of subjects (Rotander et al., 2015).

Two *low* confidence cross-sectional studies examined PFOS and TG in workers and found that PFOS was inversely associated with TG in Rotander et al. (2015), but this association was not statistically significant. Wang et al. (2012) 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, the available studies examining associations between PFOS serum concentrations and serum lipids among workers was limited to 3 *low* confidence studies. 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, 2016b) and 9 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 13 studies are shown in Figure 3-41.

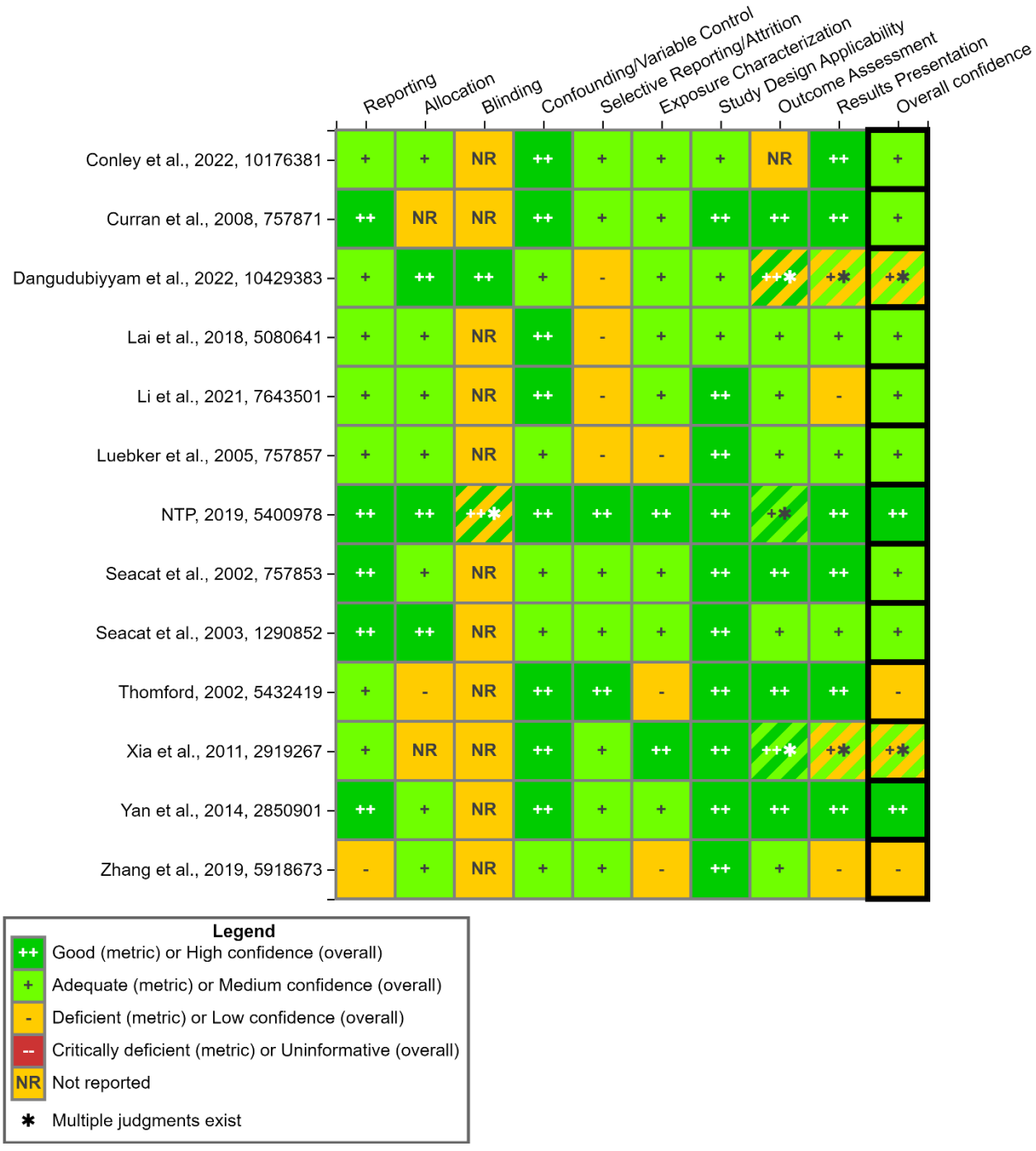


Figure 3-41. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Cardiovascular Effects

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 (Dangudubiyyam et al., 2022; Li et al., 2021c; NTP, 2019; Rogers et al., 2014; Xia et al., 2011; Curran et al., 2008). In male and female mice (sexes combined), relative heart weight was increased at PND 21 after gestational

exposure (GD 2–21) to 2 mg/kg/day PFOS; however, this was confounded by decreased body weights. Absolute heart weights were unchanged (Xia et al., 2011). 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 (NTP, 2019). 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. 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 et al., 2017). 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 et al., 2021c). In female Sprague-Dawley rats exposed to 50 µg/mL PFOS in drinking water from GD 4–20, H&E and Trichrome-Masson staining of the heart revealed a significant increase in ventricular wall thickness as well as a slight increase in the percentage of fibrotic area (approximately 1% in the control animals and 2% in the exposed animals) (Dangudubiyyam et al., 2022).

Curran et al. (2008) 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. However, in female Sprague-Dawley rats exposed to 0.5–50 µg/mL PFOS in drinking water from GD 4–20, blood pressure was significantly increased at GD 20 (Dangudubiyyam et al., 2022). Adult Sprague-Dawley offspring of dams treated with PFOS (18.75 mg/kg/day) via oral gavage from GD 2–6 had increased blood pressure measurements (Rogers et al., 2014). 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 with control animals). Rogers et al. (2014) 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), 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). 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 3-42). Decreased serum TC, triglycerides, and/or HDL levels occurred in rhesus monkeys (Goldenthal et al., 1979), cynomolgus monkeys (Seacat et al., 2002), rats (Conley et al., 2022; NTP, 2019; Curran et al., 2008; Luebker et al., 2005b; Thibodeaux et al., 2004; Seacat et al., 2003), and mice (Lai et al., 2018; Wang et al., 2014; Yan et al., 2014; Wan et al., 2012;

Bijland et al., 2011) 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). 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 et al., 2018). One study reported decreased serum HDL levels but an approximate twofold increase in serum LDL levels in male BALB/c mice following exposure to 5 mg/kg/day PFOS by gavage for 28 days (Yan et al., 2014).

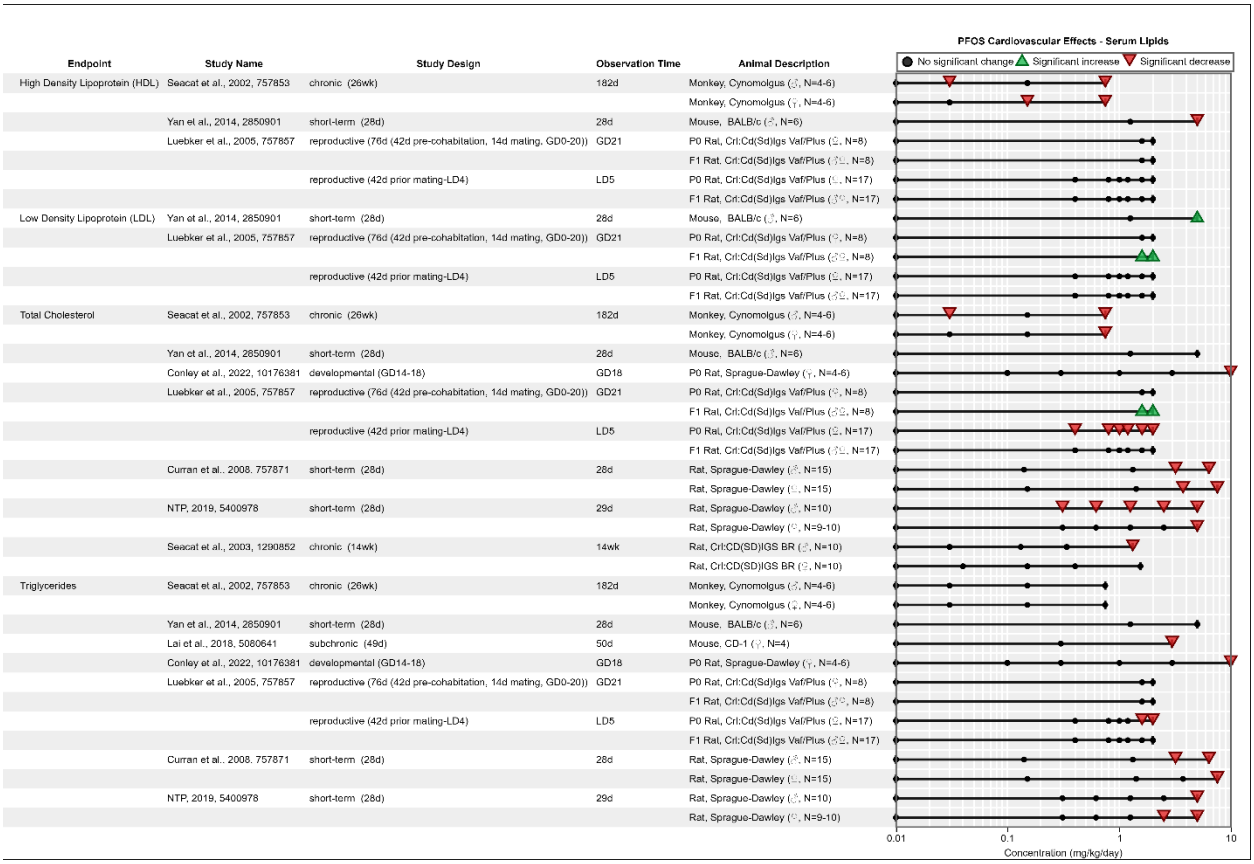


Figure 3-42. Serum Lipid Levels in Animal Models Following Exposure to PFOS

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 (Oppi et al., 2019; Getz and Reardon, 2012). Some rodent studies (Yan et al., 2014) 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 et al., 2021; Fragki et al., 2021; Schlezinger et al., 2020), 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 with 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, 2016b). There are nine 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 organized by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-43.

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 3-43. Summary of Mechanistic Studies of PFOS and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

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 et al., 2019). 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 et al., 2020a). 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 et al., 2019). 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 for Perfluorooctane Sulfonate (PFOS)*(U.S. EPA, 2016b) provides mechanistic evidence related to these outcomes (Fletcher et al., 2013). 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 et al., 2020).

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 buildup 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 et al., 2020a). One epidemiological study found no significant associations between PFOS and carotid artery atherosclerotic plaque or CIMT (Lind et al., 2017b), but two other studies found significant associations between PFOS and CIMT (Lin et al., 2016; Lin et al., 2013).

3.4.3.3.4 Endothelial Dysfunction

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 et al., 2016). 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 with 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 et al., 2016).

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 et al., 2020a); however, unlike Lin et al. (2016), 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 with 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) 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) 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 et al., 2018e; Liu et al., 2017a). 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 et al., 2018e). 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 et al., 2018e). 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 et al., 2017a). 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 et al., 2017a). This demonstrated the potential for PFOS to activate the plasma KKS, but at a relatively high concentration compared with typical human exposure levels in the general population. PFAS with longer carbon chain lengths activated the KKS at a much lower concentration compared with 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 et al., 2017a).

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. (2018e), and kallikrein-like activity was significantly increased only at 3 and 5 mM PFOS (Liu et al., 2018e; Liu et al., 2017a). 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 et al., 2017a).

Binding of PFOS with purified human FXII was further evaluated by Liu et al. (2017a) 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 et al., 2017a).

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 PFOS HESD evaluated the potential of

PFOS to affect these pathways. Bassler et al. (2019) 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) 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 TC and LDL. 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.

On the basis of this systematic review of 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 to be 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 PFOS HESD. The positive associations with TC are also supported by the recent meta-analysis restricted to general population studies in adults (U.S. EPA, 2024b). 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 et al., 2021).

The human epidemiological studies identified since the 2016 PFOS HESDs provided additional clarity regarding the association between PFOS and CVD outcomes. Most of the CVD-related 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 postpartum. 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 PFOS HESD also indicated association with blood pressure in children.

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 of 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 (NCEH1 and PPARα) in samples from women, while there were no associations in men. The results for PFOS-induced changes in serum lipid levels contrast between rodents (generally decreased) and humans (generally increased). PFOS exposure led to upregulation 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 evidenced 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 3-15). 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. The consistent findings for serum lipids are also supported by evidence of associations with blood pressure in adult populations in *high* and *medium* confidence studies.

Table 3-15. Evidence Profile Table for PFOS Exposure and 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 28 *Medium* confidence studies 21 *Low* confidence studies 12 *Mixed*[a] studies	Examination of serum lipids included measures of TC, LDL, HDL, TG, and VLDL. In studies of serum lipids in adults from the general population (33), there is evidence of positive associations with TC (13/15) in the *medium* confidence studies. Positive associations were also observed for LDL (9/11) *medium* confidence studies. Results for HDL and TG were mixed, with some positive associations for HDL (8/14) and some inverse associations for TG (8/13) in *medium* confidence studies. Evidence from studies of children (21), reported significant increases in TC (7/16) and LDL (7/16), though others observed no association. While some studies observed significantly increased HDL (3/17), others reported significant decreases or no associations. Studies examining pregnant	• *High* and *medium* confidence studies • *Consistent* findings of positive associations for LDL and TC across study populations • *Coherence* of findings across serum lipids	• *Low* confidence occupational studies	⊕⊕⊙ *Moderate* Evidence for cardiovascular effects is based on numerous *medium* confidence studies reporting positive associations with serum lipids (LDL and TC) 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. However, interpretations of changes in serum lipids for children are less clear. *High* and *medium* confidence studies reported positive associations with blood pressure and increased risk of hypertension. *Low* confidence studies reported nonsignificant associations, while most *mixed* confidence studies reported significant associations. Observed	*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. The consistent findings for serum lipids are also supported by evidence of associations with blood pressure in adult populations in *high* and *medium* confidence 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. Most occupational studies (5) were considered *low* confidence (4/5), and no association was observed for TC or HDL-C in the single *medium* confidence occupational study.			effects were inconsistent for CVD and imprecise for atherosclerotic changes across all study populations.	
Blood pressure and hypertension 2 *High* confidence studies 17 *Medium* confidence studies 7 *Low* confidence studies	Studies examining changes in blood pressure, including DBP and SBP, and risk of hypertension in general population adults showed consistent positive associations with increased risk of hypertension (4/7), positive associations for SBP (7/9) and DBP (7/8), including four *medium* or *high* confidence studies reporting significant increases (4/6). Studies in children (10) reported mostly nonsignificant associations with blood pressure and/or	• *High* and *medium* confidence studies	• *Inconsistent findings* 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**	
	hypertension, though one study in adolescents reported significantly increased DBP (1/10) and another reported decreased (1/10) SBP. No studies examined blood pressure or hypertension in occupational populations.				
Cardiovascular disease 1 *High* confidence study 4 *Medium* confidence studies 5 *Low* confidence studies	In adults from the general population (8), significantly decreased odds of stroke (1/2) and significantly increased odds of MVD (1/1), heart attack (1/1), and CVD in the third exposure group (1/4), were observed. Other studies of stroke, CHD, and CVD reported nonsignificant 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 reported a significant inverse relationship between employees in high-	• *High* and *medium* confidence studies	• *Limited number* of studies examining specific outcomes • *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**	
	exposure jobs and all heart disease mortality (1/2).				
Atherosclerotic changes 1 *High* confidence study 4 *Medium* confidence studies 3 *Low* confidence studies	In studies of children and young adults (3), two studies observed significant associations with CIMT across exposure groups (2/3), among females, and among those ages 12–19. In studies of adults from the general population (5), two focused on adults older than 70 years of age. One study reported a significant increase in left ventricular end-diastolic diameter and a significant decrease in relative wall thickness (1/2). One *medium* confidence study in prediabetic adults aged over 25 also reported significantly increased odds of an Agatatson Scores over 400, a measure of arterial calcification (1/1).	• *High* and *medium* confidence studies	• *Imprecision* of findings across children and adult study populations • *Limited number* of studies examining specific outcomes		
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	• *High* and *medium* confidence studies • *Consistency* of findings across species, sex, or study design	• *Incoherence* of findings in other cardiovascular outcomes •	⊕⊕⊙ *Moderate* Evidence based on eight *high* or *medium* confidence studies	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	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 was observed in pups. Fewer studies examined HDL and LDL, with decreases in HDL (2/3) and increases in LDL (2/2) being observed.	• *Dose-response* relationship observed within multiple studies	• *Biological significance* of the magnitude of effect is unclear	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 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	• *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		

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**	
	absolute heart weight and an increase in relative heart weight which was confounded by decreases in body weights.				
Blood pressure and heart rate 3 *Medium* confidence studies	A developmental study found increased blood pressure in dams. A short-term study found no effect on blood pressure in male and female rats. One developmental study found no effect on heart rate.	• *Medium* confidence studies	• *Limited number* of studies examining outcome		

Mechanistic Evidence and Supplemental Information (Section 3.4.3.3)	
Summary of Key Findings, Interpretation, and Limitations	**Evidence Stream Judgment**
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 20 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, 4 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.

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. Further discussion of considerations for potential confounding by co-occurring PFAS can be found in Section 5.1.1.

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 and Harding, 2006) and (Perng et al., 2016)). There is some evidence that birth weight deficits can be followed by increased weight gain that may occur especially among those with rapid growth catchup periods during childhood (Perng et al., 2016).

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 toward 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) × 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 and Shlomo, 2003). 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 Summary of Evidence From the 2016 PFOS HESD

The 2016 PFOS HESD (U.S. EPA, 2016b) summarized epidemiological studies of developmental effects in relation to PFOS exposure. There are 18 studies from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and developmental effects. Study quality evaluations for these 18 studies are shown in Figure 3-44. Studies included those conducted both in the general population as well as in communities known to have experienced relatively high PFAS exposure (e.g., the C8 population in West Virginia and Ohio). Results from studies summarized in the 2016 PFOS HESD are described in Table 3-16 and below.

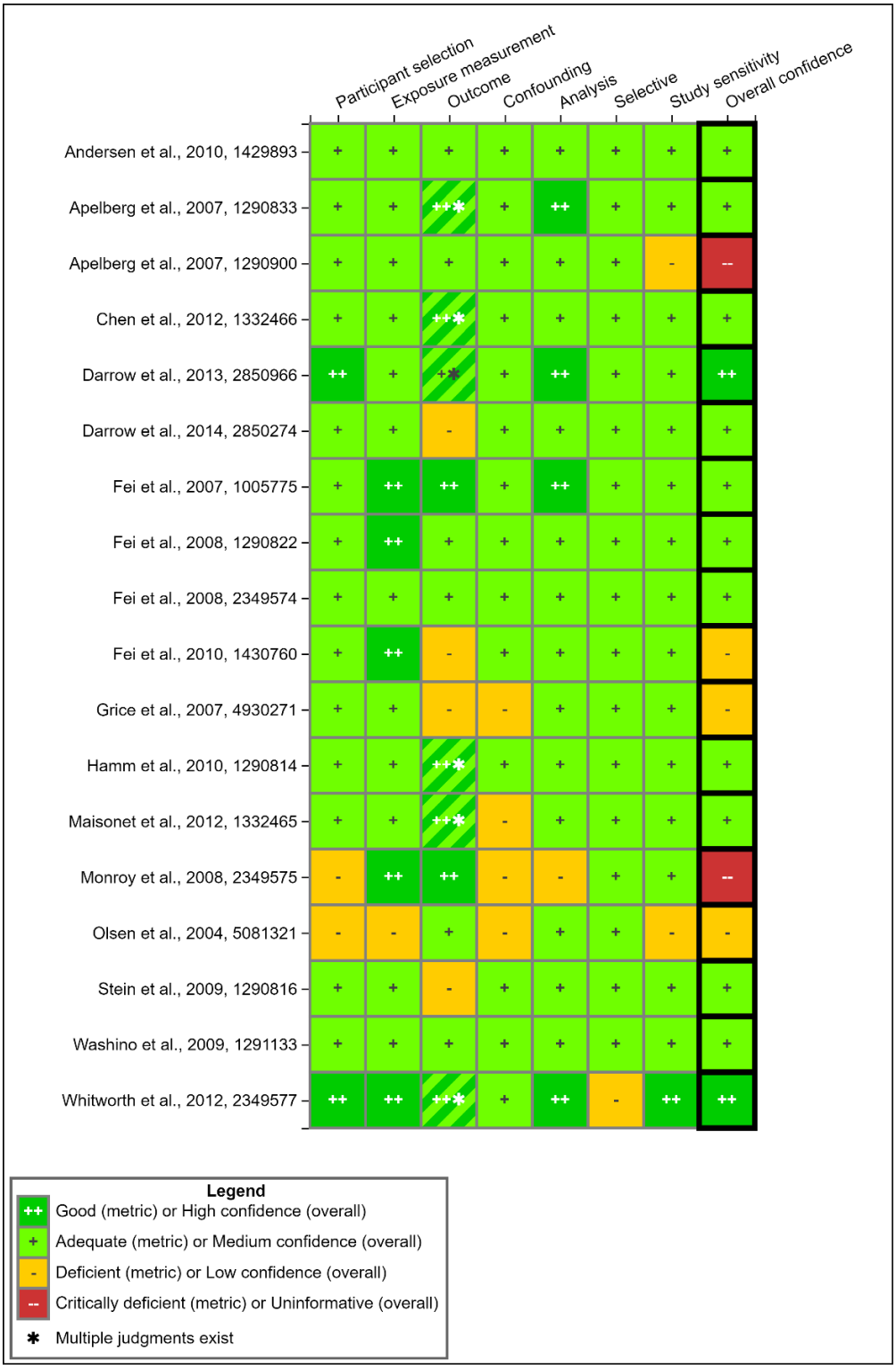


Figure 3-44. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Developmental Effects Published before 2016 (References from 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

As noted in the 2016 PFOS HESD, several available studies measured fetal growth outcomes. Apelberg et al. (2007b) 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) 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 reduced 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 et al., 2010), 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 PFOS 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. (2012a) 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 et al., 2010; Fei et al., 2008b, 2007). 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) found no associations between maternal PFOS concentration (blood samples taken in the first and second trimester) and birth weight. Also, 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 2016 PFOS HESD notes, however, that analyses in this study were limited by small cell sizes due to low incidence of these outcomes. Fei et al. (2008b) 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) 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) 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) 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) and Stein et al. (2009) found no association between maternal serum PFOS and increased risk for miscarriage in this population.

Table 3-16. Associations Between Elevated Exposure to PFOS and Developmental Outcomes in Children From Studies Identified in the 2016 PFOS HESD

Reference, confidence	Study Design	Birth Weight[a]	LBW[b]	SGA[b]	Gestational Duration[a]	Preterm Birth[b]	Birth Defects[b]	Pregnancy Loss[b]	PNG[a]
Andersen, 2010, 1429893[c] *Medium*	Cohort	↓	NA	NA	NA	NA	NA	NA	↓↓
Apelberg, 2007, 1290833 *Medium*	Cross-sectional	↓	NA	NA	↑	NA	NA	NA	NA
Chen, 2012, 1332466[c] *Medium*	Cohort	↓↓	↑	↑↑	↓↓	↑↑	NA	NA	NA
Darrow, 2014, 2850274 *Medium*	Cohort	NA	NA	NA	NA	NA	NA	↑	NA
Darrow, 2013, 2850966 *High*	Cohort	↓	↑	NA	NA	–	NA	NA	NA
Fei, 2007, 1005775[d] *Medium*	Cohort	↓	↑	–	NA	↑	NA	NA	NA
Grice, 2007, 4930271[e] *Low*	Cohort	–	NA	NA	NA	NA	NA	–	NA
Hamm, 2010, 1290814 *Medium*	Cohort	–	NA	–	–	↑	NA	NA	NA
Maisonet, 2012, 1332465 *Medium*	Cohort	↓↓	NA	NA	–	NA	NA	NA	↑
Olsen, 2004, 5081321 *Low*	Cross-sectional	NA	NA	NA	NA	↑	–	NA	NA
Stein, 2009, 1290816 *Medium*	Cohort	NA	↑↑	NA	NA	↑	↑	–	NA
Washino, 2009, 1291133[f] *Medium*	Cohort	↓↓	NA	NA	NA	NA	NA	NA	NA

Reference, confidence	Study Design	Birth Weight[a]	LBW[b]	SGA[b]	Gestational Duration[a]	Preterm Birth[b]	Birth Defects[b]	Pregnancy Loss[b]	PNG[a]
Whitworth, 2012, 2349577 *High*	Cohort	↓	NA	↑	NA	↓	NA	NA	NA

Notes: LBW = low birth weight; NA = no analysis was for this outcome was performed; PNG = postnatal growth; SGA = small-for-gestational age;↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association.

Apelberg et al. (2007a) and Monroy et al. (2008) were not included in the table due to their *uninformative* overall study confidence ratings. Fei et al. (2008a), Fei et al. (2008b), and Fei et al. (2010a) were not included in the table because the studies only analyzed other developmental outcomes that were more prone to measurement error (see Study Evaluation Considerations in Section 3.4.4.1.2) or were not as heavily studied (i.e., other measures of fetal growth restriction such as birth length and head circumference and breastfeeding duration or developmental milestones, respectively).

[a] Arrows indicate the direction in the change of the mean response of the outcome (e.g., ↓ indicates decreased mean birth weight).

[b] Arrows indicate the change in risk of the outcome (e.g., ↑ indicates an increased risk of the outcome).

[c] Chen, 2012, 1332466 reports results from a population overlapping with Chen et al. (2017b), which was considered the most updated data.

[d] Fei, 2007, 1005775 reports results from a population overlapping with Meng et al. (2018), which was considered the most updated data.

[e] Grice, 2007, 4930271 reported results from children born to women in an occupational cohort.

[f] Washino et al. (2009) reports results from a population overlapping with Kashino et al. (2020), which was considered the most updated data.

3.4.4.1.4 Study Inclusion For Updated Literature Searches

There are 78 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD (U.S. EPA, 2016b) 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). More specifically, the Rokoff et al. (2018) study overlapped with the Project Viva study by Sagiv et al. (2018). The Gennings et al. (2020) study is also not further considered here as it is a smaller subset of the Aarhus Birth Cohort described in Wikström et al. (2020). Similarly, the Li et al. (2017) Guangzhou Birth Cohort Study overlapped with a more recent study by Chu et al. (2020). Four studies (Kobayashi et al., 2022; Kobayashi et al., 2017; Minatoya et al., 2017; Kishi et al., 2015) 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). 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)). Although the Kobayashi et al. (2017) 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) study that examined a less accurate in utero growth estimate was not considered in lieu of their more accurate birth outcomes measures reported in the same cohort (Manzano-Salgado et al., 2017a). One additional study by Bae et al. (2015) was the only study to examine sex ratio and was therefore not further considered here.

In general, to best gauge consistency and magnitude of reported associations, EPA largely focused on the most accurate and most prevalent measures within each fetal growth endpoint. Studies with overlapping cohorts were included in the synthesis, as each study provided some unique data for different endpoints. Specifically, the Woods et al. (2017) publication on the Health Outcomes and Measures of the Environment (HOME) cohort overlaps with Shoaff et al. (2018) but has additional mean BWT data (received via communication with study author). The mean BWT results for singleton and twin births from Bell et al. (2018) are included in forest plots here as are the postnatal growth trajectory data in the same UPSTATE KIDS cohort by Yeung et al. (2019) as they target different developmental windows. The Bjerregaard-Olesen et al. (2019) study from the Aarhus birth cohort also overlaps with Bach et al. (2016). 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). Given that additional sex-specific data are available in the Bjerregaard-Olesen et al. (2019) study, the synthesis for head circumference and birth length are based on this subset alone. Chen et al. (2021) 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 PFOS HESD. Six additional studies (Gundacker et al., 2021; Jin et al., 2020; Maekawa et al., 2017; Alkhalawi et al., 2016; Lee et al., 2016; Lee et al., 2013) 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, 12 examined postnatal growth, 5 each examined fetal loss, and birth defects.

3.4.4.1.5 Growth Restriction: Fetal Growth

3.4.4.1.5.1 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 (Eick et al., 2020; Wikström et al., 2020; Wang et al., 2019; Workman et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Bach et al., 2016). Twenty-five of the 34 mean BWT studies shown in Figure 3-45,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 postpartum (Gao et al., 2019; Wang et al., 2019; Xu et al., 2019a; Bell et al., 2018; Gyllenhammar et al., 2018b; Shi et al., 2017; Callan et al., 2016; de Cock et al., 2016; Kwon et al., 2016).

Overall, eight of the PFOS studies relied on umbilical cord measures (Wang et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; de Cock et al., 2016; Govarts et al., 2016; Kwon et al., 2016), and one collected blood samples in infants 3 weeks following delivery (Gyllenhammar et al., 2018b). Results from the Bell et al. (2018) 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 et al., 2019). One study examined pre-conception maternal serum samples (Robledo et al., 2015). Twenty-one studies had maternal serum or plasma PFOS measures that were sampled during trimesters one (Sagiv et al., 2018; Ashley-Martin et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016), two (Lauritzen et al., 2017), or three (Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Kashino et al., 2020; Valvi et al., 2017; Callan et al., 2016), or across multiple trimesters (Chang et al., 2022; Chen et al., 2021; Eick et al., 2020; Wikström et al., 2020; Hjermitslev et al., 2019; Marks et al., 2019; Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016). The study by Meng et al. (2018) 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)) or additional information or data (e.g., Woods et al. (2017)) provided by study authors were used.

Fifteen of the 34 mean BWT studies included in the synthesis were rated *high* in overall study confidence (Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Eick et al., 2020; Wikström et

al., 2020; Bell et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Bach et al., 2016; Govarts et al., 2016), while 12 were rated *medium* (Chang et al., 2022; Chen et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018; Woods et al., 2017; de Cock et al., 2016; Kwon et al., 2016; Lenters et al., 2016; Robledo et al., 2015), and seven were classified as *low* (Gao et al., 2019; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016). Twenty-three of the 27 *high* or *medium* confidence studies detailed in this synthesis were classified as having *good* study sensitivity (Chen et al., 2021; Kashino et al., 2020; Wikström et al., 2020; Hjermitslev et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Woods et al., 2017; Bach et al., 2016; Lenters et al., 2016; Robledo et al., 2015) or *adequate* study sensitivity (Chang et al., 2022; Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Eick et al., 2020; Govarts et al., 2016), while four had *deficient* study sensitivity (Wang et al., 2019; Bell et al., 2018; de Cock et al., 2016; Kwon et al.,

2016) as shown in

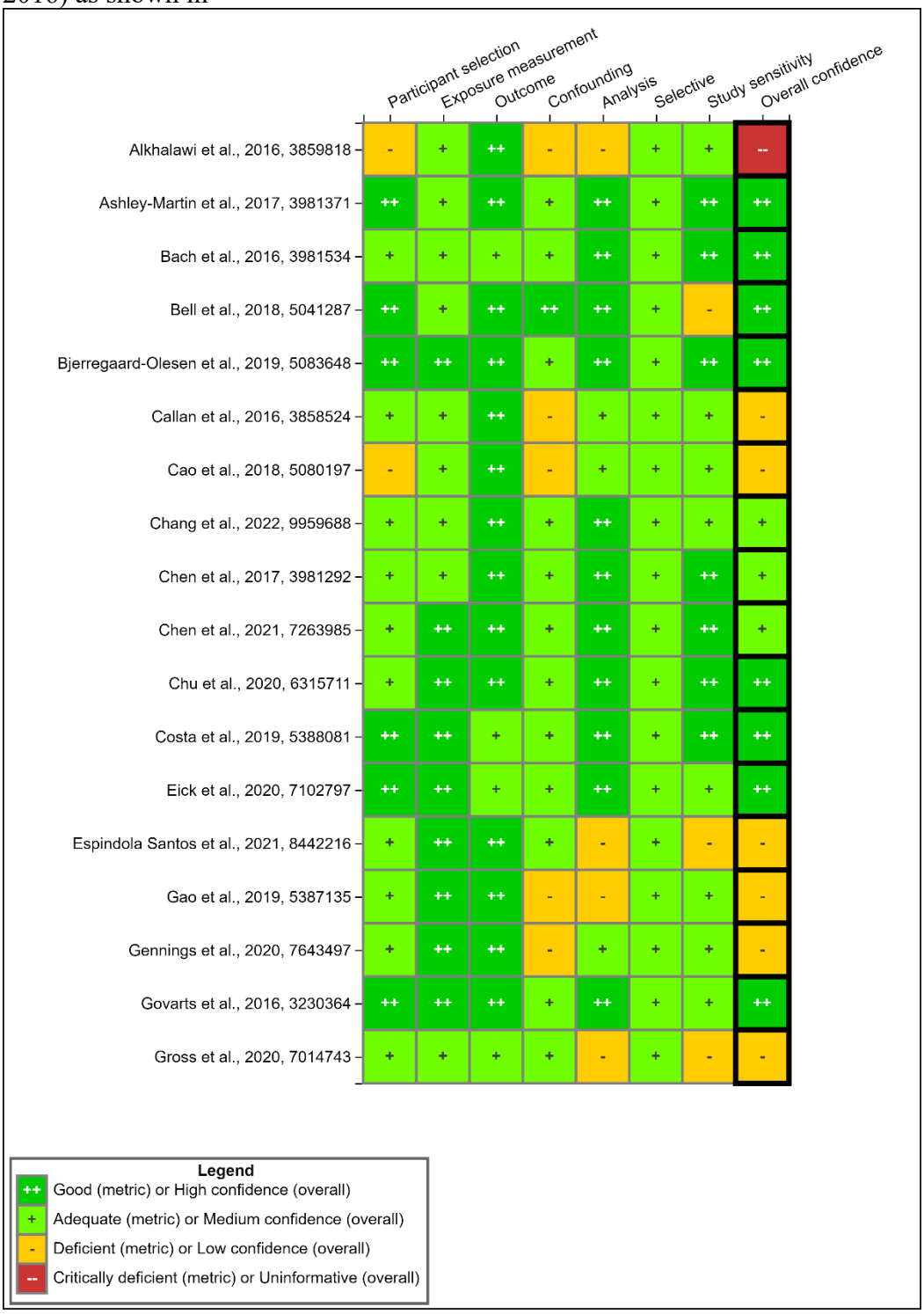


Figure 3-45, Figure 3-46, and Figure 3-47. The median PFOS exposure values across all of the studies were quite variable and ranged from 0.38 ng/mL (Kwon et al., 2016) to 30.1 ng/mL (Meng et al., 2018).

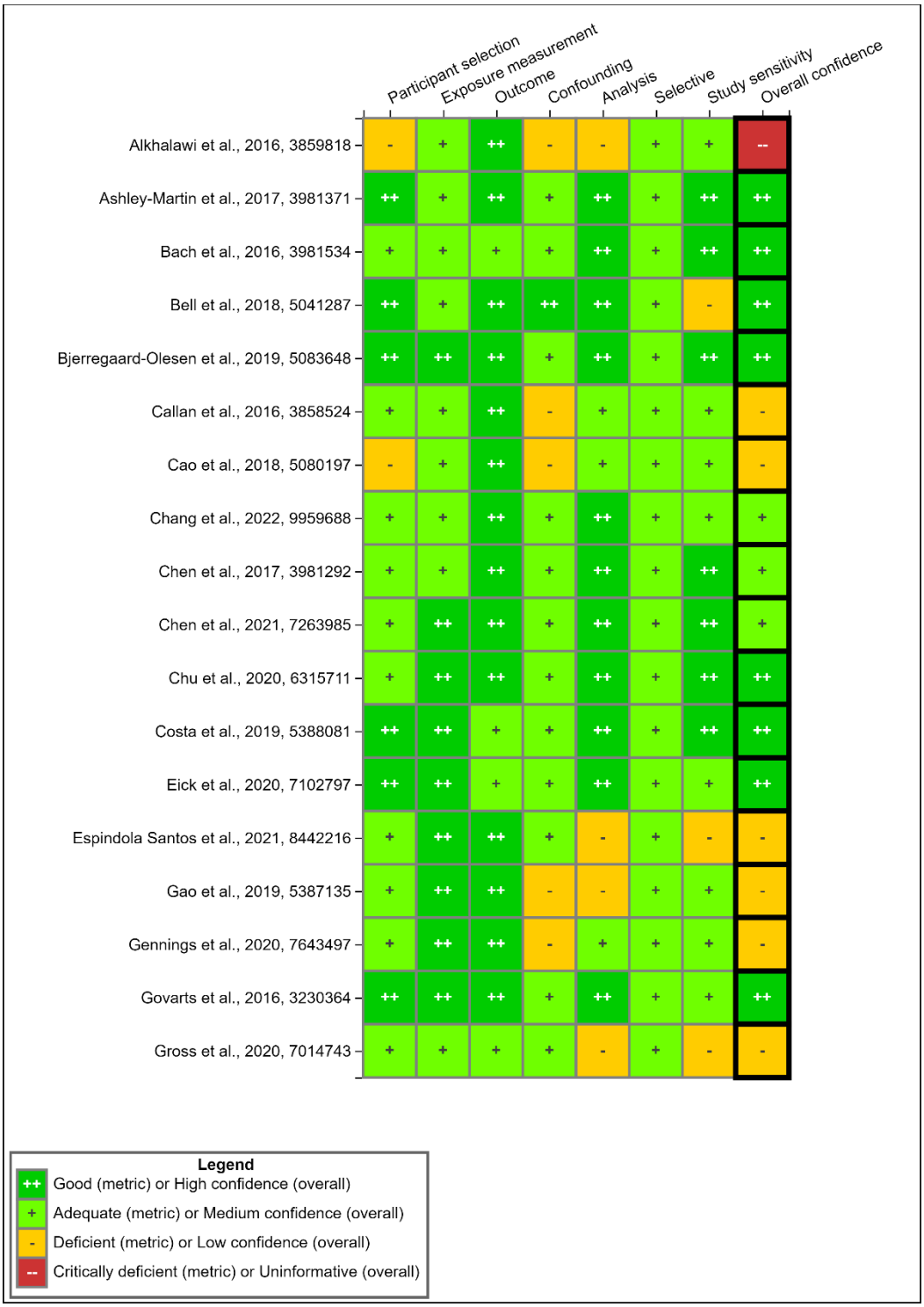


Figure 3-45. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Birth Weight Effects [a]

Interactive figure and additional study details available on HAWC.

[a] Includes six overlapping studies (Bjerregaard-Olesen et al., 2019; Rokoff et al., 2018; Kobayashi et al., 2017; Li et al., 2017; Minatoya et al., 2017; Kishi et al., 2015).

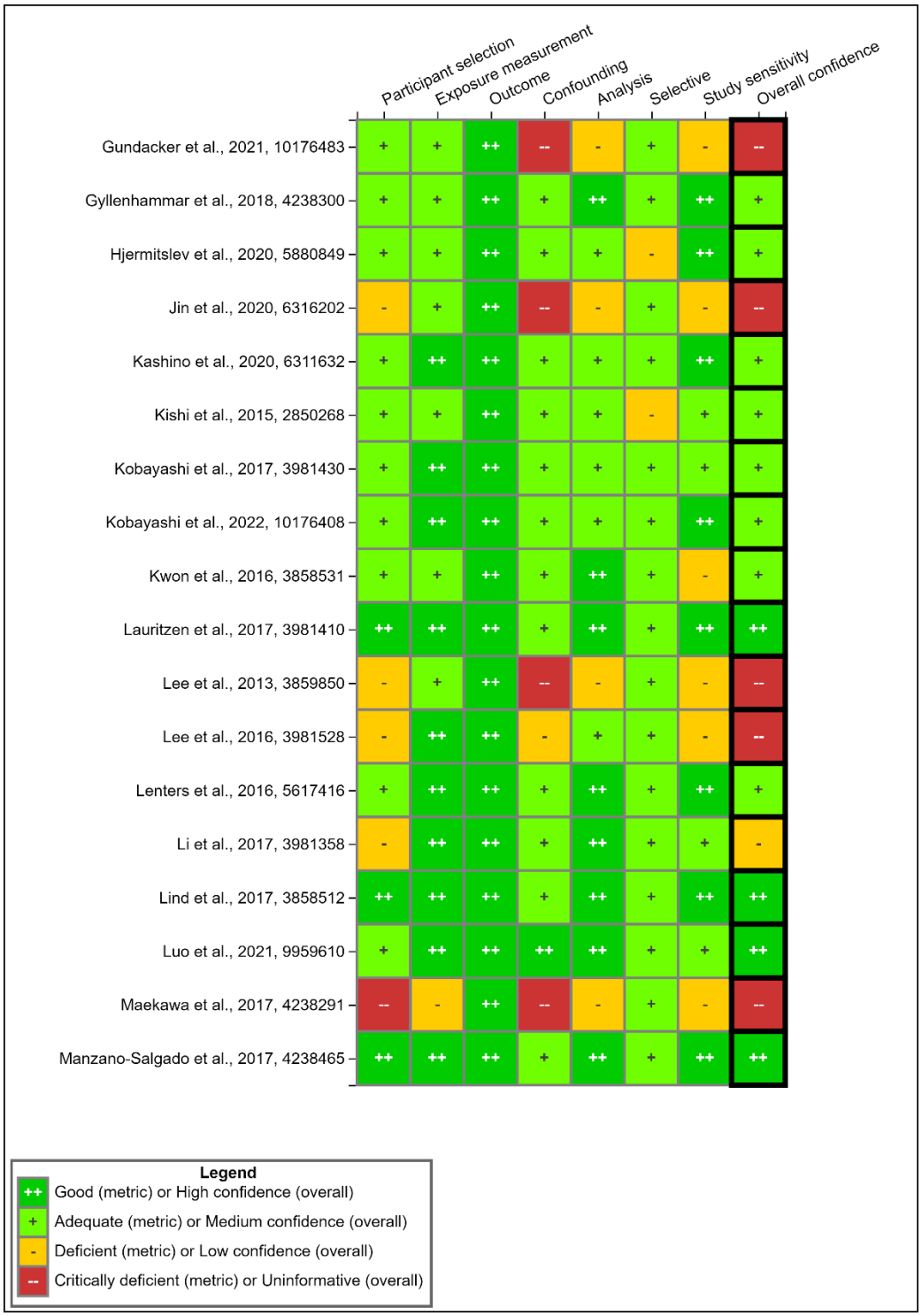


Figure 3-46. Summary of Study Evaluation for Epidemiology Studies of PFOS and Birth Weight Effects (Continued)[a]

Interactive figure and additional study details available on HAWC.

[a] Includes six overlapping studies (Bjerregaard-Olesen et al., 2019; Rokoff et al., 2018; Kobayashi et al., 2017; Li et al., 2017; Minatoya et al., 2017; Kishi et al., 2015).

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.

3.4.4.1.5.1.1 Mean Birth Weight Study Results: Overall Population Studies

Thirty of the 34 included studies that examined mean BWT data in the overall population (Chang et al., 2022; Chen et al., 2021; Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Eick et al., 2020; Kashino et al., 2020; Wikström et al., 2020; Gao et al., 2019; Hjermitslev et al., 2019; Marks et al., 2019; Xu et al., 2019a; Bell et al., 2018; Cao et al., 2018; Gyllenhammar et al., 2018b; Meng et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Shi et al., 2017; Starling et al., 2017; Valvi et al., 2017; Woods et al., 2017; Bach et al., 2016; Callan et al., 2016; de Cock et al., 2016; Govarts et al., 2016; Kwon et al., 2016; Lenters et al., 2016; Robledo et al., 2015; Wu et al., 2012), while four only reported sex-specific data only (Marks et al., 2019; Ashley-Martin et al., 2017; Lind et al., 2017a; Robledo et al., 2015). 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 3-48, Figure 3-49, Figure 3-50, Figure 3-51, and Figure 3-52**)**.

Nine mean BWT studies in the overall population reported null associations (Chang et al., 2022; Chen et al., 2021; Eick et al., 2020; Gao et al., 2019; Hjermitslev et al., 2019; Cao et al., 2018; Manzano-Salgado et al., 2017a; Woods et al., 2017; Govarts et al., 2016), while two reported increased mean BWT deficits (Shi et al., 2017; de Cock et al., 2016). Only two studies (Sagiv et al., 2018; Starling et al., 2017) out of 10 studies which examined categorical data (Chang et al., 2022; Eick et al., 2020; Wikström et al., 2020; Gao et al., 2019; Meng et al., 2018; Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Bach et al., 2016 Cao, 2018, 5080197; Govarts et al., 2016) showed inverse monotonic exposure-response relationships. Although two studies (Meng et al., 2018; Bach et al., 2016) 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 from –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. However, five (Wikström et al., 2020; Hjermitslev et al., 2019; Meng et al., 2018; Sagiv et al., 2018; Bach et al., 2016) of eight *medium* and *high* confidence studies still reported evidence of mean BWT deficits based on early pregnancy biomarker samples.

3.4.4.1.5.1.2 Mean BWT-Overall Population Summary

Eighteen of the 19 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 10 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.

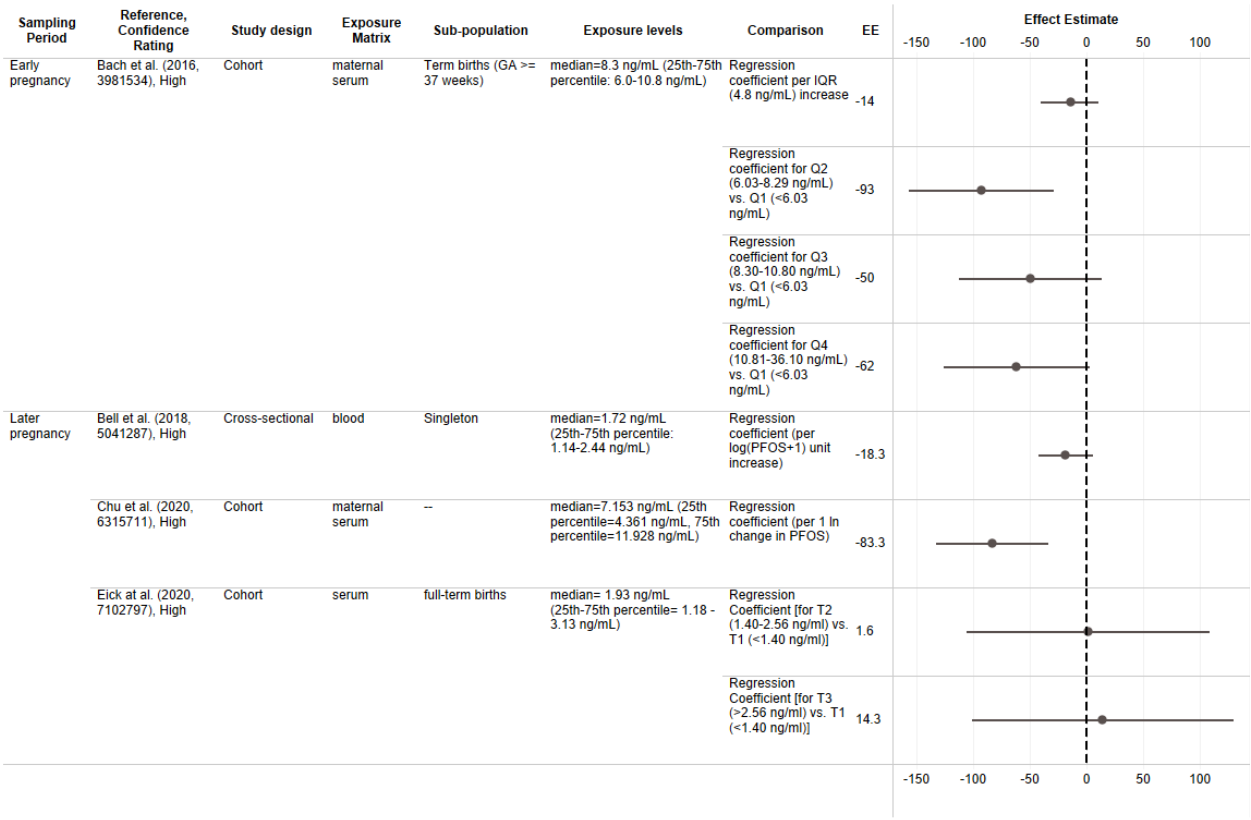


Figure 3-48. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.

Sampling Period	Reference, Confidence Rating	Study design	Exposure Matrix	Sub-population	Exposure levels	Comparison	EE
Early pregnancy	Manzano-Salgado et al. (2017, 4238465) High	Cohort	plasma, maternal blood	--	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
Later pregnancy	Govarts et al. (2016, 3230364), High	Cohort	cord blood	--	geometric mean = 2.63 ug/L (25th-75th percentile = 1.70-3.80 ug/L)	Regression coefficient (per IQR change in PFOS z-score)	10.8
	Lauritzen et al. (2017, 3981410) High	Cohort	maternal serum	--	Norway: median=9.74 ng/mL (range: 0.95-59.6 ng/mL); Sweden: median=16.4 ng/mL (range: 2.28-55.2 ng/mL)	Regression coefficient per unit increase in lnPFOS	-15.1
	Luo et al. (2021, 9959610), High	Cohort	maternal blood, cord blood	--	median (25th-75th percentile): 5.01 ng/mL (3.32-7.62)	Regression coefficient (per ln-ng/mL increase PFOS)	-93.3

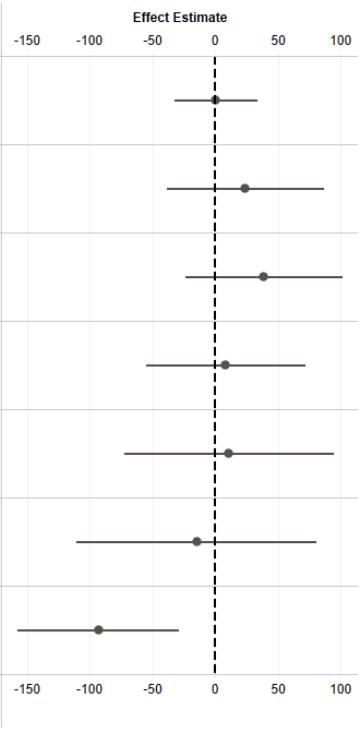


Figure 3-49. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

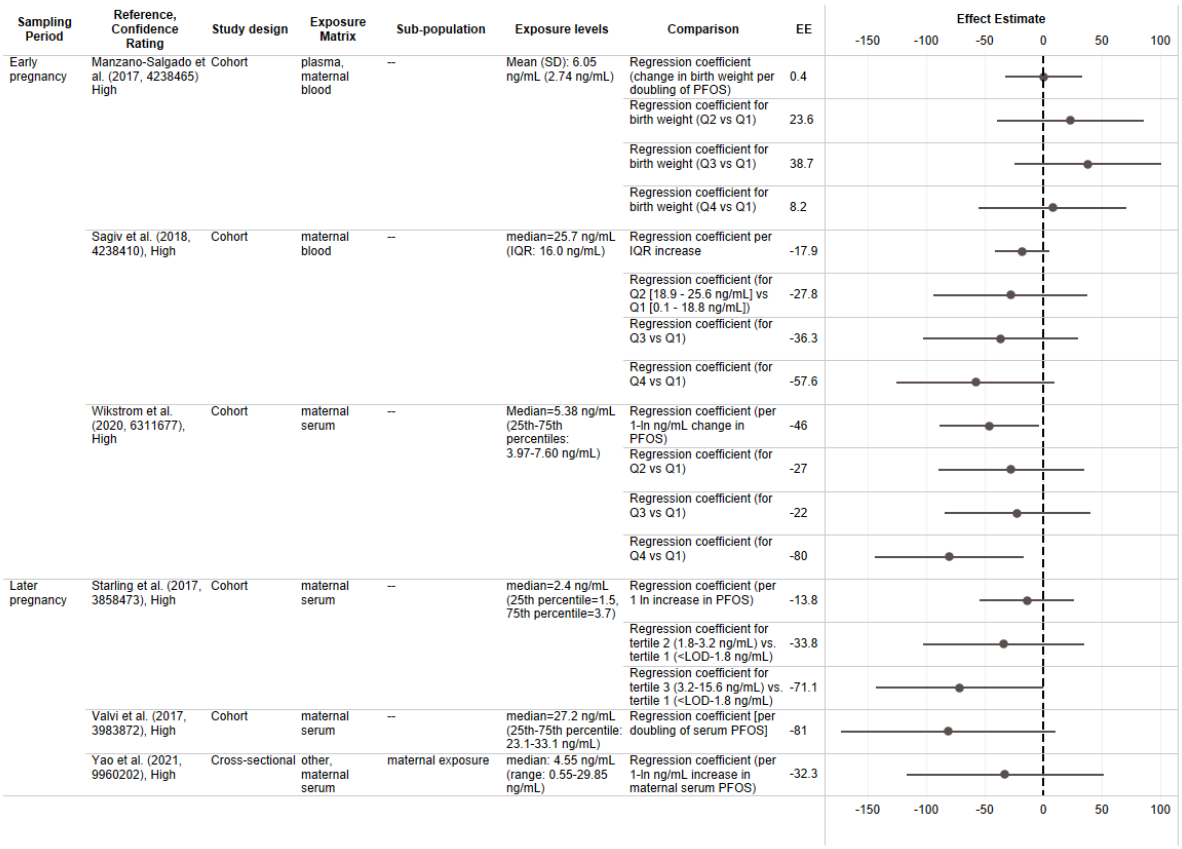


Figure 3-50. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

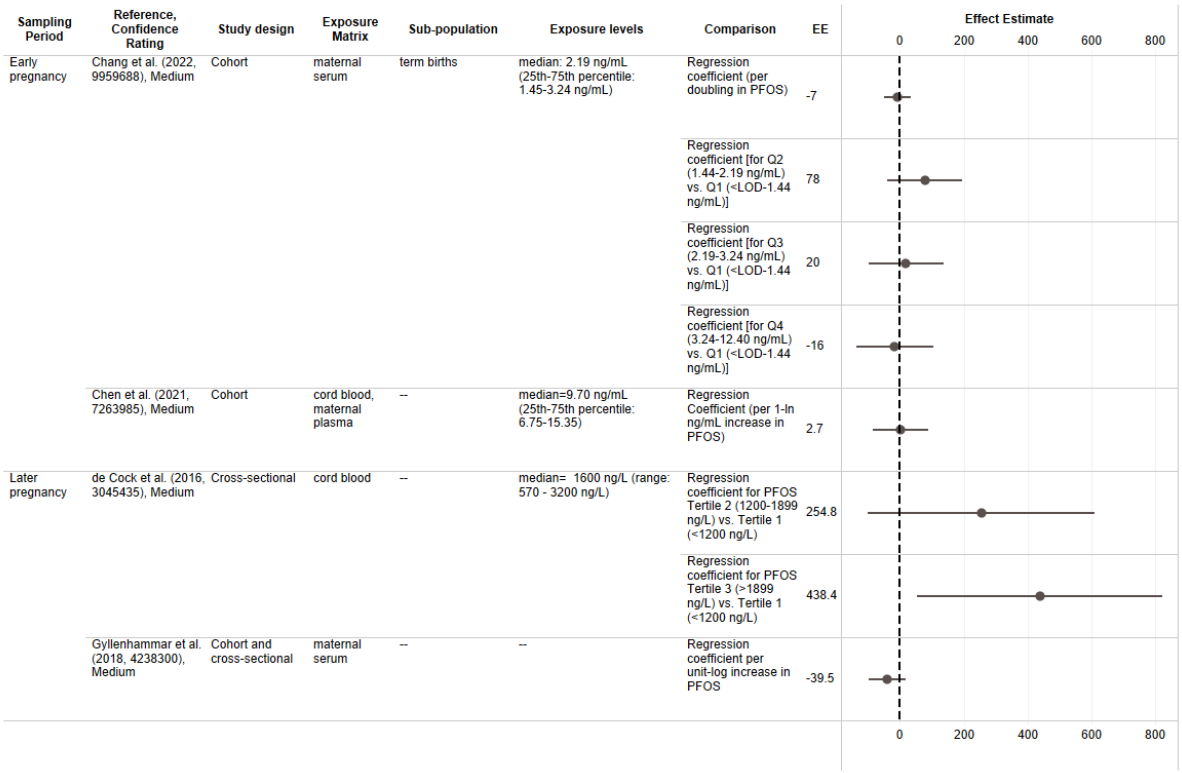


Figure 3-51. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

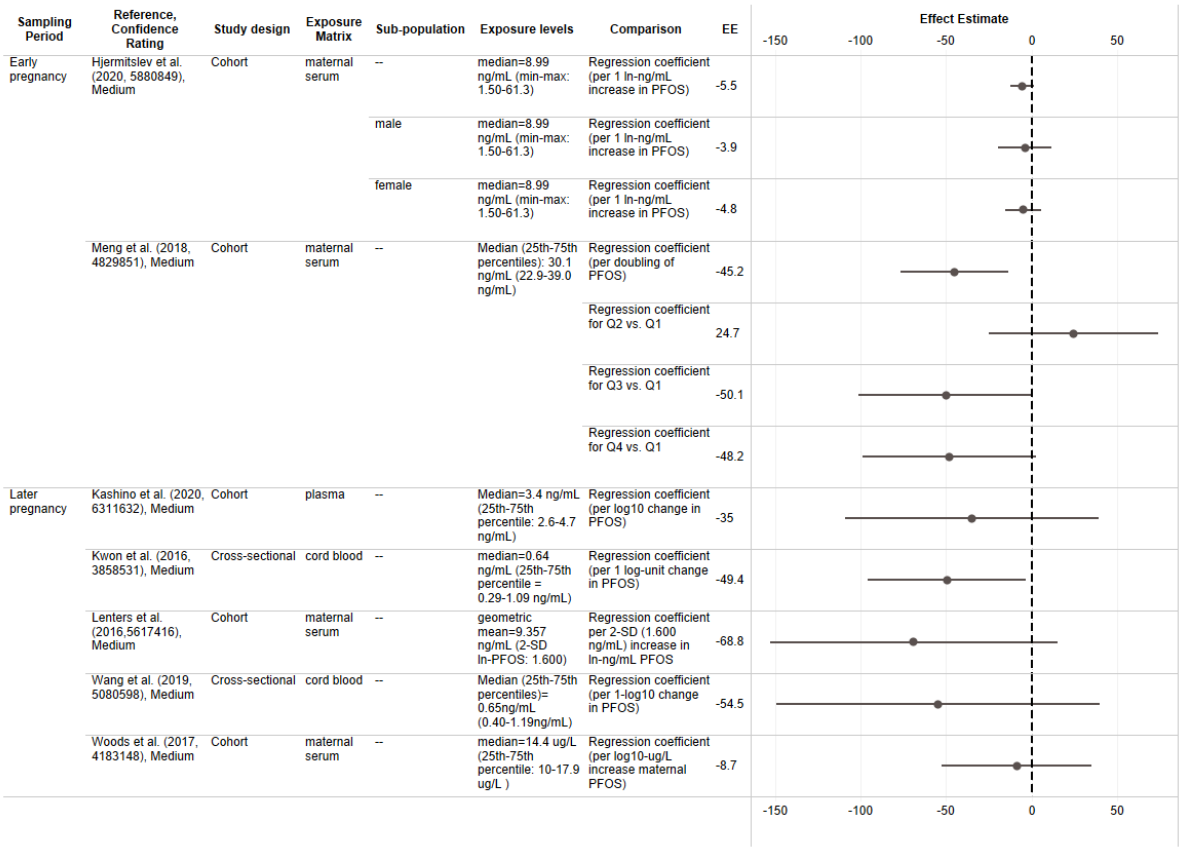


Figure 3-52. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOS (Continued)

Interactive figure and additional study details available on HAWC.

3.4.4.1.5.1.3 Mean Birth Weight Study Results: Sex-Specific Studies

Ten of 16 epidemiological studies examining sex-specific results in male neonates showed some BWT deficits. The remaining six studies (Hjermitslev et al., 2019; Cao et al., 2018; Ashley-Martin et al., 2017; Shi et al., 2017; de Cock et al., 2016; Robledo et al., 2015) in male neonates were either null or showed larger birth weights with increasing PFOS exposures. Six of 15 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 deficits comparable in magnitude among boys and girls (Chu et al., 2020; Wang et al., 2019). Three of these studies (Wikström et al., 2020; Meng et al., 2018; Bach et al., 2016) showed larger deficits among girls and one showed larger deficits among boys (Kashino et al., 2020). The *low* confidence study by Marks et al. (2019) of males only detected a small statistically significant association (β per each ln-unit PFOS increase: −8.5 g; 95% CI: −15.9, −1.1) and showed an exposure-response with reported

large deficits in PFOS tertile 2 (β: −26.6 g; 95% CI: −147.3, 94.2) and tertile 3 (β: −83.9 g; 95% CI: −201.4, 33.7) compared with the tertile 1 referent. Four other studies reported mean BWT deficits only in boys (Lind et al., 2017a; Manzano-Salgado et al., 2017a; Valvi et al., 2017); no studies reported deficits in girls only.

Overall, there was more evidence of inverse 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 with early sampled studies (38%). Study sensitivity was not an explanatory factor among the null studies in either sex.

3.4.4.1.5.1.4 Standardized Birth Weight Measures

Fifteen studies examined standardized BWT measures including 14 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 (Gardener et al., 2021; Eick et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Sagiv et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Bach et al., 2016), four were *medium* (Wang et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018; Chen et al., 2017b) and three were *low* confidence (Espindola-Santos et al., 2021; Gross et al., 2020; Workman et al., 2019) (Figure 3-45, Figure 3-46, Figure 3-47).

Nine of the 15 studies showed some evidence of inverse associations between PFOS exposures and BWT z-scores. Six of these were *high* confidence (Gardener et al., 2021; Wikström et al., 2020; Xiao et al., 2019; Sagiv et al., 2018; Shoaff et al., 2018; Bach et al., 2016), two were *medium* confidence (Wang et al., 2019; Chen et al., 2017b) and one was *low* confidence (Gross et al., 2020). 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) reported that participants in the highest PFOS exposure quartile (relative to the lowest quartile) had a higher odds ratio (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 et al., 2021; Gyllenhammar et al., 2018b) also reported that there were no statistically significant interactions for their BWT-z measures by sex.

Among the 14 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) reported a statistically significant association between mean BWT z-score and PFOS quartile 2 (β: −0.15; 95% CI: −0.29, −0.02) and quartile 4 (β: −0.11; 95% CI: −0.25, 0.02) only, with no exposure-response relationship detected. Although not statistically significant, both Wang et al. (2019) (β: −0.15; 95% CI: −0.41, 0.11) and Shoaff et al. (2018) reported associations similar in magnitude for their overall population (β: −0.12; 95% CI: −0.36, 0.13). The *medium* confidence study by Chen et al. (2017b) reported inverse associations in the overall population (β: −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) reported

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

3.4.4.1.5.1.5 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 studies in general. Twice as many studies showing inverse 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, 9 different studies out of 15 showed some suggestion of inverse associations in the overall population or either or both sexes.

3.4.4.1.5.2 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. (2017a)). Overall, 11 studies examined either or both LBW or SGA in relation to PFOS exposure with 4 classified as *high* confidence (Chu et al., 2020; Wikström et al., 2020; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a), three as *medium* confidence (Hjermitslev et al., 2019; Govarts et al., 2018; Meng et al., 2018), three as *low* confidence, (Chang et al., 2022; Souza et al., 2020; Xu et al., 2019a) and one as *uninformative* (Arbuckle et al., 2013). Six of these studies had *good* sensitivity (Chu et al., 2020; Wikström et al., 2020; Hjermitslev et al., 2019; Meng et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a), while five were considered

adequate (Chang et al., 2022; Souza et al., 2020; Xu et al., 2019a; Govarts et al., 2018; Arbuckle et al., 2013).

Four (Souza et al., 2020; Wikström et al., 2020; Xu et al., 2019a; Lauritzen et al., 2017) of the seven SGA studies reporting main effects showed some increased risk, while three studies were null (Chang et al., 2022; Govarts et al., 2018; Manzano-Salgado et al., 2017a). The magnitude of odds ratios (ORs) across the four studies showing increased risk 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 3-53 and Figure 3-54) with the two low confidence studies showing the largest risks. For example, Xu et al. (2019a) reported an OR of 4.14 (95% CI: 1.07, 16.0) for each log10 unit increase in PFOS. Souza et al. (2020) reported an OR of 3.67 (1.38–9.74) in quartile 4 relative to quartile 1. The *high* confidence Lauritzen et al. (2017) 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) 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 per each ln-unit change = 1.19; 95% CI: 0.87, 1.64) 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 (Figure 3-53 and Figure 3-54). SGA findings from *low* confidence studies are not included in figures. 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. Few patterns were discernible across study characteristics or study confidence for these SGA findings, although the number of studies was small.

Sampling Period	Reference, Confidence Rating	Exposure Matrix	Study Design	Exposure Levels	Sub-population	Comparison	EE
Early pregnancy	Manzano-Salgado et al. (2017, 4238465) High	plasma, maternal blood	Cohort	Mean (SD): 6.05 ng/mL (2.74 ng/mL)	Boys	OR (per doubling in maternal plasma PF..	1.01
					Girls	OR (per doubling in maternal plasma PF..	0.84
					--	OR (per doubling in maternal plasma PF..	0.92
	Wikstrom et al. (2020, 6311677), High	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, 3981410) High	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); Sweden: median=16.4 ng/mL (range: 2.28-55.2 ng/mL)	--	OR (per ln unit increase in PFOS)	0.95

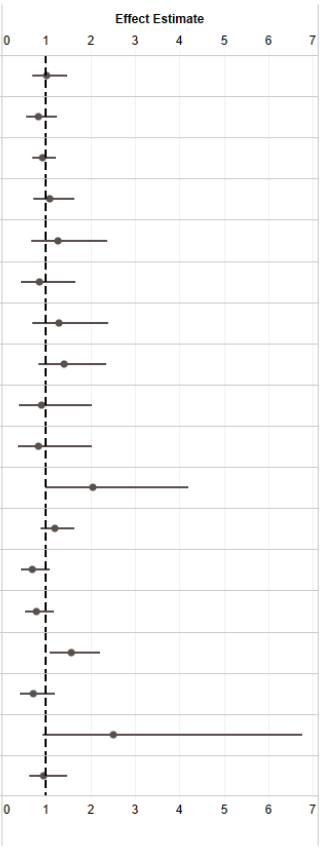


Figure 3-53. Odds of Small for Gestational Age in Children from High Confidence Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
Small for gestational age defined as birthweight below the 10th percentile for the reference population.

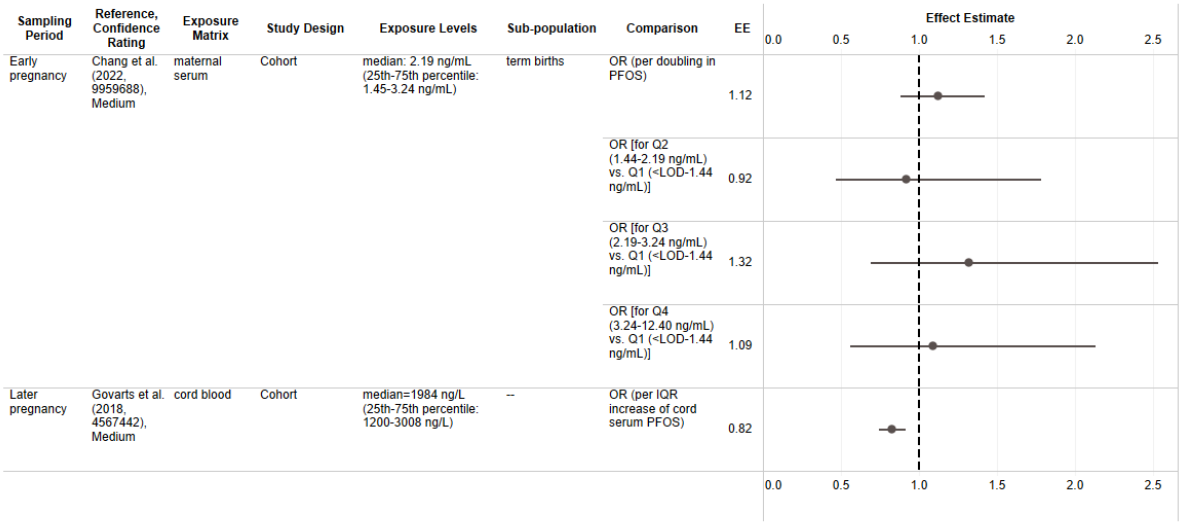


Figure 3-54. Odds of Small for Gestational Age in Children from Medium Confidence Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
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 et al., 2013) and two each that were either *high* (Chu et al., 2020; Manzano-Salgado et al., 2017a) or *medium* confidence (Hjermitslev et al., 2019; Meng et al., 2018). All but two (Hjermitslev et al., 2019; Arbuckle et al., 2013) of the five LBW studies reported some associations with either the overall population, or in either boys or girls (Figure 3-55) 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 (Figure 3-55). The *high* confidence study by Manzano-Salgado et al. (2017a) 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) reported nonsignificant 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) 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 with the quartile one referent.

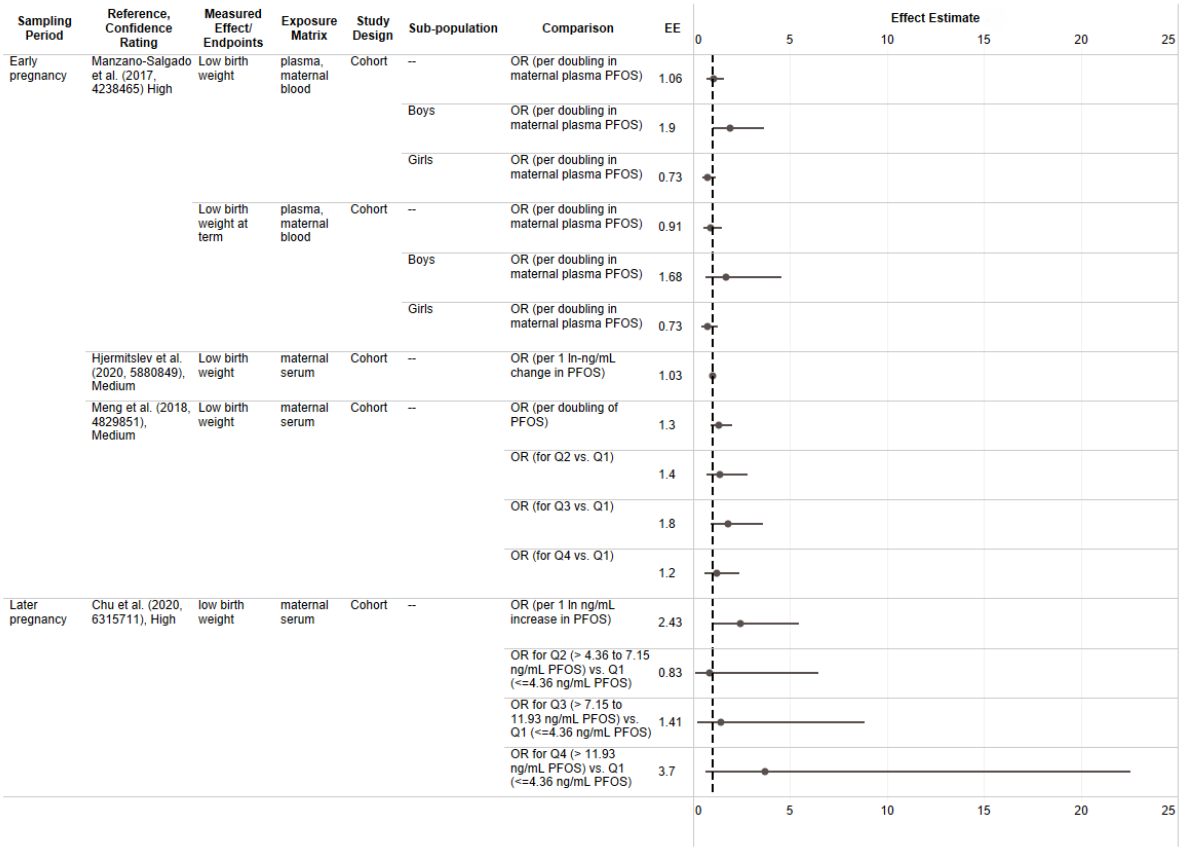


Figure 3-55. Odds of Low Birthweight in Children from Epidemiology Studies Following Exposure to PFOS

Interactive figure and additional study details available on HAWC.
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.

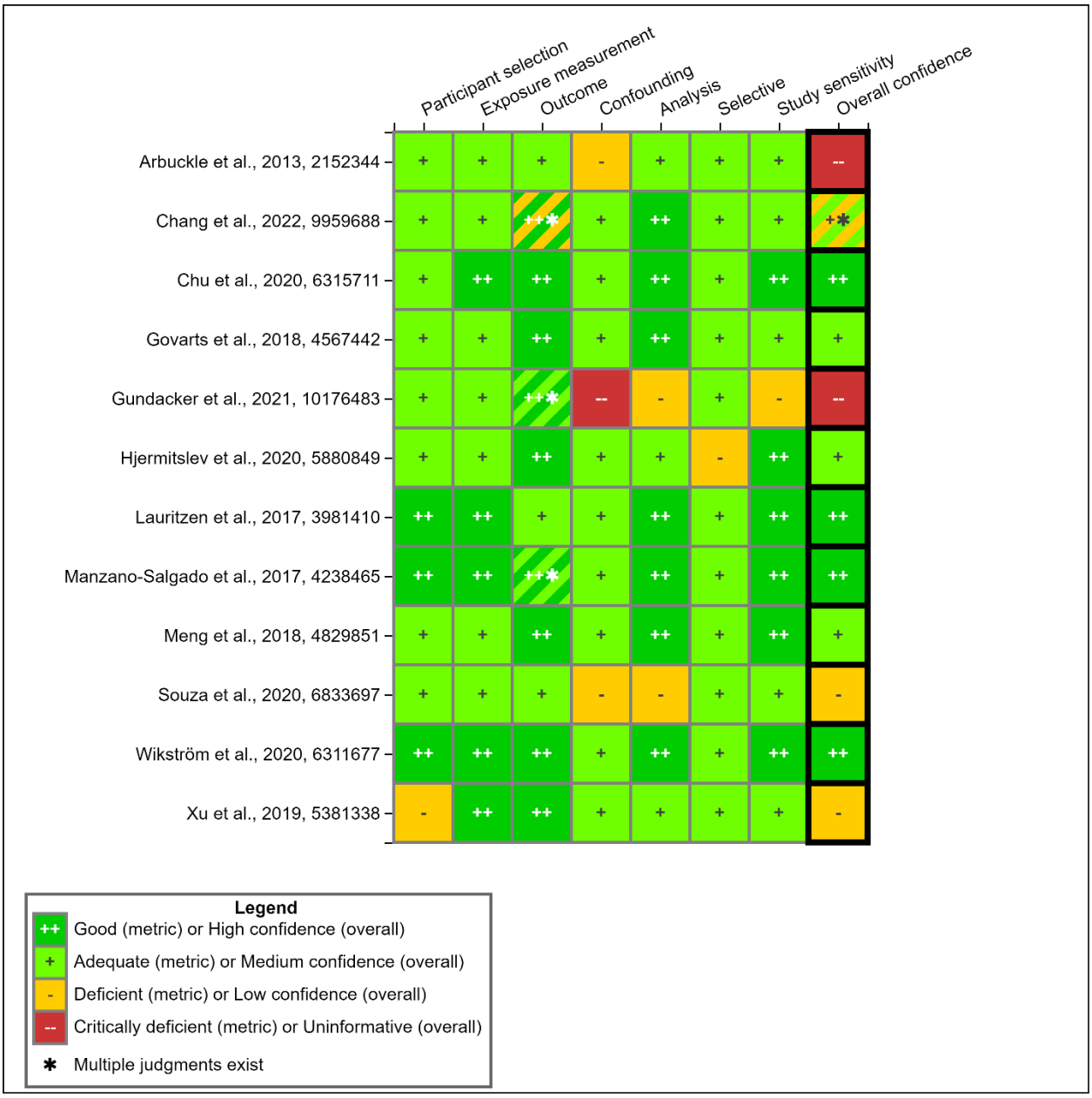


Figure 3-56. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Small for Gestational Age and Low Birth Weight Effects

Interactive figure and additional study details available on HAWC.

3.4.4.1.5.3 Birth Length

Thirty-one birth length studies were considered as part of the study evaluation as shown in Figure 3-57. and Figure 3-58. Four studies were considered *uninformative* (Gundacker et al., 2021; Jin et al., 2020; Alkhalawi et al., 2016; Lee et al., 2013) and four more studies noted above (Kobayashi et al., 2022; Bach et al., 2016; Kishi et al., 2015 Kobayashi, 2017, 3981430) 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 (Espindola-Santos et al., 2021; Xiao et al., 2019; Gyllenhammar et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b), and one evaluating both measures (Workman et al., 2019). Twelve studies examined sex-specific data with two studies (Marks et al., 2019; Robledo et al., 2015) 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 (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Bell et al., 2018; Shoaff et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017), eight were *medium* confidence (Chen et al., 2021; Luo et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018b; Chen et al., 2017b; Robledo et al., 2015) and eight were *low* confidence studies (Espindola-Santos et al., 2021; Gao et al., 2019; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016). Twelve PFOS studies had *good* study sensitivity (Chen et al., 2021; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Xiao et al., 2019; Gyllenhammar et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Robledo et al., 2015), while eight had *adequate* sensitivity (Luo et al., 2021; Gao et al., 2019; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Shi et al., 2017; Callan et al., 2016) and three (Espindola-Santos et al., 2021; Wang et al., 2019; Bell et al., 2018) were considered *deficient*.

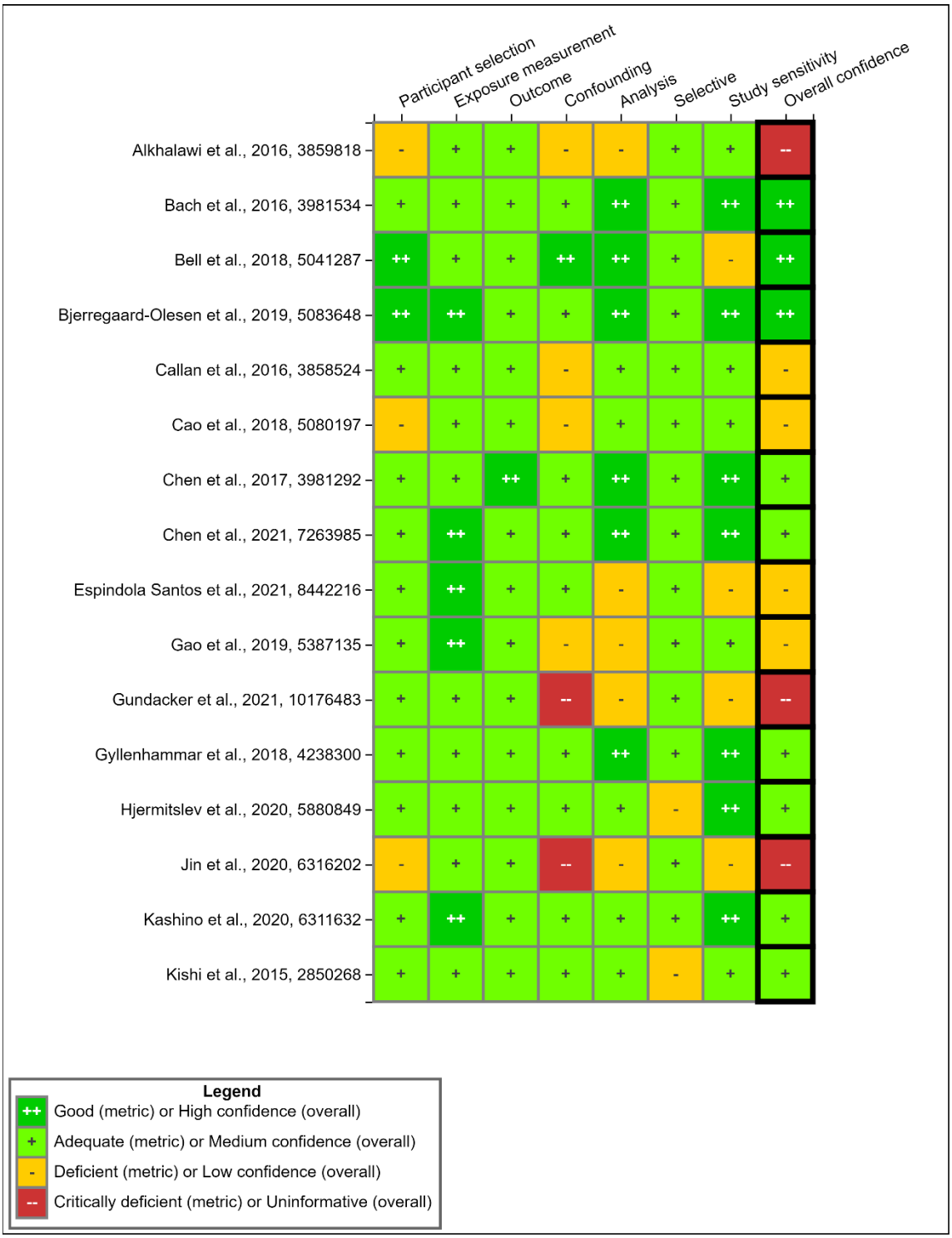


Figure 3-57. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Birth Length Effects [a]

Interactive figure and additional study details available on HAWC.

[a] Includes three overlapping studies: Bjerregaard-Olsen et al. (2019); Kishi et al. (2015); Kobayashi et al. (2017).

Figure 3-58. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure 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); Kishi et al. (2015); Kobayashi et al. (2017).

Of the 23 studies examining either standardized birth length or mean birth length measures, seven studies showed some inverse associations based on the overall population. This included three of the six (Espindola-Santos et al., 2021; Workman et al., 2019; Xiao et al., 2019; Gyllenhammar et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b) studies that reported standardized birth length data. The *high* confidence study by Xiao et al. (2019) reported reduced birth length z-scores (β per each log2 increase in PFOS: −0.33; 95% CI: −0.69, 0.03) in the overall population, as well as for both male (β: −0.41; 95% CI: −0.87, 0.05) and female neonates (β: −0.23; 95% CI: −0.75, 0.30). Although smaller in magnitude, the *medium* confidence study by Chen et al. (2017b) also reported a birth length deficit of −0.16 per each ln-unit PFOS increase (95% CI: −0.31, −0.02) in the overall population as well as male (β: −0.15; 95% CI: −0.33, 0.03) and female neonates (β: −0.20; 95% CI: −0.44, 0.05). The other *high* confidence study by Shoaff et al. (2018) of standardized birth length measures showed a deficit only for tertile 3 (β: −0.24; 95% CI: −0.64, 0.15) compared with tertile 1.

Four (Chen et al., 2021; Workman et al., 2019; Lauritzen et al., 2017; Callan et al., 2016) of the 16 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) showed a small deficit in the overall population (β: –0.3 cm; 95% CI: −0.7, 0.1), but detected the strongest association when restricted to the Swedish population (β: −1.2 cm; 95% CI: −2.1, −0.3). The *medium* confidence study by Chen et al. (2021) reported birth length deficits in the overall population (β per each PFOS ln-unit increase: –0.27 cm; 95% CI: –0.51, –0.02), males (β: –0.14 cm; 95% CI: –0.55, 0.26), and females (β: –0.40 cm; 95% CI: –0.74, –0.06). The *low* confidence study by Workman et al. (2019) reported a non-statistically significant birth length reduction (β per each ln-unit PFOS increase: –0.16 cm; 95% CI: –0.92, 0.60). The *low* confidence study by Callan et al. (2016) 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) results noted above. Among the two sex-specific only studies (Marks et al., 2019; Robledo et al., 2015), the Marks et al. (Marks et al., 2019) *low* confidence study of boys only showed inverse associations (β for tertile 3 vs. tertile 1: −0.52 cm; 95% CI: −1.05, 0.01). The *high* confidence study by Valvi et al. (2017) reported no associations in the overall population but did detect a nonsignificant birth length deficit in male neonates (β per each PFOS log2 exposure increase: −0.18 cm; 95% CI: −0.60, 0.23). The *low* confidence study Wang et al. (2019) study also reported a nonsignificant birth length deficit in males that was similar in magnitude (β: −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) detected a difference in mean birth length among girls only (β per each IQR PFOS increase: −0.3 cm; 95% CI: −0.7, 0.0). 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 et al., 2018b).

In summary, of the 23 birth length studies, 11 different ones showed some inverse 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 with 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.

3.4.4.1.5.4 Head Circumference at Birth

Nineteen informative studies that examined head circumference were considered in the synthesis. Seven studies were rated as *medium* (Chen et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018b; Lind et al., 2017a; Robledo et al., 2015) confidence, while six were *high* confidence (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Bell et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017) and six were *low* confidence (Espindola-Santos et al., 2021; Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Callan et al., 2016). Three studies were *deficient* in study sensitivity (Espindola-Santos et al., 2021; Wang et al., 2019; Bell et al., 2018), while 11 had *good* (Chen et al., 2021; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Xiao et al., 2019; Gyllenhammar et al., 2018b; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Robledo et al., 2015) and five had *adequate* study sensitivity (Marks et al., 2019; Workman et al., 2019; Xu et al., 2019a; Cao et al., 2018; Callan et al., 2016).

Figure 3-59. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Birth 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 (Marks et al., 2019; Lind et al., 2017a; Robledo et al., 2015) only reported sex-specific data, including the *low* confidence study by Marks et al. (2019) which only examined male neonates. The three remaining studies (Espindola-Santos et al., 2021; Xiao et al., 2019; Gyllenhammar et al., 2018b) 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) showed consistent head circumference z-score deficits across their overall population (β: −0.26; 95% CI: −0.68, 0.16), as well as male (β: −0.15; 95% CI: −0.68, 0.39) and female neonates (β: −0.42; 95% CI: −1.05, 0.21) per each log2 increase in PFOS. Although the *high* confidence study by Lauritzen et al. (2017) reported a null association in the combined Norwegian and Swedish population, they did detect a large head circumference reduction amongst their Swedish population only (β per each ln-unit PFOS change: −0.4 cm; 95% CI: −0.9, 0.04).

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) showed a small, nonsignificant head circumference differences (β per each PFOS IQR increase: –0.1 cm; 95% CI: −0.2, 0.1). In their *low* confidence study, Cao et al. (2018) reported a nonsignificant inverse association in the overall population (β per each ln-unit PFOS: −0.23 cm; 95% CI: −1.19, 0.73) as did the *low* confidence study by Callan et al. (2016) (β per each ln-unit PFOS: −0.39 cm; 95% CI: −0.98, 0.20).

Two of 10 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 et al., 2018b). 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. (2017a) 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) also reported deficits only in male neonates (β per each doubling of serum PFOS: −0.28 cm; 95% CI: −0.65, 0.09), while head circumference increases were found for female neonates (β: 0.48 cm; 95% CI: 0.05, 0.90). The *low* confidence study of boys only by Marks et al. (2019) reported monotonic deficits across PFOS tertiles 2 (β: −0.13 cm; 95% CI: −0.45, 0.19) and 3 (β: −0.31 cm; 95% CI: −0.62, 0.01) compared with tertile 1. The *medium* confidence study by Kashino et al. (2020) reported smaller deficits only in male neonates (β per each log10 PFOS: −0.14 cm; 95% CI: −0.61, 0.32). Although it was not statistically significant, the *high* confidence study by Bjerregaard-Olesen et al. (2019) detected a small difference in mean head circumference among girls only (β per each IQR PFOS increase: −0.1 cm; 95% CI: −0.3, 0.1). The low confidence study by Cao et al. (2018) found a large head circumference difference (β for tertile 3 vs. 1: –1.22 cm; 95% CI: −2.70, 0.25) 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 subgroups precluded a detailed assessment, few patterns were evident across the 10 different studies that showed some inverse associations with head circumference. Only two (Bjerregaard-Olesen et al., 2019; Lind et al., 2017a) of these nine studies had any early pregnancy (i.e., trimester 1) samples, with seven studies (Kashino et al., 2020; Marks et al., 2019; Xiao et al., 2019; Cao et al., 2018; Lauritzen et al., 2017; Valvi et al., 2017; Callan et al., 2016) based on either second and/or third trimester maternal samples or later. Overall, nine of 19 studies showing some evidence of inverse 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.

3.4.4.1.5.5 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. However, there were also inverse associations observed in multiple studies based on early pregnancy biomarker samples. 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 inverse 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 sizable 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 (studies published since the 2016 PFOS HESD) is fairly strong 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 the meta-analyses of PFOS by Dzierlenga et al. (2020a) and PFOA by Steenland et al. (2018a) 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 postpartum maternal samples) that may be more prone to pregnancy hemodynamic impacts. This observation is in agreement with the results of Dzierlenga et al. (2020a), though there was also evidence of associations in studies less likely to be biased by pregnancy hemodynamics (i.e., preconception or trimester 1 sampling). Therefore, despite consistency in evidence across some of 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.

3.4.4.1.6 Postnatal growth

Eleven studies examined PFOS exposure in relation to postnatal growth measures (Figure 3-60). The synthesis here is focused on postnatal growth measures including mean and standardized weight (Starling et al., 2019; Yeung et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018b; Lee et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b; Manzano-Salgado et al., 2017b; de Cock et al., 2014) and height (Yeung et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018b; Lee et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b; de Cock et al., 2014), as well as body mass index (BMI)/adiposity measures (Gross et al., 2020; Jensen et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Chen et al., 2017b; de Cock et al., 2014) and estimates of rapid growth during infancy (Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b).

Four postnatal growth studies were *high* confidence (Jensen et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018), four were *medium* confidence (Gyllenhammar et al., 2018b; Chen et al., 2017b; Manzano-Salgado et al., 2017b; de Cock et al., 2014), and three were *low* confidence (Gross et al., 2020; Cao et al., 2018; Lee et al., 2018b). As shown in Figure 3-60, seven postnatal growth studies had good study sensitivity (Jensen et al., 2020; Starling et al., 2019; Gyllenhammar et al., 2018b; Lee et al., 2018b; Shoaff et al., 2018; Chen et al., 2017b; Manzano-Salgado et al., 2017b), two each were adequate (Yeung et al., 2019; Cao et al., 2018) or deficient (Gross et al., 2020; de Cock et al., 2014). The *medium* confidence study by de Cock et al. (2014) 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. (2017b) 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. (2018b) reported statistically significant inverse associations for height at age 2 years (β per each PFOS ln-unit increase: −0.77 cm; 95% CI: −1.27, −0.15) as well as height change from birth to 2 years (β: −0.71 cm; 95% CI: −1.27, −0.15). Small differences were seen for mean weight differences at age 2 years (β: −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 with lower exposures. Although a statistically significant birth length association was detected, the *medium* confidence study by Chen et al. (2017b) 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 (β per each ln-unit PFOS increase: −0.25; 95% CI: −0.47, −0.04) or 12 to 24 months (β: −0.25; 95% CI: −0.41, −0.06). Females seemed to drive the deficit detected in the overall population (β per each ln-unit PFOS increase: −0.13; 95% CI: −0.32, 0.07) for the 6-to-12-month window. The *medium* confidence study by Gyllenhammar et al. (2018b) 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 with 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) reported slight increases in postnatal length (i.e., height) (β: 1.37 cm; 95% CI: −0.5, 3.28), while large postnatal weight deficits were reported for PFOS tertiles 2 (β: −138 g; 95% CI: −574, 298) and 3 (β: −78 g; 95% CI: −532, 375).

Associations at five months of age in the overall population (β: –0.28; 95% CI: –0.51, –0.05) and females (β: –0.56; 95% CI: –0.87, –0.26) from the *high* confidence study by Starling et al. (2019) were detected for weight-for-age z-scores, as well as weight-for-length z-scores (β: 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) detected statistically significant deficits and exposure-response relationships for infant weight-for-age z-score (β: −0.33; 95% CI: −0.65, −0.01) and weight-for-length z-score (β: −0.34; 95% CI: −0.59, −0.08) in PFOS tertile 3 compared with tertile 1. Small deficits that were not statistically significant were observed in tertile 3 for length for age z-score (β: −0.22; 95% CI: −0.49, 0.04). In their *high* confidence study, Yeung et al. (2019) 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 3 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 (β: −0.10; 95% CI: −0.16, −0.05) and weight z-score (β: −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 (β: –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 inverse associations with PFOS. Study quality ratings, including study sensitivity and overall confidence, did not appear to be explanatory factors for heterogeneous results across studies.

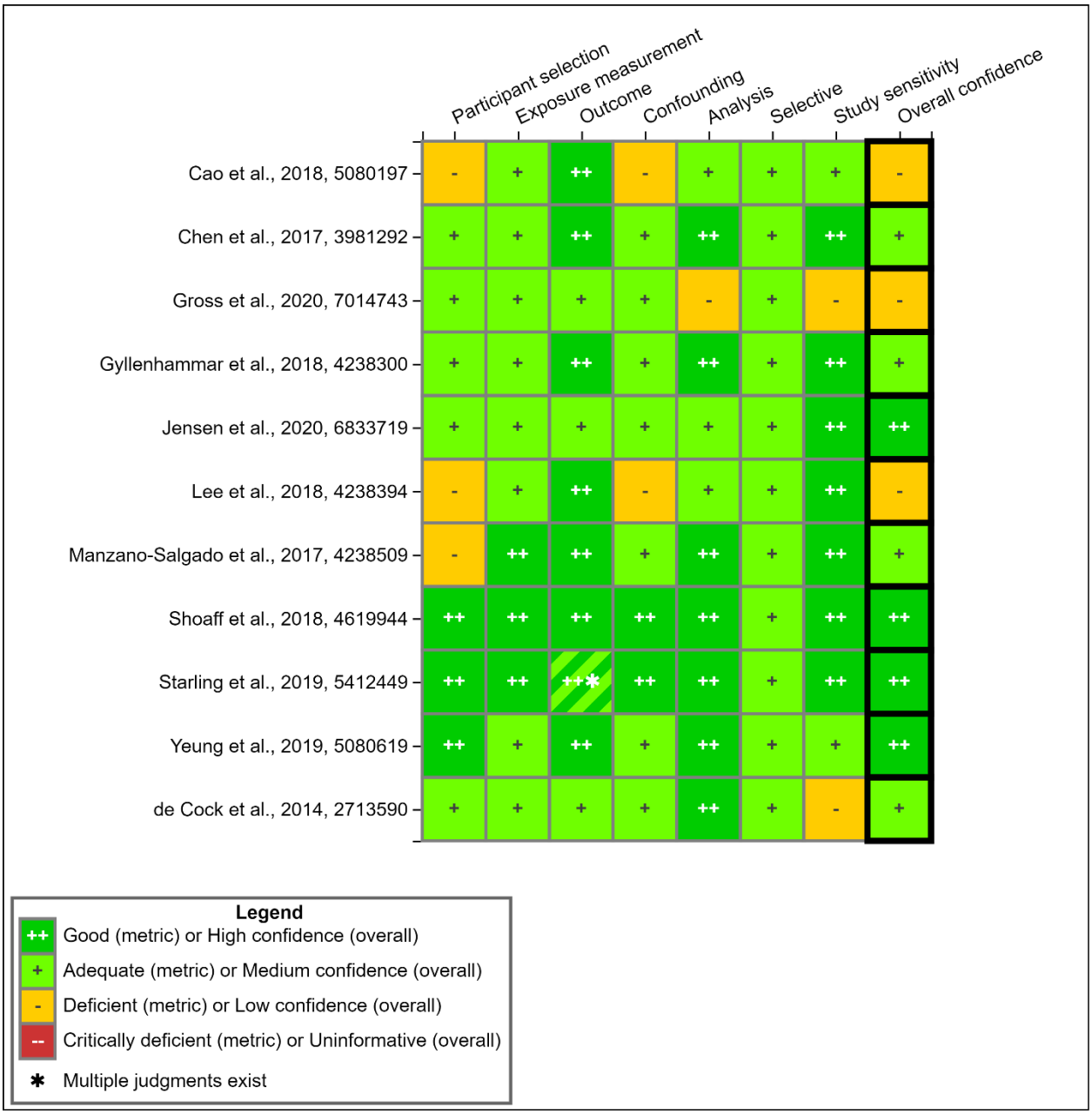


Figure 3-60. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Postnatal Growth

Interactive figure and additional study details available on HAWC.

3.4.4.1.6.1 Adiposity/BMI

In their *high* confidence study of repeated measures at 4 weeks, 1 year and 2 years of age, Shoaff et al. (2018) detected statistically significant decreases in infant BMI z-score (β: −0.36; 95% CI: −0.60, −0.12). Although they were not statistically significant, the *medium* confidence Chen et al. (2017b) 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 (β: −0.33; 95% CI: −0.59, −0.08) and 12 to 24 months

(β: −0.25; 95% CI: −0.45, −0.05) developmental periods. In their *high* confidence study, Yeung et al. (2019) 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 3 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 (β: −0.11; 95% CI: –0.17, –0.05) and BMI (β: –0.16 kg/m2; 95% CI: –0.24, –0.08). In the *high* confidence study by Starling et al. (2019), decreased adiposity (β: –2.08; 95% CI: –3.81, –0.35) among girls was detected in PFOS tertile 3 compared with the tertile 1 referent. The *high* confidence study by Jensen et al. (2020) 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) 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.

3.4.4.1.6.2 Rapid Weight Gain

Four *high* confidence studies (Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) examined rapid infant growth. Limited evidence of associations was reported, as only one (Starling et al., 2019) of four studies (Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) showed increased odds or rapid weight gain with increasing PFOS. For example, Starling et al. (2019) 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.

3.4.4.1.6.3 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 inverse 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 postnatal growth measures up to 2 years of age.

3.4.4.1.7 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 (Gardener et al., 2021; Liu et al., 2020d).

3.4.4.1.7.1 Gestational Age

Seventeen of the 20 studies reporting gestational age estimates in relation to PFOS exposures were considered (Figure 3-61). Two studies were deemed *uninformative* (Gundacker et al., 2021; Lee et al., 2013) and were excluded and one study was excluded based on an overlapping cohort (Li et al., 2017). Sixteen non-overlapping and informative studies examined mean gestational age (in weeks) in relation to PFOS exposures and one study reported sex-specific results only (Lind et al., 2017a).

Among the 17 different studies included here, nine were *high* confidence (Chu et al., 2020; Eick et al., 2020; Huo et al., 2020; Bell et al., 2018; Sagiv et al., 2018; Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Bach et al., 2016), four were *medium* (Yang et al., 2022In Press; Hjermitslev et al., 2019; Gyllenhammar et al., 2018b; Meng et al., 2018) and four were *low* confidence (Bangma et al., 2020; Gao et al., 2019; Workman et al., 2019; Xu et al., 2019a). Ten of these studies had good study sensitivity, six were adequate (Yang et al., 2022In Press; Bangma et al., 2020; Eick et al., 2020; Gao et al., 2019; Workman et al., 2019; Xu et al., 2019a) and one was deficient (Bell et al., 2018).

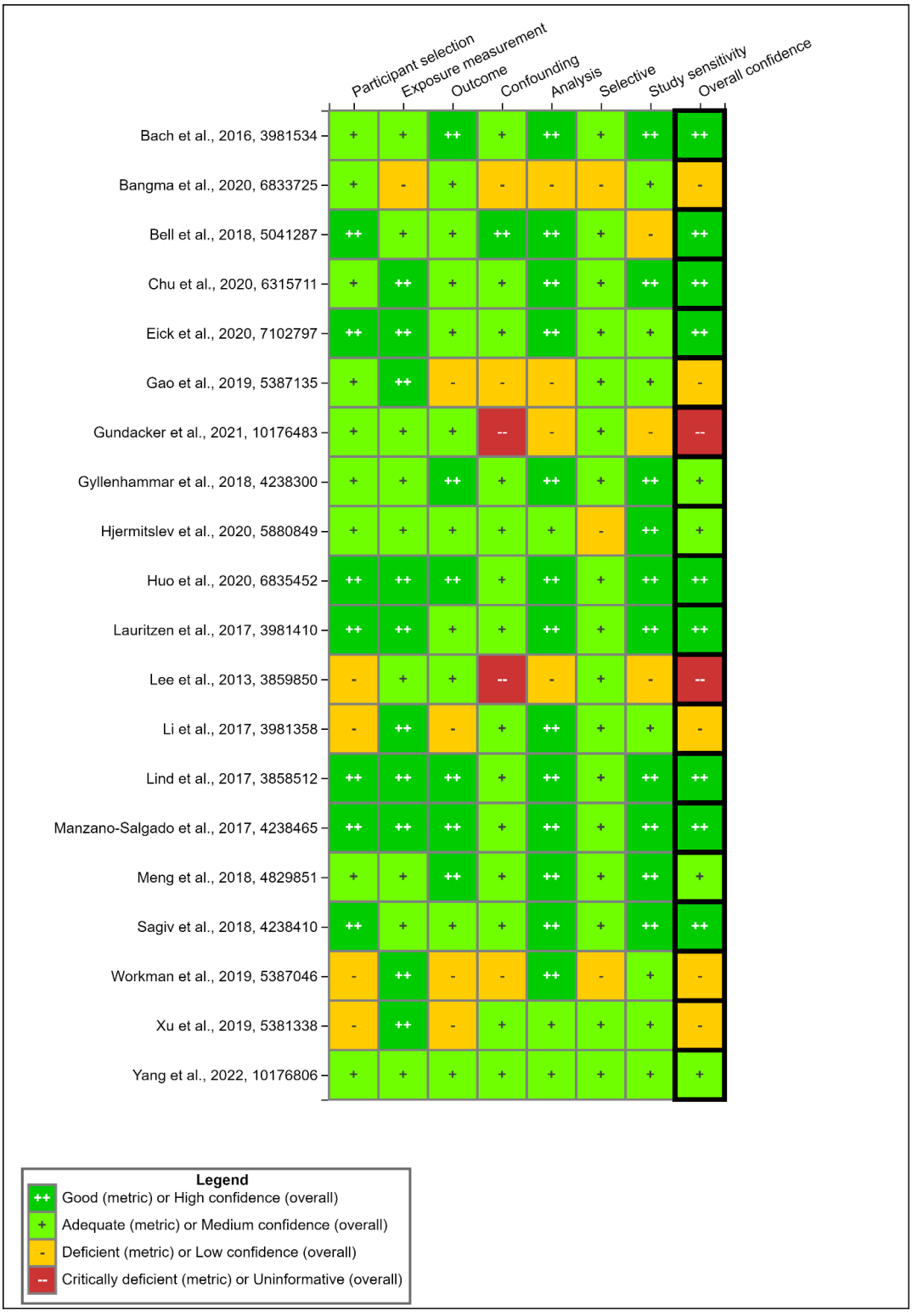


Figure 3-61. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Gestational Age

Interactive figure and additional study details available on HAWC.

Nine of the 16 studies examining mean gestational age change in the overall population reported some deficits. Among these nine studies, four were *high* confidence, and three were *medium* and two were *low* confidence. The *medium* confidence study by Gyllenhammar et al. (2018b) 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) reported a similar gestational age reduction in the overall population (β: –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 (β per each IQR increase: −0.19 weeks; 95% CI: −0.33, −0.05). The *high* confidence study by Chu et al. (2020) reported similar deficits in the overall population (β: –0.32 weeks; 95% CI: –0.53, –0.11) which was driven by female neonates (β: –0.61 weeks; 95% CI: –0.90, –0.32). The *high* confidence study by Lauritzen et al. (2017) only showed deficits among their Swedish population (β: –0.4 weeks; 95% CI: –0.9, 0.2). Compared with tertile 1, the *low* confidence study by Gao et al. (2019) reported deficits in tertile 2 (β: –0.40 weeks; 95% CI: –0.92, 0.12) and tertile 3 (β: –0.20; 95% CI: –0.61, 0.20). The *high* confidence study by Manzano-Salgado et al. (2017a) reported deficits in quartile 4 among the overall population (β: –0.31 weeks; 95% CI: –0.55, –0.06) compared with quartile 1. Despite relatively low overall PFOS concentrations, the *medium* confidence study by Yang et al. (2022In Press) showed reduced gestational age only among pre-term births for both total PFOS (β: −1.26 weeks; 95% CI: − 2.46, –0.05) and linear PFOS (β per each IQR increase: −1.80 weeks; 95% CI: −3.24, −0.37), with results larger results in female (β: −1.06 weeks; 95% CI: −2.87, 0.74) than male neonates (β: −0.41 weeks; 95% CI: −2.20, 1.37). The *medium* confidence study by Meng et al. (2018) 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) reported a nonsignificant decrease (β per each ln-unit PFOS change: –0.17 weeks; 95% CI: –0.52, 0.18).

Lind et al. (2017a) reported sex-specific changes in mean gestational age only. Inverse associations were observed for both boys (β per ln-unit increase: –0.5 days, 95% CI: –3.4, 2.3) and girls (β: –1.0, 95% CI: –4.2, 2.1), but neither was significant.

Overall, nine of the 16 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 to –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 inverse 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.

3.4.4.1.7.2 Preterm Birth

As shown in Figure 3-62, 11 studies examined the relationship between PFOS and preterm birth (PTB); all of the studies were either *medium* (Yang et al., 2022In Press; Liu et al., 2020d;

Hjermitslev et al., 2019; Meng et al., 2018) or *high* confidence (Gardener et al., 2021; Chu et al., 2020; Eick et al., 2020; Huo et al., 2020; Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016). Nine of the 11 studies were prospective birth cohort studies, while the two studies by Liu et al. (2020d) and Yang et al. (2022In Press) were case-control studies nested with prospective birth cohorts. Four studies had maternal exposure measures that were sampled during trimester one (Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016), or trimester three (Gardener et al., 2021). The *high* confidence study by Chu et al. (2020) sampled during the late third trimester or within three days of delivery. Four studies collected samples across multiple trimesters (Eick et al., 2020; Huo et al., 2020; Liu et al., 2020d; Hjermitslev et al., 2019). One study used umbilical cord serum samples (Yang et al., 2022In Press). The *medium* confidence study by Meng et al. (2018) 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 (Yang et al., 2022In Press; Gardener et al., 2021; Eick et al., 2020; Liu et al., 2020d) with the median exposure values in the overall population ranging from 1.79 ng/mL (Liu et al., 2020d) to 30.1 ng/mL (Meng et al., 2018). Lower levels were also seen for a total PFOS measure in Yang et al. (2022In Press) for both cases (median (IQR) = 0.27 (0.30) ng/mL) and controls (0.21 (0.37) ng/mL).

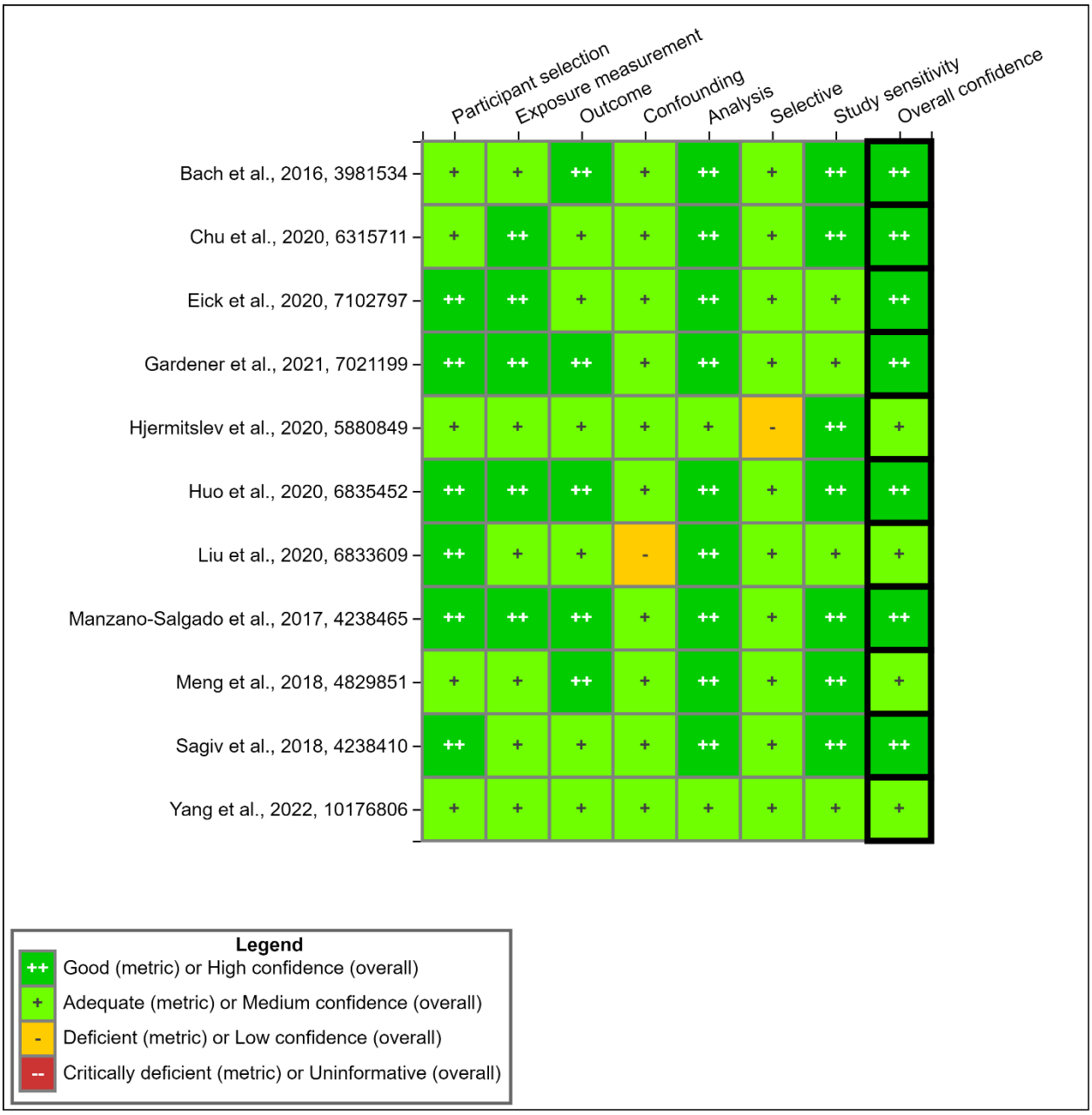


Figure 3-62. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Preterm Birth Effects

Interactive figure and additional study details available on HAWC.

An increased risk was 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) 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) 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 with the referent. The *high* confidence study by Eick et al. (2020) reported an exposure-response relationship as well (tertile 2 OR = 1.21; 95% CI: 0.50, 2.91; tertile 3 OR = 1.87; 95% CI: 0.72, 4.88, compared with tertile 1). Although they were not statistically significant, the *medium* confidence study by Liu et al. (2020d) 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 with the referent. The *high* confidence study by Sagiv et al. (2018) 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) 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. (2022In Press) 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), Huo et al. (2020), Manzano-Salgado et al. (2017a) and Hjermitslev et al. (2019). Overall, only two (Chu et al., 2020; Eick et al., 2020) 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 sizable 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 strong with respect to an increased risk 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 increased risk had limited exposure contrasts.

3.4.4.1.7.3 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 studies showed some increased risk of gestational duration deficits. 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 increased risk), 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 the qualitative analysis.

3.4.4.1.8 Fetal Loss

As shown in Figure 3-63, five (two *high*, two *medium,* and one *low* confidence) studies examined PFOS exposure and fetal loss. All of these studies had good study sensitivity owing largely to very large sample sizes (Wang et al., 2021; Wikström et al., 2021; Liew et al., 2020; Buck Louis et al., 2016; Jensen et al., 2015).

The *high* confidence study by Wikström et al. (2021) 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 nonsignificant 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), there was some suggestion of an exposure-response relationship for miscarriages across PFOS quartiles (OR range: 1.1–1.4). Similarly, the *low* confidence study by Jensen et al. (2015) reported increased nonsignificant 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) (OR = 0.95; 95% CI: 0.87, 1.04) or the *medium* confidence study by Buck Louis et al. (2016) (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.

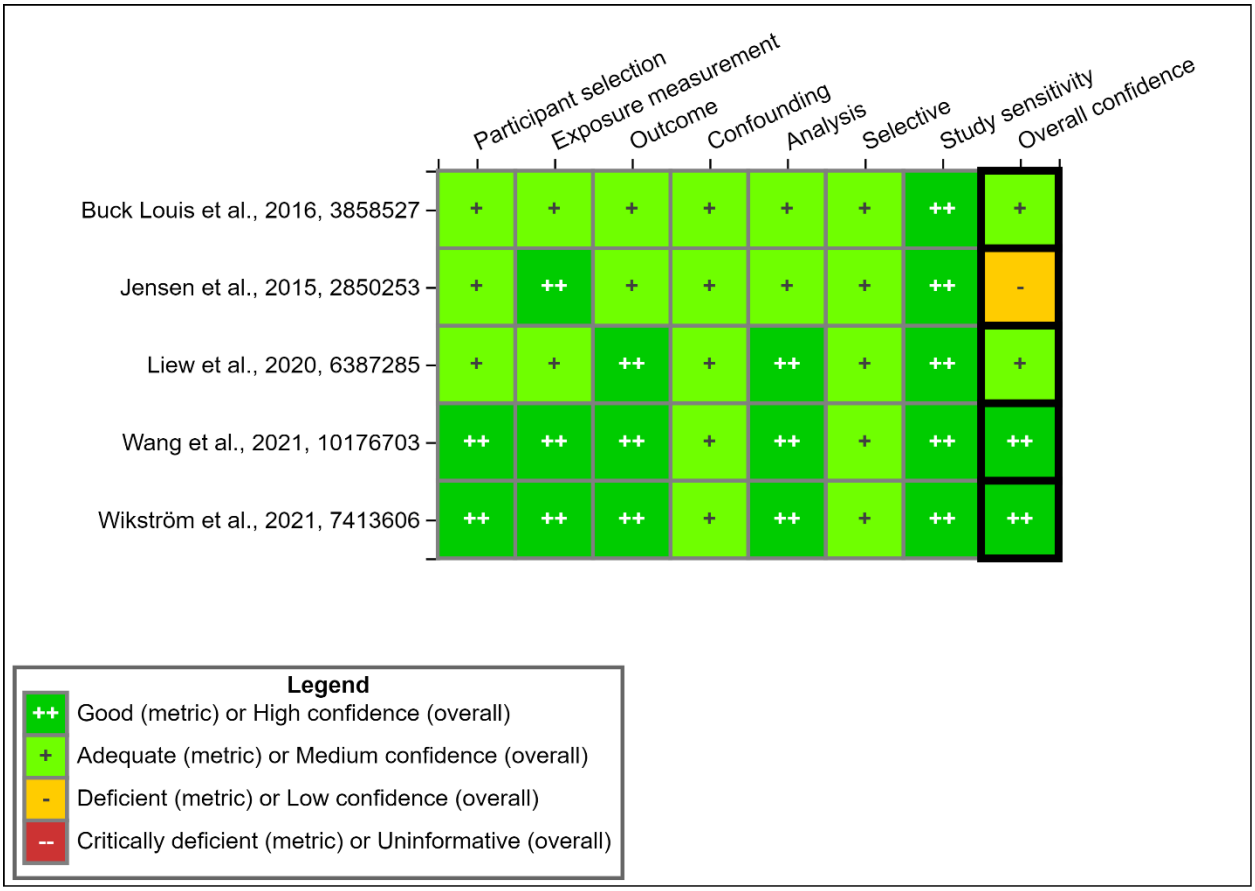


Figure 3-63. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Fetal Loss

Interactive figure and additional study details available on HAWC.

3.4.4.1.9 Birth Defects

As shown in Figure 3-64, five (three *medium* and two *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) 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 the 75th PFOS percentile level (relative to those <75th percentile). A *low* confidence study of a non-specific grouping of all birth defects (Cao et al., 2018) 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) detected an inverse association for cryptorchidism (OR per each ln-unit increase in PFOS = 0.51; 95% CI: 0.21–1.20). This risk seemed to be largely driven by boys from Finland. The *medium* confidence study by Toft et al. (2016) 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) did not find statistically significant PFOS exposure differences among cryptorchidism or hypospadia cases compared with 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.

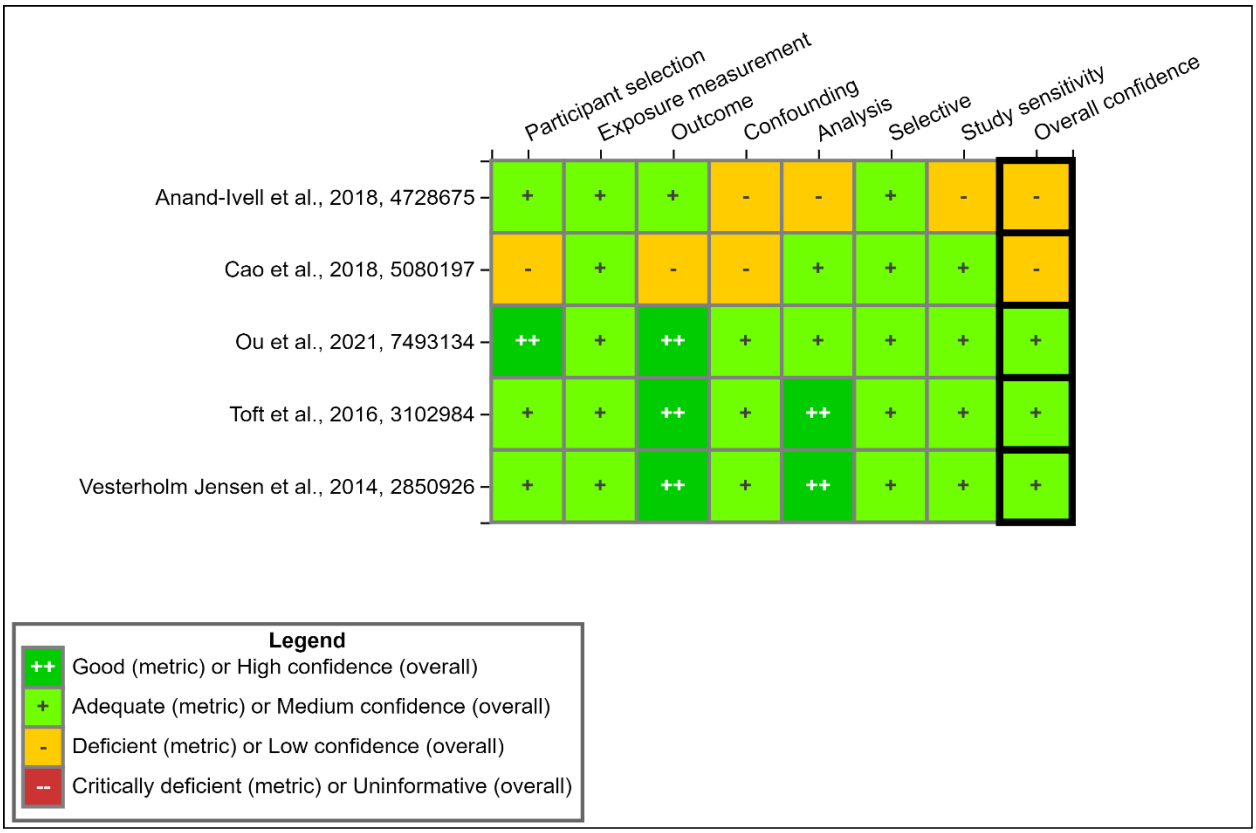


Figure 3-64. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Defects

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, 2016b) and 16 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOS HESD that investigated the association between PFOS and developmental effects. Study quality evaluations for these 20 studies are shown in Figure 3-65.

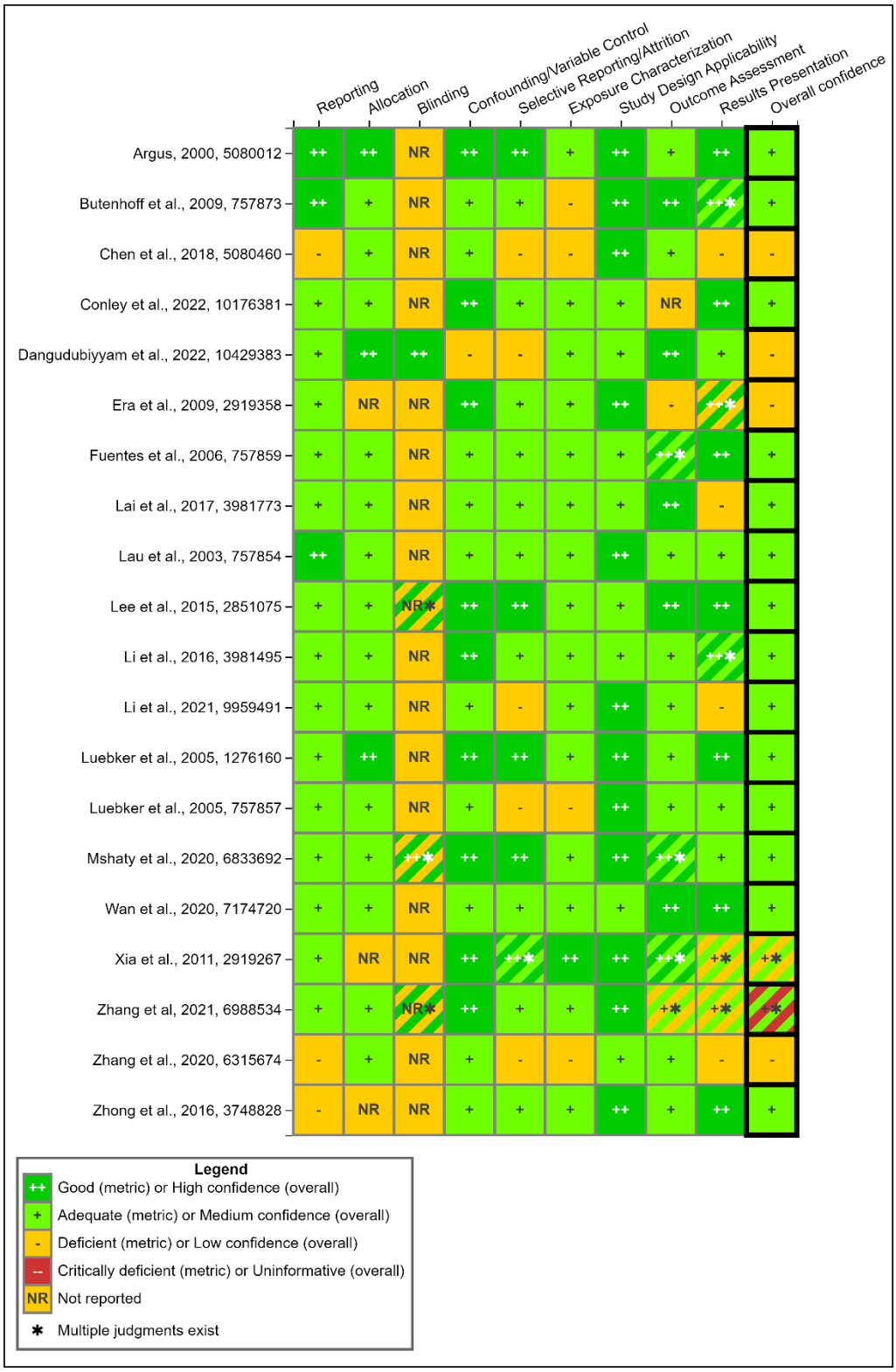


Figure 3-65. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Developmental Effects

Interactive figure and additional study details available on HAWC.

Evidence indicates 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 Appendix C (U.S. EPA, 2024a)).

3.4.4.2.1 Maternal Effects

Multiple developmental studies evaluated maternal weight outcomes in rats, mice, and rabbits (Figure 3-66). Yahia et al. (2008) 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) 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) 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 with controls. Dams in the 2 and 8 mg/kg/day dose groups had significantly lower mean body weights on GD 14–17. In contrast, Lai et al. (2017a) 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 1–20). The authors determined that there were no observable maternal effects related to PFOS exposure at the relatively low doses evaluated. Wan et al. (2020) 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) 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) 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) 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) 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. (2005a) 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 with 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 pre-cohabitation 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. (2005b) 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 et al., 2005b). 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) also reported reduced mean maternal body weights in the 20 mg/kg/day dose group. In another study, Conley et al. (2022) 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) 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) 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 4–20) 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) 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.

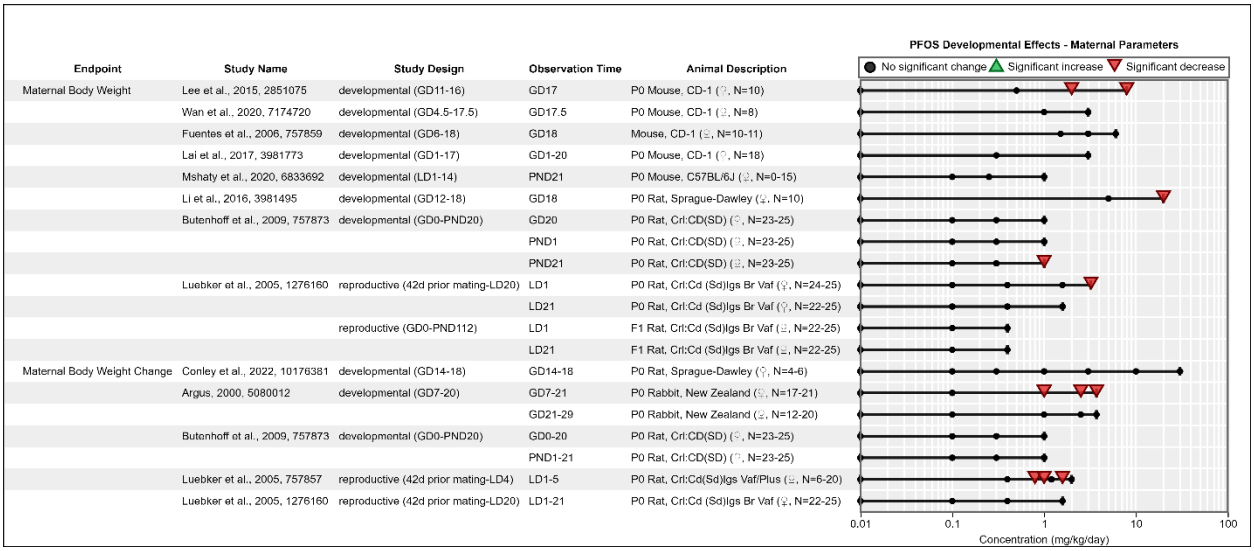


Figure 3-66. Maternal Body Weight in Mice, Rats, and Rabbits Following Exposure to PFOS

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 3-67). Lee et al. (2015) 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 versus controls. A decrease in the mean number of live fetuses was 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 et al., 2003). 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 et al., 2006).

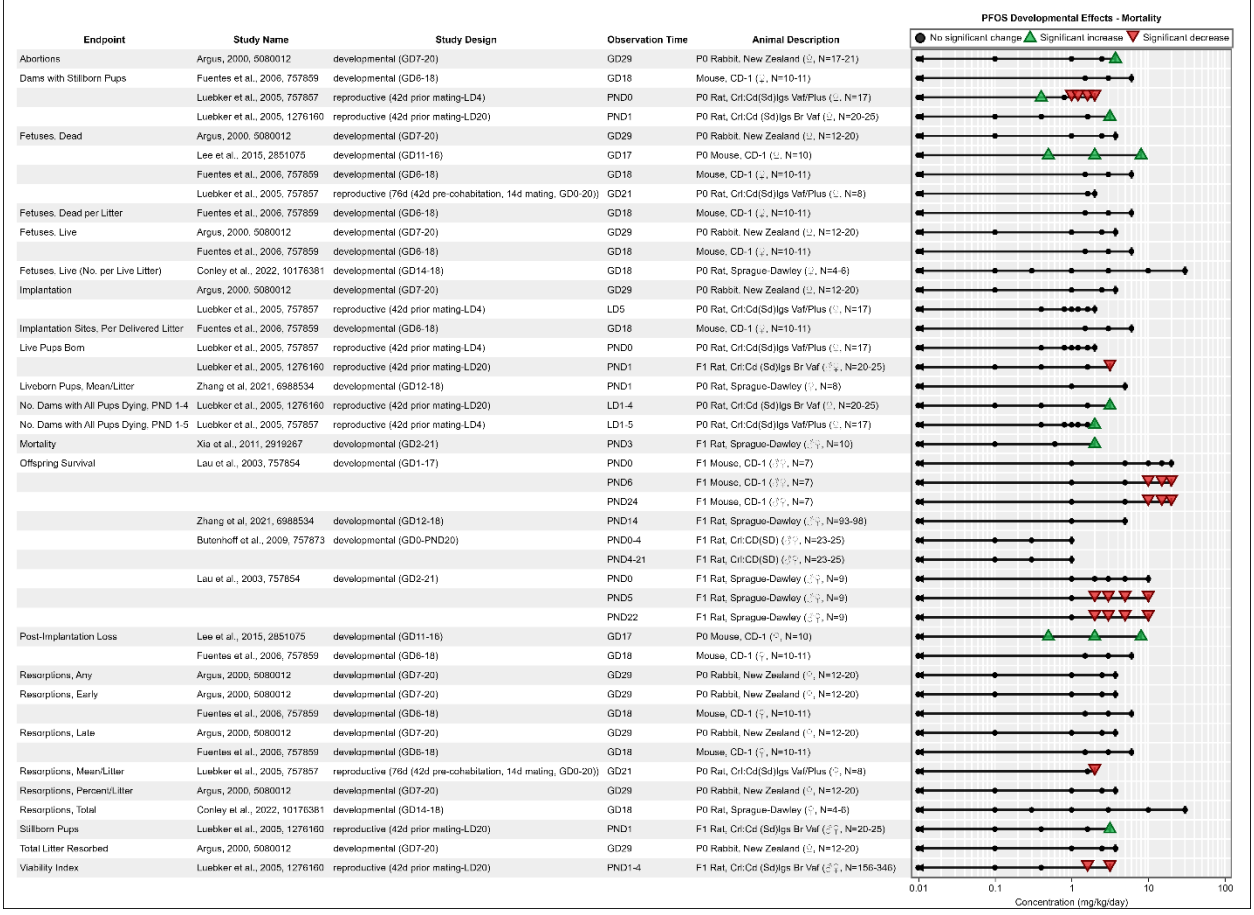
Mice appear to be more sensitive to alterations in fetal viability than rats. Thibodeaux et al. (2003) dosed pregnant Sprague-Dawley rats with 0, 1, 2, 3, 5, or 10 mg/kg PFOS daily by gavage from GD 2 to GD 20. The number of implantations was not affected by treatment and there were no treatment-related effects observed on the live rat fetuses at term. Likewise, Zhang et al. (Zhang et al., 2021) 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) 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) 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) reported no significant effects on implantations or resorptions. However, Argus Research Laboratories (2000) 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 statistically greater relative to control for the 3.75 mg/kg/day dose group. Argus Research Laboratories (2000) reported no significant effects on the mean number of live fetuses per doe, number of dead fetuses per 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. (2005b; 2005a) observed reduced pup viability index (ratio of the number of pups alive at PND 5 to the number of live pups born) with 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 et al., 2005b); 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 et al., 2005a); 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 19–20, Grasty et al. (2006) 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) 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. (2012b) 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) and Lau et al. (2003) 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) 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) 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) evaluated the impact of lactational (PND 1–14) PFOS exposure on pup survival. Mshaty et al. (2020) 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).



Endpoint	Study Name	Study Design	Observation Time	Animal Description
Abortions	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=17-21)
Dams with Stillborn Pups	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
	Luebker et al., 2005, 757857	reproductive (42d prior mating-LD4)	PND0	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=17)
	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	PND1	P0 Rat, Crl:Cd (Sd)Igs Br Vaf (♀, N=20-25)
Fetuses, Dead	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
	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)
	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)
Fetuses, Dead per Litter	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Fetuses, Live	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)
Fetuses, Live (No. per Live Litter)	Conley et al., 2022, 10176381	developmental (GD14-18)	GD18	P0 Rat, Sprague-Dawley (♀, N=4-6)
Implantation	Argus, 2000, 5080012	developmental (GD7-20)	GD29	P0 Rabbit, New Zealand (♀, N=12-20)
	Luebker et al., 2005, 757857	reproductive (42d prior mating-LD4)	LD5	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=17)
Implantation Sites, Per Delivered Litter	Fuentes et al., 2006, 757859	developmental (GD6-18)	GD18	Mouse, CD-1 (♀, N=10-11)
Live Pups Born	Luebker et al., 2005, 757857	reproductive (42d prior mating-LD4)	PND0	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=17)
	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	PND1	F1 Rat, Crl:Cd (Sd)Igs Br Vaf (♂♀, N=20-25)
Liveborn Pups, Mean/Litter	Zhang et al, 2021, 6988534	developmental (GD12-18)	PND1	P0 Rat, Sprague-Dawley (♀, N=8)
No. Dams with All Pups Dying, PND 1-4	Luebker et al., 2005, 1276160	reproductive (42d prior mating-LD20)	LD1-4	P0 Rat, Crl:Cd (Sd)Igs Br Vaf (♀, N=20-25)
No. Dams with All Pups Dying, PND 1-5	Luebker et al., 2005, 757857	reproductive (42d prior mating-LD4)	LD1-5	P0 Rat, Crl:Cd(Sd)Igs Vaf/Plus (♀, N=17)
Mortality	Xia et al., 2011, 2919267	developmental (GD2-21)	PND3	F1 Rat, Sprague-Dawley (♂♀, N=10)
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 3-67. 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.

3.4.4.2.3 Skeletal, Soft Tissue, and Gross Effects

Skeletal defects in offspring, including bone ossification, have been observed in mice, rats, and rabbits gestationally exposed to PFOS. 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 et al., 2008). 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) 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) 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 et al., 2006). 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 et al., 2006).

Skeletal malformations in fetal and neonatal rabbits were reported in Argus Research Laboratories (2000) at comparatively lower PFOS doses than those described in rat and mouse studies. A significant decrease in the mean number of isolated ossification sites of the metacarpal per fetus per litter was observed in the 3.75 mg/kg/day dose group versus control (4.82 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 1 mg/kg/day (3.95) dose groups. A significant difference in fetal incidence of irregular ossification of the skull was reported in both the 2.5 and 3.75 mg/kg/day dose groups relative to control (0.8% and 9.2% incidence respectively, relative to 4% in the control); no significant difference was observed in the 0.1 (5.6%) and 1 mg/kg/day (2%) dose groups. There were no significant differences in litter incidence of irregular ossification of the skull in the 0.1, 1, 2.5, and 3.75 dose groups versus control (38.9%, 15.8%, 6.2%, and 25%, respectively, 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) versus Control (1); no change in mean number of hyoid ossification sites was reported in other dose groups (mean of 1 for the 0.1, 1, and 2.5 mg/kg/day dose groups). A significant increase in fetal incidence of a hole in the parietal bone was observed in the 3.75 mg/kg/day dose group versus Control (6.5% 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 versus 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 versus Control (16.7% vs. 0%). The rest of the dose groups exhibited 0% litter incidence of unossified pubis. However, fetal alterations were observed in a similar percentage of litters across all dose groups (70%, 61.1%, 47.4%, 25%, and 66.7% in the 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively). No significant difference was seen in the mean percentage of fetuses per litter with any alteration (14.1%, 17%, 9.5%, 3.6%, and 17.4% in the 0, 0.1, 1, 2.5, and 3.75 mg/kg/day dose groups, respectively).

3.4.4.2.4 Fetal or Pup Body Weight

Several studies in different species reported data on fetal body weight (Figure 3-68). In a study in CD-1 mice with gestational PFOS exposure from GD 11 to GD 16, Lee et al. (2015) 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) reported a significant reduction in fetal body weight in the 3 mg/kg/day group compared

with controls. In contrast, Fuentes et al. (2006) 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. (2021a) 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 statistical significance, though it should be noted that the sample size was small (n = 3 litters/group). Li et al. (2016) 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) 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. (2005b) reported no effect on pooled fetal body weights with PFOS doses up to 2 mg/kg/day. Similarly, Conley et al. (2022) 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) 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 3-68). 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 et al., 2005b; Luebker et al., 2005a). 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 et al., 2005a); the lowest dose of 0.1 mg/kg/day was not tested in the one-generation study (Luebker et al., 2005b) where the LOAEL was the lowest dose tested of 0.4 mg/kg/day for decreased pup body weight, decreased maternal body weight, and decreased gestation length. In both the one- and two-generation studies, the decreased pup body weight was observed across multiple time points (PND 0 and LD 5 and PND 1, 4, 7, 14, and 21, respectively) in the first generation. In the second generation, decreased pup weight was only observed in the highest dose group tested (0.4 mg/kg/day) on PND 7 and 14 (Luebker et al., 2005a). Lau et al. (2003) 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) 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. In contrast, Zhang et al. (2021) 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.

For this endpoint, rats appear to be more sensitive than mice. Yahia et al. (2008) reported significant decreases in ICR mouse neonatal weight at relatively high doses of 10 and 20 mg/kg/day. Lau et al. (2003) 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) 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) 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.

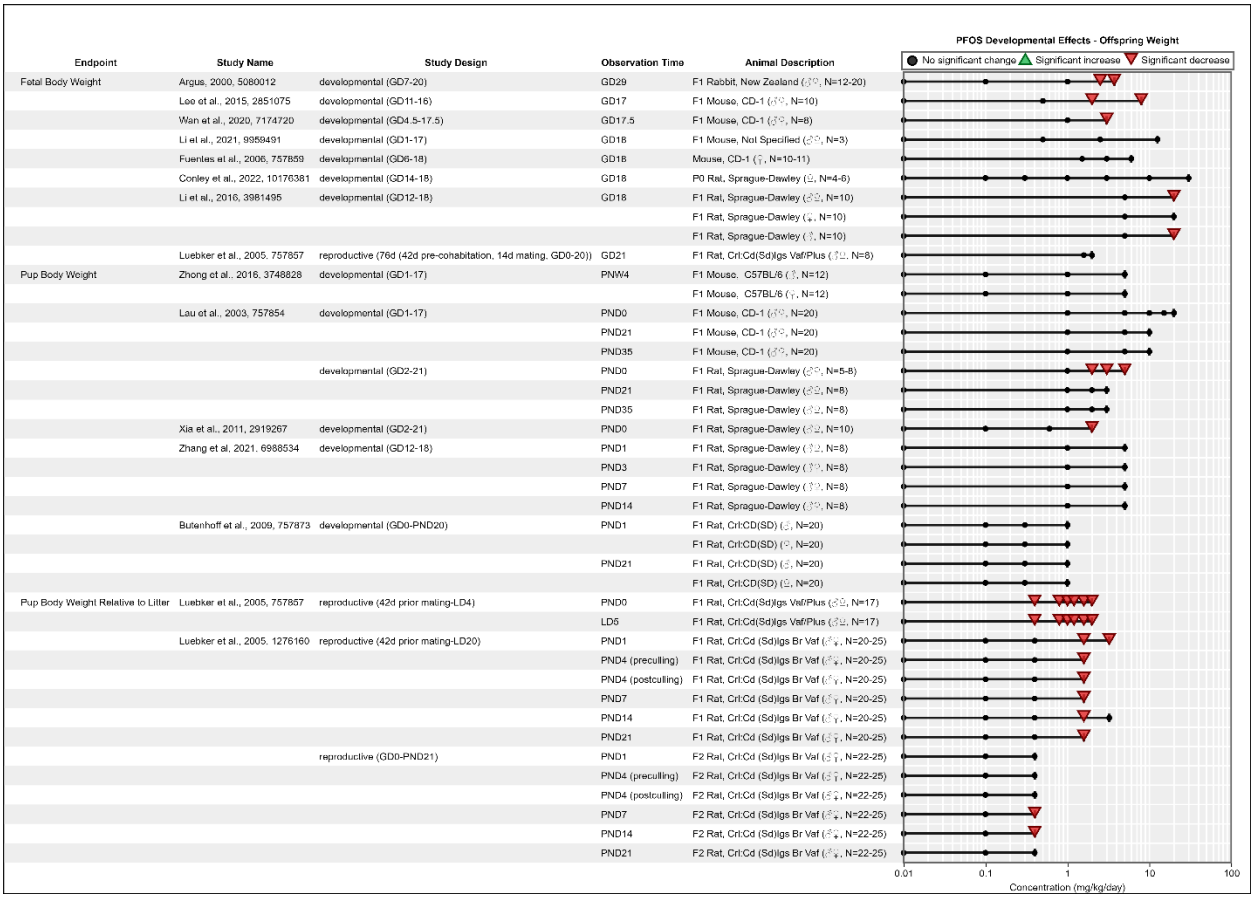


Figure 3-68. Offspring Body Weight in Mice, Rats, and Rabbits Following Exposure to PFOS (Logarithmic Scale, Sorted by Observation Time)

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) 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 in controls). No significant difference in placental weights was detected in dams exposed to 5 mg/kg/day PFOS relative to control. At ≥0.5 mg/kg/day, Lee et al. (2015) 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 et al., 2015). Li et al. (2021a) 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 with controls, though the biological significance of that effect is unclear. Wan et al. (2020) 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) did not observe any placental effects in exposed rabbits and Luebker et al. (2005b) 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) 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. A two-generation reproduction study in rats (Luebker et al., 2005a) 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. Mshaty et al. (2020) evaluated age at eye opening in mice exposed to 1 mg/kg/day from PND 1 through PND 14 and found no significant effects.

Developmental PFOS exposure also had adverse effects on lung development, further described in the Respiratory Section of Appendix C (U.S. EPA, 2024a).

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 (U.S. EPA, 2016b). There are 34 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 by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-69.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	1	0	0	1
Big Data, Non-Targeted Analysis	5	6	4	14
Cell Growth, Differentiation, Proliferation, Or Viability	8	0	15	20
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	14	7	16	34

Figure 3-69. Summary of Mechanistic Studies of PFOS and Developmental Effects

Interactive figure and additional study details available on HAWC.

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 et al., 2020). In general, PFOS can lead to embryo and/or larva malformation, delays in hatching, and decreases in body length. Wang et al. (2017) 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) 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) 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). 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 et al., 2019). Lastly, Chen et al. (2014) 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. (2015; 2013) 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) 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), 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 et al., 2013). Xu et al. (2015) 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) 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) 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. (2018a) 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 et al., 2018).

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 programming role of PFOS on telomere length during gestation (Liu et al., 2018c).

3.4.4.3.2 Fetal Growth and Placental Development

Growth was measured in developing zebrafish larvae in three studies. Wang et al. (2017), 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. (2016a), 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) 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) 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) 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. (2020b) 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 et al., 2013), 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 et al., 2020b).

Sonkar et al. (2019) 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 et al., 2014). In Yang et al. (2016), 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 and Aras, 2014).

Lastly, in humans, Ouidir et al. (2020) 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 Louis et al., 2018). These data suggest that the prenatal toxicity of PFOS might be driven by epigenetic changes in the placenta (Ouidir et al., 2020).

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 et al., 2019). 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 et al., 2019a). 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 et al., 2019a).

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 and Aras, 2014). 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) studied the effects of developmental exposure to PFOS on metabolic liver function in Kunming mice, in postnatal 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 (Liu et al., 2020a; Yang et al., 2020c; Tang et al., 2017; Zhou et al., 2017a; Zhang et al., 2016; Cheng et al., 2013). Most of the studies utilized mouse ESC-CMs but one study, Yang et al. (2020c), 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 (Tang et al., 2017; Zhou et al., 2017a; Zhang et al., 2016; Cheng et al., 2013) and a decreased proportion of α-actinin-positive cells (a marker of cardiomyocytes) on differentiation day 10 (Tang et al., 2017; Zhang et al., 2016). 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) to 73 μM (Zhou et al., 2017a). 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 et al., 2020c). 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 et al., 2016). 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 (Zhou et al., 2017a; Cheng et al., 2013). Cheng et al. (2013) 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 with controls on day 9, but expression of *Nkx2.5* and *Mlc1a* were significantly higher in PFOS-treated cells by day 14 of differentiation (Cheng et al., 2013).

Proteomic analysis during cardiac differentiation of mouse ESCs revealed 176 differentially expressed proteins (67 upregulated and 109 downregulated) (Zhang et al., 2016). 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. (2020c) 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 et al., 2020c) found that PFOS did not affect mitochondrial integrity on day 12 of differentiation.

Cheng et al. (2013) found that PFOS reduced ATP production, increased accumulation of ROS, and stimulated apoptosis in mouse ESC-CMs. However, Tang et al. (2017) 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. (2020a) 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 et al., 2020a). 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, 2016b) 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 et al., 2014). 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. (2013a) 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. (2018a) 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. (2016a), 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) 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 upregulated in brain cortical tissue (Wan Ibrahim et al., 2013).

Lastly, Leung et al. (2018) 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 epidemiological literature reviewed in the 2016 PFOS HESD and the updated literature searches. As noted in the epidemiological fetal growth restriction summary, there is *robust* 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 (Yang et al., 2022; Cao et al., 2021; Dzierlenga et al., 2020a; Negri et al., 2017; Verner et al., 2015) (see Appendix A, (U.S. EPA, 2024a)). Comparing the postnatal growth results in infants with birth-related measures is challenging due to complex growth dynamics including rapid growth catchup periods for those with fetal restriction. Nonetheless, the evidence for postnatal weight deficits was comparable to that seen for BWT.

The consistent and strong evidence for decreases in birth weight in PFOS-exposed population is further supported by coherent evidence for other developmental effects. There is evidence of an impact of PFOS exposure on gestational duration measures (i.e., either preterm birth or gestational age measures) with most of the studies showing increased risk of gestational duration deficits. 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 increased risk), 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.

Overall, there was inconsistent evidence of PFOS impacts on rapid growth measures, postnatal height and postnatal adiposity measures up to age 2. There was less evidence available for studies of associations between PFOS exposure and 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. Several meta-analyses also show associations between PFOS and preterm birth (Yang et al., 2022; Deji et al., 2021; Gao et al., 2021) (see Appendix A, (U.S. EPA, 2024a)).

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 et al., 2018a). Additional uncertainty exists due to the potential for confounding by other PFAS, and Section 5.1.1 provides a further discussion on considerations for potential confounding by co-occurring PFAS. Very few of the existing studies performed multipollutant modeling in comparison with single pollutant estimates of PFOS associations. For studies that provided this comparison, the results were often mixed, 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 et al., 2018). 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 in rodents 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 (e.g., ossification), and developmental delays (e.g., 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 all metrics of fetal or pup viability).

Reductions in litter size or fetal 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 stillbirth, and others. However, Argus Research Laboratories (2000) 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. (2005b; 2005a) 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) 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 et al., 2015) 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. It is unclear at this time whether one effect should be considered a precursor for the other.

The mechanistic data are primarily focused on gene expression changes and epigenetic alterations related to exposure to PFOS during developmental stages. The PFOS-induced alterations to the expression of genes related to growth and development support the observations of developmental effects in animals and humans (e.g., fetal growth restriction). Molecular alterations (primarily epigenetic alterations) measured in human cord blood were related to PFOS levels in the same biological samples. Specifically, global DNA 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). The authors of these studies of epigenetic

alterations did not measure gene expression changes to confirm that the epigenetic alterations indeed affected gene expression, nor were adverse postnatal outcomes measured in the same study. 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.

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 3-17). 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 measures of decreased gestational duration and other 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 3-17. Evidence Profile Table for PFOS Exposure and 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 21 *High* confidence studies 26 *Medium* confidence studies 11 *Low* confidence studies 2 *Mixed* confidence studies	Deficits in mean birth weight were observed in most studies (27/39) in the overall population. Studies on changes in standardized birth weight measures reported some inverse associations (12/18) in the overall population or among boys or girls. Ten of 17 studies observed increased risk of low birth weight or SGA. Deficits in birth weight-related measures were supported by decreases in related FGR outcomes such as birth length (15/28) and head circumference (12/23).	• *High* and *medium* confidence studies • *Coherence* across 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. Inverse associations 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 arises due to the potential impact of hemodynamics in later pregnancy due to use of biomonitoring samples from the second and third trimester or postpartum. However,	*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). Further support is provided by consistent inverse associations with gestational age measures in *high* or *medium* confidence epidemiological studies in the overall population and consistent findings of dose-dependent decreases in fetal weight in animal models gestationally exposed to PFOS. *Human relevance and other inferences:* The available mechanistic information provides support for the biological plausibility of the
Gestational duration 10 *High* confidence studies 11 *Medium* confidence studies 7 *Low* confidence studies	Some inverse associations with gestational age measures were observed in *high* or *medium* confidence studies in the overall population (10/18). Increased risk of preterm birth was also observed in *high* or *medium* confidence studies	• *High* and *medium* confidence studies • *Consistency* in the magnitude of gestational age deficits	• *Limited* evidence of exposure-response relationships in studies examining preterm birth		

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**	
	(12/17).			several studies present associations for samples collected pre-pregnancy or in the first trimester.	phenotypic effects observed in exposed animals in support of the human relevance of the animal findings.
Fetal loss 3 *High* confidence studies 3 *Medium* confidence studies 1 *Low* confidence study	Increased risk of fetal loss was observed (4/7) although results were mostly nonsignificant. One *high* confidence study observed a significant increase in risk for miscarriage for some quintiles of exposure in subgroup analyses. One *medium* confidence study reported an inverse association.	• *High* and *medium* confidence studies • *Good sensitivity* across all studies • *Consistent* magnitude of effect • *Exposure-response* relationship	• No factors noted		
Postnatal growth 4 *High* confidence studies 7 *Medium* confidence studies 3 *Low* confidence studies	Most studies (8/10) reported an inverse association for infant weight or BMI changes. There was some evidence of an exposure-response relationship in two studies (2/4) reporting categorical exposures. Decreases in infant height were mixed (2/4). Inverse associations were observed for infant weight in most *medium* and *high* confidence studies (6/10), while two studies observed positive associations (2/10). In	• *High* and *medium* confidence studies • *Exposure-response* relationship • *Good* or *adequate sensitivity* for most studies	• *Inconsistent* timing of follow-up evaluation		

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**	
	medium and *high* confidence studies, inverse associations with infant BMI or adiposity were observed in some studies (4/9), but other studies reported positive associations (1/8) or mixed associations by sex and timepoint (2/8).				
Birth defects 4 *Medium* confidence studies 3 *Low* confidence studies	One *low* confidence study (1/2) 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 nonsignificant 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 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 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 • *Exposure-response* relationship	• *Inconsistent direction* of effects across species	⊕⊕⊙ *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 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 to mating through lactation, and the study with higher doses	• *Medium* confidence studies • *Consistent direction* of effects • *Dose-dependent* response	• No factors noted		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	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 capacity (1/1) were	• *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
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	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
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream 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 Judgment**	
Key findings and interpretation: • Evidence from zebrafish embryo assays demonstrate that PFOS exposure can lead to embryonic and/or larval 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,	

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	
				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 = deoxyribonucleic acid; mRNA = messenger ribonucleic acid.

3.4.5 Evidence Synthesis and Integration for Other Noncancer Health Outcomes

Consistent with the SAB's recommendation (U.S. EPA, 2022e), EPA concluded that the noncancer 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 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 (U.S. EPA, 2021b). Based on this analysis, these outcomes were not prioritized for the subsequent literature search update efforts; the evidence synthesis and integration for these outcomes are presented in Appendix C (U.S. EPA, 2024a). In addition, Section 5.5 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 17 epidemiological and 1 animal toxicological study (2 overlapping publications) that investigated the association between PFOS and cancer. Of the epidemiological studies, eight were classified as *medium* confidence, seven as *low* confidence, and two were 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 and Summary of Evidence from the 2016 PFOS HESD

There are eight epidemiological studies (nine publications[15]) from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and cancer effects. Study quality evaluations for these seven studies are shown in Figure 3-70.

The 2016 PFOS HESD (U.S. EPA, 2016b) concluded that there was no evidence of carcinogenic effects for PFOS from human studies, 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 et al., 2003), a subsequent study to ascertain cancer incidence in the cohort observed elevated but nonsignificant incidence ratios that were 1.7- to twofold higher among

[15] Ghisari, 2014, 2920449 analyzes interactions between gene polymorphisms and PFOS exposure on breast cancer risk in the same population analyzed in Bonefeld-Jørgensen, 2011, 2150988.

exposed workers (Alexander and Olsen, 2007). Mean PFOS serum levels were 94.1 ng/mL. In the same 3M cohort, Grice et al. (2007) 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 risk was observed for bladder, liver, or pancreatic cancer in a nested case-control study in a Danish cohort with plasma PFOS concentrations at enrollment ranging 1–130.5 ng/mL (Eriksen et al., 2009). No elevated risk of colorectal cancer was observed in community participants of the C8 Health Project (Innes et al., 2014). Elevated nonsignificant ORs for prostate cancer were reported for the occupational cohort examined by Alexander and Olsen (2007) and the Danish population-based cohort examined by Eriksen et al. (2009), and no association was reported by another case-control study in Denmark (Hardell et al., 2014). 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 et al., 2014; Bonefeld-Jorgensen et al., 2011). 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 (Hardell et al., 2014; Bonefeld-Jorgensen et al., 2011). No associations were adjusted for other perfluorinated chemicals in serum in any of the occupational and population-based studies.

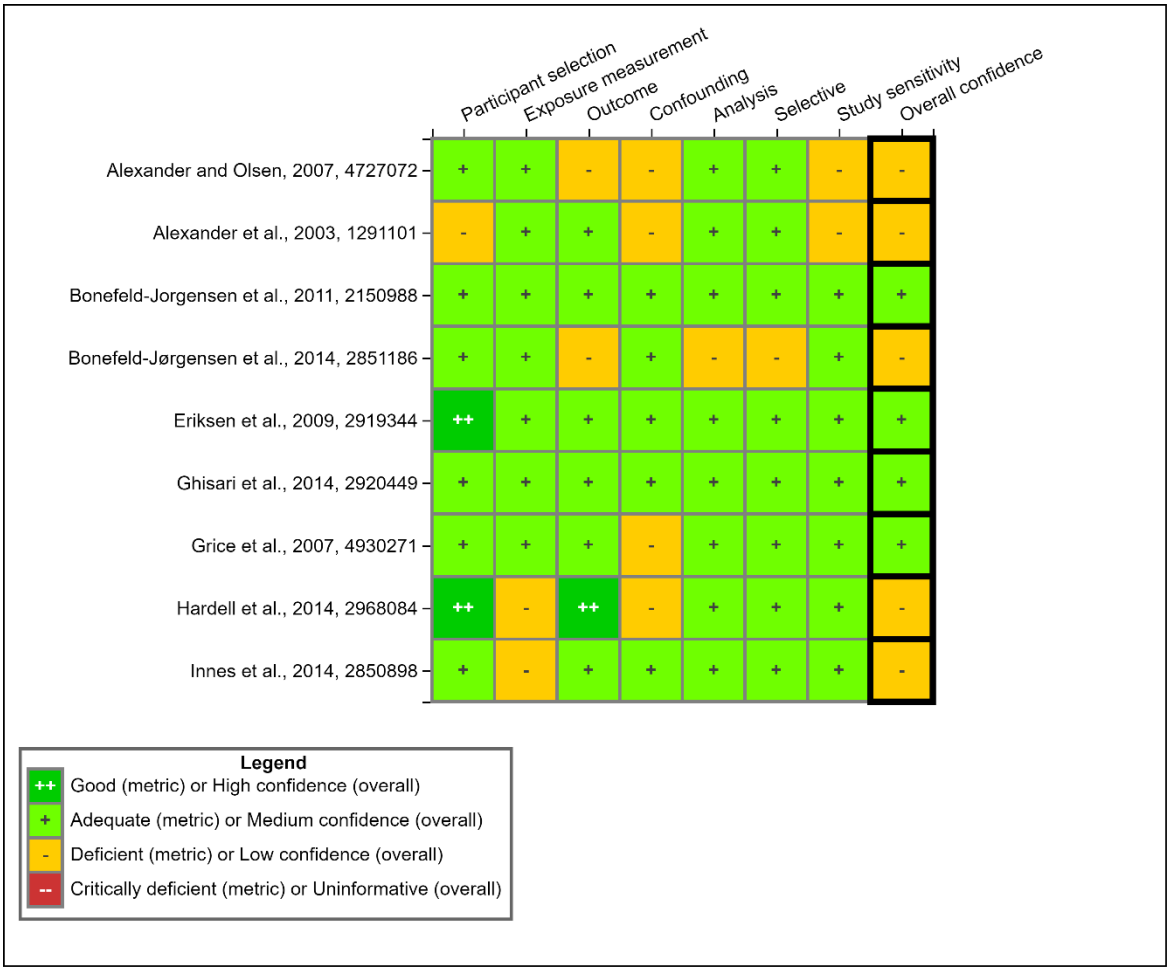


Figure 3-70. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Cancer Effects Published Before 2016 (References from 2016 PFOS HESD)

Interactive figure and additional study details available on HAWC.

Since publication of the 2016 PFOS HESD (U.S. EPA, 2016b), 17 studies have been published that investigated the association between PFOS and cancer (see Appendix D, (U.S. EPA, 2024a)). 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 (Li et al., 2022; Fry and Power, 2017), six case-control studies (Cao et al., 2022; Itoh et al., 2021; Liu et al., 2021; Lin et al., 2020b; Tsai et al., 2020; Wielsøe et al., 2017), six nested case-control studies (Goodrich et al., 2022; Shearer et al., 2021; Cohn et al., 2020; Mancini et al., 2020; Hurley et al., 2018; Ghisari et al., 2017), and three cross-sectional studies (Omoike et al., 2021; Christensen et al., 2016; Ducatman et al., 2015). The studies were conducted in different study populations including populations from China (Cao et al., 2022; Liu et al., 2021; Lin et al., 2020b), Denmark (Ghisari et al., 2017), France (Mancini et al., 2020), Greenland (Wielsøe et al., 2017), Japan (Itoh et al., 2021), Sweden (Li et al., 2022), Taiwan (Tsai et al., 2020), and the United States (Goodrich et al., 2022; Omoike et al., 2021; Shearer et al., 2021; Cohn et al., 2020; Hurley et al., 2018; Fry and Power, 2017; Christensen et al., 2016; Ducatman et al., 2015). 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 et al., 2020). Cancers evaluated included breast (Li et al., 2022; Itoh et al., 2021; Omoike et al., 2021; Cohn et al., 2020; Mancini et al., 2020; Tsai et al., 2020; Hurley et al., 2018; Ghisari et al., 2017; Wielsøe et al., 2017), germ cell tumors (Lin et al., 2020b), kidney (Shearer et al., 2021), liver (Cao et al., 2022; Goodrich et al., 2022), melanoma (Li et al., 2022), ovarian (Omoike et al., 2021), prostate (Omoike et al., 2021; Ducatman et al., 2015), thyroid (Liu et al., 2021) uterine (Omoike et al., 2021), and any cancer (Li et al., 2022; Fry and Power, 2017; Christensen et al., 2016).

3.5.1.2 Study Quality

Study quality evaluations for the 17 studies identified since the 2016 PFOS HESD are shown in Figure 3-71. Of these 17 studies, eight were considered *medium* confidence and seven were *low* confidence (Cao et al., 2022; Itoh et al., 2021; Liu et al., 2021; Omoike et al., 2021; Lin et al., 2020b; Tsai et al., 2020; Christensen et al., 2016). One study conducted in the high exposure to PFAS Ronneby Register Cohort in Sweden was *uninformative* (Li et al., 2022) because of concerns about exposure assessment and lack of data on important covariates. One study conducted in Greenland was considered *uninformative* (Wielsøe et al., 2017) because of concerns about exposure assessment and participant selection. As a result, these two studies are not further considered in this review. Concerns in 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 and other lifestyle factors, which could be associated with both exposure and cancer outcome. Although PFOS has a relatively 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 was noted to be a concern in several *low* confidence studies (Itoh et al., 2021; Liu et al., 2021; Omoike et al., 2021; Tsai et al., 2020). Many of the *low* confidence studies also had sensitivity issues due to small sample sizes. Lack of details or reporting issues were also a concern for some *low* confidence studies which resulted in difficulty in quantitatively interpreting analysis results (Cao et al., 2022). Cao et al. (2022) was determined to have *mixed* confidence (*low* and *uninformative*). The *uninformative* metric was the liver cancer biomarker analysis included in this study which did not provide sufficient information on biomarker measurement methods (Cao et al., 2022). The biomarker analysis portion of this study is not further considered in this review.

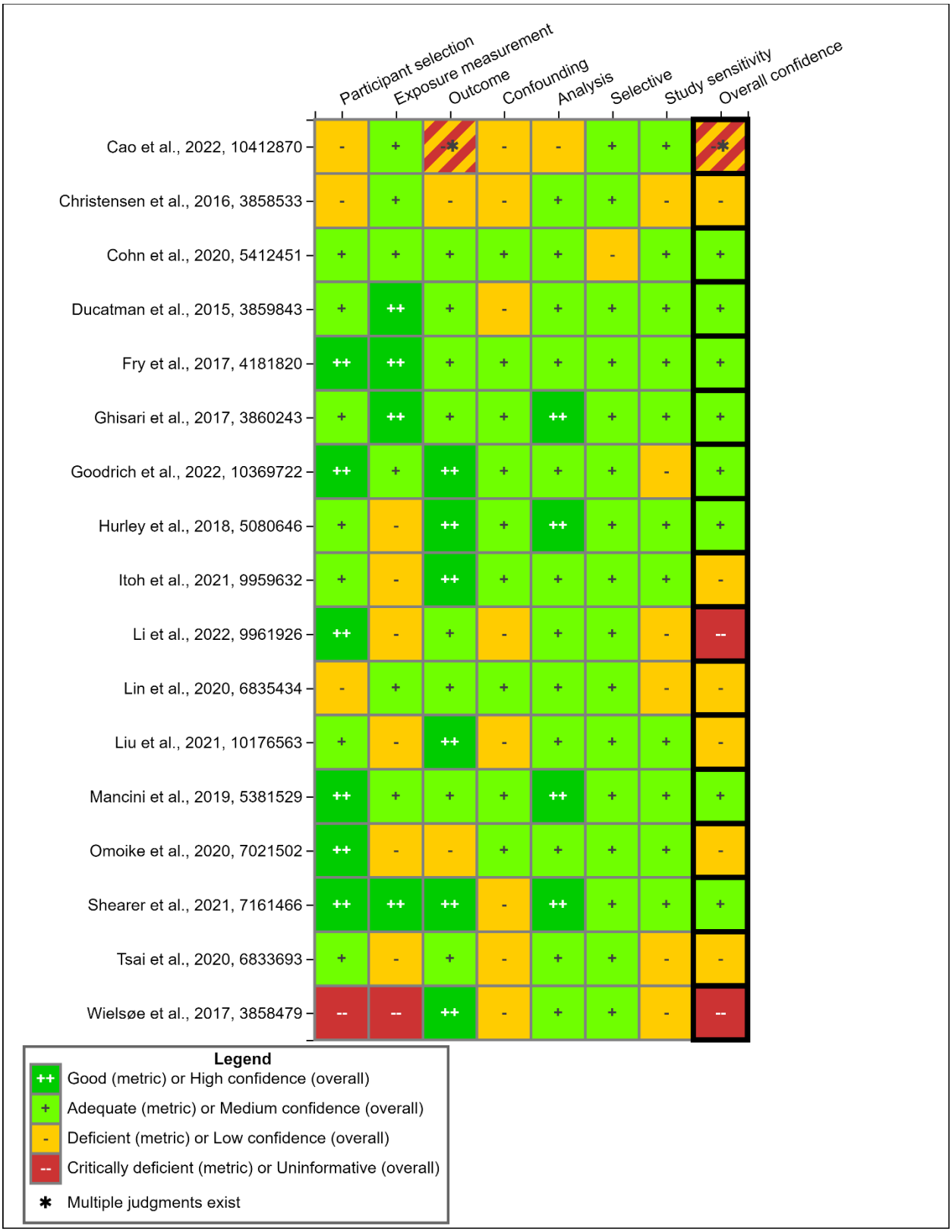


Figure 3-71. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOS Exposure and Cancer Effects

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 et al., 2020b) 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 1 week after diagnosis. However, the study did not observe an increased risk of germ cell tumors association with a per ng/mL increase in blood PFOS. One *low* confidence study examined liver cancers in children and adults (Cao et al., 2022), but since results are not presented separately by age group, this study will be reviewed in the following section.

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 et al., 2021). 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 et al., 2021). 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 with a per doubling change in PFOS after adjusting for other PFAS.

Seven general population studies published since the 2016 PFOS HESD, evaluated PFOS and risk for breast cancer (Itoh et al., 2021; Omoike et al., 2021; Cohn et al., 2020; Mancini et al., 2020; Tsai et al., 2020; Hurley et al., 2018; Ghisari et al., 2017) with mixed results. All studies were case-control studies (with some nested case-controls), except for one cross-sectional NHANES-based study (Omoike et al., 2021). Three studies were considered *low* confidence (Itoh et al., 2021; Omoike et al., 2021; Tsai et al., 2020) because of concerns about temporality of exposure measurements and breast cancer development, the control status was not confirmed via examination or medical records (Tsai et al., 2020), and potential for residual confounding due to SES, lifestyle factors and exposure to 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 et al., 2018). A nested case-control study in a prospective (pregnancy) cohort study, the CHDS, suggested that maternal PFOS was associated with a decrease in the daughters' breast cancer risk in the first or fourth quartile of TC (Cohn et al., 2020), 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 (Mancini et al., 2020; Tsai et al., 2020; Ghisari et al., 2017). Ghisari et al. (2017) 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 with 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 et al., 2020). Mancini et al. (2020) 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 et al., 2020) 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 et al., 2021) 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 with the lowest (OR = 0.87; 95% CI: 0.86, 0.89). One *low* confidence case-control study conducted in Japanese women (Itoh et al., 2021) observed a significant inverse association across serum PFOS quartiles with a significant dose-response trend (p-value < 0.0001) (see Appendix D, (U.S. EPA, 2024a)). 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).

Two general population studies published since the 2016 PFOS HESD examined liver cancer (Cao et al., 2022; Goodrich et al., 2022). One study was considered *medium* confidence (Goodrich et al., 2022) and one study was considered *low* confidence (Cao et al., 2022). The *medium* confidence nested case-control study of U.S. adults observed a significant increase in risk of liver cancer when comparing participants with PFOS exposures above the 85th percentile (54.9 ng/mL) compared with those at or below (OR = 4.50, 95% CI: 1.20, 16.00) (Goodrich et al., 2022). The association remained elevated but not statistically significant in analyses of continuous PFOS exposure. The study was nested in the large Multiethnic Cohort study of California and Hawaii; however, the sample size was small (n = 50 cases and controls each) which likely limited study sensitivity. A significantly elevated risk of liver cancer was also observed in a *low* confidence case-control study of Chinese children and adults (OR per log-ng/mL increase in PFOS exposure = 2.609; 95% CI: 1.179, 4.029) (Cao et al., 2022). However, confidence in the study results was considered *low* due to limited or lacking information regarding selection of controls, diagnosis method for liver cancer, adjustment for potential confounding, and details on the statistical analysis.

One *medium* confidence study based on the C8 Health Project (Ducatman et al., 2015) 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) observed a significantly inverse association with prostate cancer (OR = 0.994; 95% CI: 0.994, 0.994).

Omoike et al. (2021) 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 Appendix D, (U.S. EPA, 2024a)). 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 et al., 2021).

One *low* confidence study conducted in Shandong Province, in eastern China (Liu et al., 2021) 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 excrete PFAS could change when the thyroid becomes cancerous by causing abnormal thyroid hormone levels which can affect the glomerular filtration rate (Dzierlenga et al., 2020b), thereby changing the PFAS concentrations.

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 and Power, 2017); 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 et al., 2016). Christensen et al. (2016) 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 (2 overlapping publications) from the 2016 PFOS HESD (U.S. EPA, 2016b) that investigated the association between PFOS and cancer effects. Study quality evaluation for this one study is shown in Figure 3-72.

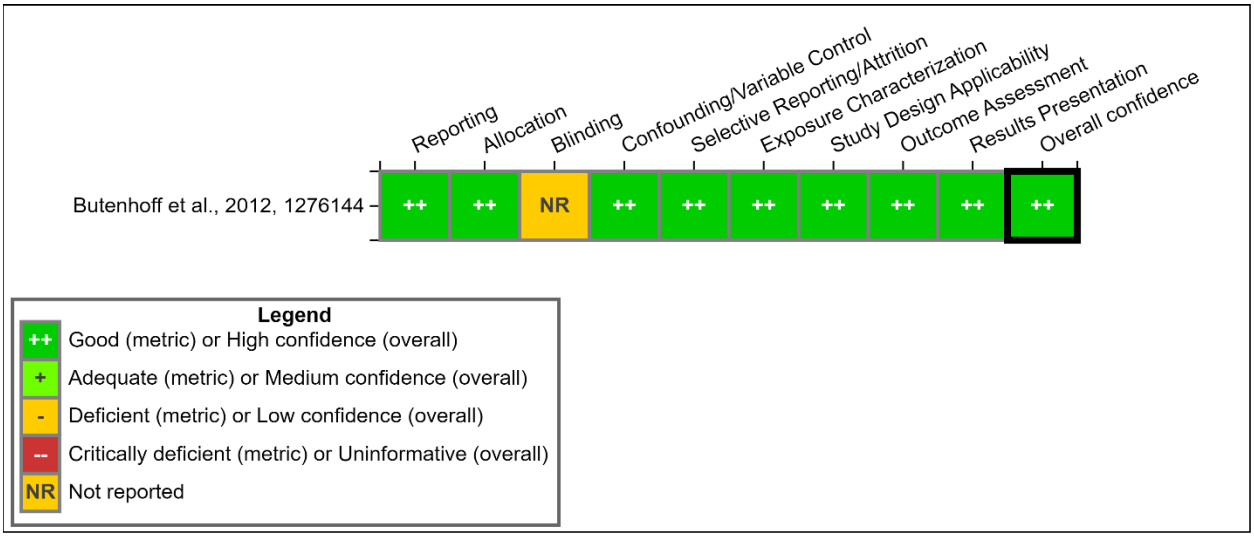


Figure 3-72. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOS Exposure and Cancer Effects

Interactive figure and additional study details available on HAWC.

A single chronic cancer bioassay in animals was previously identified for PFOS (Butenhoff et al., 2012; Thomford, 2002a). In this study, conducted by Thomford (2002a) and published in part by Butenhoff et al. (2012), male and female Crl:CD®(SD)IGS BR rats were administered diets containing 0, 0.5, 2, 5, or 20 ppm PFOS for 103–104 weeks. Increased incidence of hepatocellular adenomas in the high-dose groups for male (7/43; 16%) and female rats (5/31; 16%) and combined adenomas/carcinomas in high-dose group females (6/32; 19%) were observed (Table 3-18). There was also a statistically significant positive trend of each of these responses in both male and female rats (all $p \leq 0.01$). At 105 weeks there was an accompanying increase in eosinophilic clear cell foci, and cystic hepatocellular degeneration in males given 2, 5, and 20 ppm PFOS. Low levels of single cell necrosis in all dose groups for both males and females were identified, though the increase compared with controls was significant only at the highest dose in each sex.

Table 3-18. Incidences[a] of Hepatocellular and Pancreatic Tumors in Male and Female Sprague-Dawley Rats as Reported by Thomford (2002b)

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%)*

Sex	Tumor Type	Treatment group				
		0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
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[b]	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 (17%)	9/44 (20%)

Notes: *Statistically significant compared with the control group at $p \leq 0.05$. **Statistically significant compared with 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.
[b] Statistical significance determined by EPA using the Cochran-Armitage test.

In addition to hepatocellular tumors, Thomford (2002b) reported increased incidences of pancreatic islet cell carcinomas in male rats (Table 3-18). Though the 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 ($p \leq 0.05$; Cochran-Armitage test).

Thyroid and mammary gland tumors were also observed but did not exhibit linear dose-response relationships (Butenhoff et al., 2012; Thomford, 2002b). 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, 2016b). There are 27 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 by data source is shown in Figure 3-73.

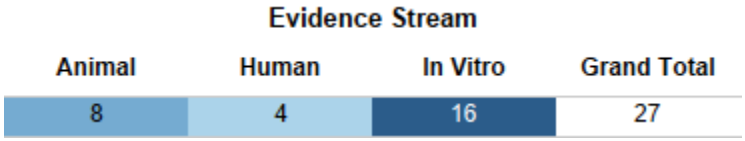


Figure 3-73. Summary of Mechanistic Studies of PFOS and Cancer Effects

Interactive figure and additional study details available on HAWC..

In 2016, 10 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 et al., 2016b). In contrast to the

"Hallmarks of cancer" as presented by Hanahan and Weinberg (Hanahan, 2022; Hanahan and Weinberg, 2011, 2000), the key characteristics focus on the properties of human carcinogens that induce cancer, not the phenotypic or genotypic traits of cancers. The 10 key characteristics provide a framework to systematically identify, organize, and summarize mechanistic information for cancer hazard evaluations (Smith et al., 2016b).

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 7 of the 10 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, 2016b) 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-characterized 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 Gene Mutation

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 et al., 2015b). 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 3-19).

3.5.3.1.1.2 In Vitro Evidence

Several studies have demonstrated that PFOS is not mutagenic in vitro (Table 3-20). Of the four publications that tested PFOS for mutagenicity in *Salmonella typhimurium, Saccharomyces cerevisiae,* and *Escherichia coli* (NTP, 2019; Mecchi, 1999; Litton Bionetics, 1979 10228135; Simmon, 1978), no evidence of DNA mutagenesis has been described in the presence or absence of metabolic activation. In contrast, Wang et al. (2015b) 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

3.5.3.1.2.1.1 Human Studies

One study reported on the genotoxic potential of PFOS exposure in humans (Table 3-21). Governini et al. (2015) collected semen samples from healthy nonsmoking men and evaluated aneuploidy, diploidy, and DNA fragmentation. The occurrence of aneuploidy and diploidy in sperm cells, which are normally haploid, was significantly higher in the PFAS-positive samples (PFOS was detected in 25% of the samples) when compared with PFAS-negative samples. This suggests that PFAS exposure is related to errors in cell division leading to aneugenicity. Additionally, fragmented chromatin levels were also significantly increased for the PFAS-positive group compared with the PFAS-negative group.

3.5.3.1.2.1.2 Animal Toxicological Studies

Evaluations of PFOS exposure in rat, mouse, and zebrafish models were identified, which predominantly demonstrated evidence of genotoxicity (Table 3-21). 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. Quantifying micronuclei formation in rats via optimal and reliable methods has been previously described (WHO & FAO, 2020; WHO and FAO, 2009; Witt et al., 2000).

NTP (2019) 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) 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 with controls indicating a positive test, the response was nonetheless within the range of historical control levels. NTP (2019) 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 (Eke et al., 2017; Eke and Çelik, 2016; Çelik et al., 2013). Çelik et al. (2013) 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, similar to the results from NTP (2019), the study also demonstrated that PFOS exposure decreased the ratio of PCEs to normochromic erythrocytes (NCEs), indicating 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) reported increased micronuclei formation and genetic damage indices (calculated using results of a comet assay) in peripheral blood, while Eke et al. (2017) 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. Given the results from Çelik et al. (2013) 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.

Micronucleus frequency was slightly elevated 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 et al., 2015b). Similarly, EPA's 2016 PFOS HESD (U.S. EPA, 2016b) reported mouse bone marrow micronucleus assays to be negative after high-dose acute exposures (237.5, 475, and 950 mg/kg; measured after approximately 24, 48, and 72 hours) to PFOS (Murli, 1996). 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).

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 et al., 2014). Following exposure, Du et al. (2014) found significant dose-dependent increases in micronucleus formation. Du et al. (2014) 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.

3.5.3.1.2.2 In Vitro Evidence

3.5.3.1.2.2.1 Chromosomal aberrations

EPA's 2016 PFOS HESD (U.S. EPA, 2016b) reports that PFOS exposure did not induce chromosomal aberrations in human lymphocytes (Table 3-22) (Murli, 1999). 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) 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 HAWC visual 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 3-22). Wang et al. (2015b) 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 et al., 2012).

In contrast, several studies found no evidence of DNA damage after exposure. Jacquet et al. (2012) 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 et al., 2010).

Florentin et al. (2011) 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) 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 3-19. Mutagenicity Data From In Vivo Studies

Reference	Species, Strain (Sex)	Tissue	Results	PFOS Concentration (Dosing Regimen)
Wang et al. (2015b)	Mouse, *Gpt* delta transgenic (Male)	Liver	Negative	1–10 mg/kg/day (daily via gavage for 28 days)

Table 3-20. 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)	*Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100)	Negative	Negative	0.1–1,000 µg/plate
Litton Bionetics, Inc. (1979)	*Saccharomyces cerevisiae* (D4)	Not Reported	Negative	0.1–1,000 µg/plate
Mecchi (1999)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537)	Negative	Negative	0.333–5,000 µg/plate
Mecchi (1999)	*Escherichia coli* (WP2*uvr*A)	Negative	Negative	33.3–5,000 µg/plate
NTP (2019)	*Salmonella typhimurium* (TA98, TA100)	Negative	Negative	100–5,000 µg/plate
NTP (2019)	*Escherichia coli* (WP2*uvr*A/pkM101)	Negative	Negative	100–10,000 µg/plate
Simmon (1978)	*Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100)	Negative	Negative	10–5,000 µg/plate
Simmon (1978)	*Salmonella cerevisiae* (D3)	Negative	Negative	0.1–5 µg/plate
Wang et al. (2015b)	*gpt* Delta transgenic mouse embryonic fibroblasts	Not reported	Positive[a]	1–20 µM (24 hours)

Notes:
[a] Mutagens were present in cells exposed ≥10 µM.

Table 3-21. DNA Damage Data From In Vivo Studies

Reference	Species, Strain (Sex)	Tissue	Results	PFOS Concentration (Dosing Regimen)
DNA Strand Breakage				
Governini et al. (2015)	Human (Male)	Semen	Positive	Average Seminal Plasma Concentration of 5.37 ng/g f.w.
DNA Damage via Comet Assay				
Çelik et al. (2013)	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)	Zebrafish, AB (Male and female)	Peripheral blood cells	Negative	0.4–1.6 mg/L (single dose to rearing water)
Eke and Çelik (2016)	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)	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)	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)	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)	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)	Rat, Swiss Albino (Male)	Liver	Positive	0.6–2.5 mg/kg/day (every other day via gavage for 30 days)
Murli (1996)	Mouse, Crl:CD-1 (Male and female)	Bone marrow	Negative	—[a]
NTP (2019)	Rat, Sprague-Dawley (Male)	Peripheral blood cells	Negative	0.312–5 mg/kg/day (daily via gavage for 28 days)
NTP (2019)	Rat, Sprague-Dawley (Female)	Peripheral blood cells	Equivocal	0.312–5 mg/kg/day (daily via gavage for 28 days)
Wang et al. (2015b)	Mouse, *Gpt* delta transgenic (Male)	Bone marrow	Negative	1–10 mg/kg/day (daily via gavage for 28 days)

Notes: f.w. = formula weight.

[a] Findings based on the 2016 EPA's Health Effects Support Document for Perfluorooctane Sulfonate (PFOS) (U.S. EPA, 2016b), concentration(s) unknown.

Table 3-22. DNA Damage Data From In Vitro Studies

Reference	In Vitro Model (Assay)	Results	Concentration (Duration of Exposure)
	Chromosomal Aberrations		
Murli (1999)	Human lymphocytes	Negative	10–470 µg/mL (3 hours)
	Unscheduled DNA Synthesis		
Cifone (1999)	Fisher 344 male rat hepatocytes	Negative	0.25–4,000 µg/mL
	DNA Damage		
Wang et al. (2015b)	*gpt* Delta transgenic mouse embryonic fibroblasts (γ-H2AX foci)	Positive	0–30 µM (24 hours)
Jacquet et al. (2012)	Syrian hamster embryo cells (comet assay)	Negative	2×10^{-4}–50 µg/mL (7 days)
Kawamoto et al. (2010)	*Paramecium caudatum* (comet assay)	Negative	10–100 µM (1–24 hours)
Lu et al. (2012)	Calf thymus DNA (X-ray photoelectron spectroscopic and electrochemical impedance spectroscopy)	Positive	10 µmol/L (30 minutes)
Wielsoe et al. (2015)	HepG2 (comet assay)	Positive	2×10^{-7}–2×10^{-5} M (24 hours)
Florentin et al. (2011)	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 et al., 2016b).

3.5.3.2.1 In Vivo Evidence

3.5.3.2.1.1 Humans

A cohort of singleton term births were recruited from Faroese hospitals over an eighteen-month period from 1986 to 1987 (Leung et al., 2018). 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 et al., 2014). 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 HAWC visual for additional supporting information and study details).

3.5.3.2.1.2 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 HAWC visual 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 HAWC visual for additional supporting information and study details).

3.5.3.2.2 In Vitro Evidence

Pierozan et al. (2020) 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 HAWC visual 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., 2016b).

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 HAWC visual 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 et al., 2018a). 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) 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 versus 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 HAWC visual 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) 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) 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. (2015b) 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 with cells exposed to PFOS alone. These results in Wang et al. (2015b) suggest that PFOS-induced genotoxicity is mediated by the induction of ROS.

Wielsoe et al. (2015) 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 HAWC visual 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 HAWC visual 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 et al., 2016b). 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 HAWC visual 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 et al., 2016b). 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 HAWC visual 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 et al., 2016b).

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 HAWC visual 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 (Wolf et al., 2014; Wolf et al., 2008; Martin et al., 2007; Shipley et al., 2004).

Jacquet et al. (2012) 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 threefold 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 (twofold 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 and Fair, 2013).

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 et al., 2016b).

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 HAWC visual 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 (Rao and Reddy, 1996; Ashby et al., 1994), 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 et

al., 2003). However, the same authors continued this same dietary PFOS exposure in Sprague-Dawley rats for up to 2 years and found liver effects consistent with PPAR activation (Butenhoff et al., 2012; Thomford, 2002b). This 2-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 et al., 2019). 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 et al., 2020a; Gogola et al., 2020b).

The potential of PFOS to induce tumorigenic activity (proliferation, cell-cycle progression, and malignant phenotype) was evaluated in MCF-10A breast epithelial cells (Pierozan and Karlsson, 2018). 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 et al., 2020). 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 and Fair, 2013). 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 et al., 2006). 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 et al., 2017). 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 HAWC visual for additional supporting information and study details).

3.5.4 Weight Of Evidence for Carcinogenicity

3.5.4.1 Summary of Evidence

The carcinogenicity of PFOS has been documented in both epidemiological and animal toxicological studies. The available epidemiology studies report elevated risk of liver, bladder, kidney, prostate, and breast cancers after chronic PFOS exposure in some studies, though limited evidence for some tumor types (i.e., liver and renal) and mixed results for other tumor types (i.e., bladder, prostate, breast) provide plausible but not definitively causal evidence of a relationship between PFOS exposure and cancer outcomes from the epidemiological evidence alone. The animal chronic cancer bioassay provides additional support for carcinogenicity with the identification of multi-site tumorigenesis (liver and pancreas) in both male and female rats. The available mechanistic data suggest that multiple MOAs could play role in the hepatic and pancreatic tumorigenesis associated with PFOS exposure based on animal model study findings.

3.5.4.1.1 Evidence From Epidemiological Studies

Results for liver cancer from one *low* confidence occupational (Alexander et al., 2003) and one *medium* confidence general population-based (Eriksen et al., 2009) study of PFOS exposure published approximately 15–20 years ago were generally imprecise (i.e., null results with wide confidence intervals), but more recent studies have reported statistically significant increased risk of liver cancer associated with increased PFOS exposure (Cao et al., 2022; Goodrich et al., 2022). A *medium* confidence nested case-control study of adults from the Multiethnic Cohort (MEC) study reported a significant increased risk of liver cancer when comparing those in the 85th percentile of PFOS exposure to those at or below the 85th percentile (Goodrich et al., 2022). Positive, but not statistically significant, associations were observed in analyses of continuous PFOS exposure which supported the study's overall conclusion of an increased risk of liver cancer with increasing PFOS exposure. The study's sensitivity was limited by the small number of cases and controls (n = 50 each). Consistent with this finding, a Chinese general population case-control study of children and adults reported a significant increase in risk of liver cancer in analyses of continuous PFOS exposure; however, the study was considered *low* confidence due to lack of information on control selection, outcome ascertainment, and statistical analysis (Cao et al., 2022).

Studies of the association between PFOS serum concentrations and bladder cancer have mixed (positive and null) findings. An elevated risk of bladder cancer mortality was associated with PFOS exposure in an occupational study (Alexander et al., 2003) but a subsequent study to ascertain cancer incidence in this cohort with four additional years of observation observed elevated but not statistically significant incidence ratios that were 1.7- to twofold higher among workers with higher cumulative exposure to PFOS (Alexander and Olsen, 2007). Some of the limitations of these studies include the lack of precision of the risk estimates due to the small number of cases, and the lack of 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 et al., 2009). Overall, there is plausible evidence of a relationship between PFOS exposure and bladder cancer, particularly for high-exposure communities.

One study in the general population reported a statistically significant increase in risk of RCC in the highest PFOS exposure quartile and in continuous analyses of PFOS exposure (i.e., per

doubling of PFOS concentration) (Shearer et al., 2021). 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 reported association when evaluated on a per doubling of PFOS after adjusting for other PFAS.

Elevated nonsignificant ORs for prostate cancer were reported for the occupationally exposed cohort examined by Alexander and Olsen (2007) and the Danish population-based cohort examined by Eriksen et al. (2009). In the same occupational cohort studied by Alexander and Olsen (2007), Grice et al. (2007) observed that prostate cancers were among the most frequently reported cancers. When cumulative PFOS 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 (Grice et al., 2007). No association between PFOS exposure and prostate cancer was reported in either a second case-control study in Denmark (Hardell et al., 2014) 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 et al., 2015). In an NHANES population, Omoike et al. (2021) observed a significantly inverse association between PFOS exposure and prostate cancer.

The majority of studies examining associations between PFOS exposure and cancer outcomes were on breast cancer. One study of Inuit females in Greenland observed positive associations between PFOS levels and risk for breast cancer (Bonefeld-Jorgensen et al., 2011), although the association was of a low magnitude and could not be separated from the effects of other perfluorosulfonated compound exposures (i.e., PFHxS and PFOSA). 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) reported that increased breast cancer risk was associated with increased PFOS serum concentrations in Danish individuals with a specific polymorphism in the CYP19 gene (for aromatase, associated with estrogen biosynthesis and metabolism). Mancini et al. (2020) 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) observed a statistically significant increased risk of breast cancer in all women 50 years old or younger (including ER+ and ER– participants), and in ER+ participants aged 50 years or younger. Statistically significant increases in breast cancer risk were also observed in an NHANES population in the two highest quartiles of exposure, but the association was inverse in the second quartile (Omoike et al., 2021). No association was identified between PFOS and breast cancer in either case-control or nested case-control studies of Danish and California cancer registry populations, respectively (Hurley et al., 2018; Bonefeld-Jørgensen et al., 2014). Another general population study in the United States 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 individuals with genetic variants that may have increased susceptibility (Cohn et al., 2020). A recent study in a Japanese population observed an inverse association across serum PFOS quartiles with a significant dose-response trend (Itoh et al., 2021). 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 (Omoike et al., 2021; Tsai et al., 2020). 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 exposure and subsequent cancer assessment is not available. Overall, study design limitations with specific studies, lack of replication of the results, and a lack of mechanistic understanding of specific breast cancer subtypes or susceptibilities of specific populations limit firm conclusions regarding PFOS and breast cancer. However, there is suggestive evidence that PFOS exposure may be associated with an increased breast cancer risk based on studies in susceptible populations, such as those with specific polymorphisms and for specific types of breast tumors.

3.5.4.1.2 Evidence From Animal Bioassays

One available chronic toxicity/carcinogenicity bioassay for PFOS, a 104-week dietary study in rats, provides evidence of multi-sex and multi-site tumorigenesis resulting from PFOS exposure (Butenhoff et al., 2012; Thomford, 2002b). This study was originally published as a 3M-sponsored report by Thomford (2002b) and some of the data were later published in a peer-reviewed study by Butenhoff et al. (2012). Statistically significant increases in the incidence of hepatocellular adenomas in the high-dose (20 ppm) male (7/43; 16%) and female (5/31; 16%) rat groups and combined adenomas/carcinomas in the females (6/32; 19%; five adenomas, one carcinoma) were observed. The observation of one 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, 2020a). Historical control incidence rates for these tumor types were not provided by Thomford (2002b). Additionally, there were statistically significant dose-related 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, 2020a).

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 (Butenhoff et al., 2012; Thomford, 2002b). 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 (2002b) 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 (2020a) has reported for its laboratories (3/637 combined follicular cell adenoma or carcinoma). There were few follicular cell adenomas/carcinomas in the females (4 total, excluding the recovery group) with a nonlinear dose response. Mammary gland tumors, primarily combined fibroma adenoma and adenoma, were also observed in females, though there was a high background incidence of mammary gland tumors in the control animals, and the incidence lacked dose response for all tumor classifications.

3.5.4.2 Mode of Action Analysis

As PFOS has been associated with multi-site tumorigenesis in both epidemiological studies and animal toxicological studies, not always with site concordance, it is reasonable to assume that it may act through multiple carcinogenic MOAs. In the 2016 PFOS HESD (U.S. EPA, 2016b), EPA suggested that the induction of tumors may be related to nuclear receptor activation, mitochondrial effects, and gap junction intercellular communication. As described in the following subsections, the available mechanistic data continue to suggest that multiple MOAs could play a role in the tumorigenesis associated with PFOS exposure in animal models and human populations.

3.5.4.2.1 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 et al., 2012; Thomford, 2002b). These findings in rats are supported by recent epidemiological studies that have reported associations between PFOS and hepatocellular carcinoma in humans (Cao et al., 2022; Goodrich et al., 2022).

The EPA previously concluded that, "the data are inadequate to support a PPARα-linked MOA for the liver and thyroid adenomas observed by Thomford (2002)/Butenhoff et al. (2012)" (U.S. EPA, 2016b). As described in the subsections below, the available mechanistic data continue to 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.

3.5.4.2.1.1 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, 2016b). 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 (Corton et al., 2018; Klaunig et al., 2003). 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 (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003).

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 (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003) (Table 3-23, Table 3-24). This AOP is associated with but not necessarily causally related to nonneoplastic 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 (Corton et al., 2018; Huck et al., 2018; Rosen et al., 2017; Beggs et al., 2016).

Table 3-23. Evidence of Key Events Associated With the PPARα Mode of Action for Hepatic Tumors[a] in Male Sprague-Dawley Rats Exposed to PFOS

Canonical MOA	Key Event 1: PPARα Activation	Key Event 2: Altered Cell Growth Signaling	Key Event 3a: Increased Hepatic Cell Proliferation	Key Event 3b: Inhibition of Apoptosis	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**PPARα Activation[c]**	**Altered Cell Growth Signaling**	**Hepatic Cell Proliferation**	**Apoptosis**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
0.024	– (4, 14w)	– (4w)	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.098	– (4, 14w)	– (4w)	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.242	– (4, 14w)	– (4w)	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.312	**↑ (4w)**	NR	NR	– (4w)	NR	NR
0.625	**↑ (4w)**	NR	NR	– (4w)	NR	NR
0.984	**↑ (4w) – (14w)**	**↑ (4w)**	**↑ (4w) – (14, 53w)**	**↓ (103w) – (14, 53w)**	NR	**↑ (103w)**
1	**↑ (F_1 PND 21)**	NR	NR	NR	NR	NR
1.25	**↑ (4w)**	NR	NR	– (4w)	NR	NR
1.33/1.51	**– (4, 14w)**	NR	– (4w)	NR	NR	NR
1.66	**↑ (28d) – (1, 7d)**	NR	**↑ (7d) – (1, 28d)**	↑ (7d) – (1, 28d)	NR	NR
1.93	– (7d)	NR	**↑ (7d)**	**↓ (7d)**	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; NR = not reported; d = day(s); w = week(s); F_1 = first generation of offspring; PND = postnatal day.

Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from NTP (2019); Chang et al. (2009); Elcombe et al. (2012b); Elcombe et al. (2012a); Seacat et al. (2003); and Butenhoff et al. (2012)/Thomford (2002b).

[a] Reviewed in Klaunig et al. (2003); Corton et al. (2014); and Corton et al. (2018).

[b] Doses for 0.024, 0.098, 0.242, and 0.984 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012). Dose for 1.33/1.51 mg/kg corresponds to 20 ppm in feed for animals exposed for 14 and 4 weeks, respectively, in Seacat et al., (2003). Dose for 1.66 mg/kg corresponds to 20 ppm in feed in Elcombe et al. (2012a). Dose for 1.93 mg/kg corresponds to 20 ppm in feed in Elcombe et al. (2012b).

[c] Indirect measurement of PPARα induction provided as *Cyp4a1*, *Cyp2b2*, or *ACoA* mRNA expression in Chang et al. (2009); as hepatic palmitoyl-CoA oxidase activity in Butenhoff et al. (2012)/Thomford (2002b), Seacat et al. (2003), Elcombe et al. (2012b), and Elcombe et al. (2012a); and as *Cyp4a1*, *Cyp2b1*, *Cyp2b2*, and *Acox1* gene expression or hepatic acyl-CoA oxidase activity in NTP (2019).

Table 3-24. Evidence of Key Events Associated With the PPARα Mode of Action for Hepatic Tumors[a] in Female Sprague-Dawley Rats Exposed to PFOS

Canonical MOA	Key Event 1: PPARα Activation	Key Event 2: Altered Cell Growth Signaling	Key Event 3a: Increased Hepatic Cell Proliferation	Key Event 3b: Inhibition of Apoptosis	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**PPARα Activation[c]**	**Altered Cell Growth Signaling**	**Hepatic Cell Proliferation**	**Apoptosis**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
0.029	– (4, 14w)	NR	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.120	↓ (4w) – (14w)	NR	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.299	– (4, 14w)	NR	– (4, 14w)	– (14, 103w)	NR	– (103w)
0.312	**↑ (4w)**	NR	NR	– (4w)	NR	NR
0.47	↓ (4w)	NR	– (4w)	NR	NR	NR
0.625	**↑ (4w)**	NR	NR	– (4w)	NR	NR
1	**↑ (P_0 GD 1–20)**	NR	NR	NR	NR	NR
1.25	**↑ (4w)**	NR	NR	– (4w)	NR	NR
1.251	– (4, 14w)	NR	– (4, 14, 53w)	**↓ (103w) – (14, 53w)**	NR	**↑ (103w)**
1.56/1.77	– (4, 14w)	NR	– (4w)	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; NR = not reported; w = week(s); P_0 = parental generation; GD = gestational day.

Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from NTP (2019); Chang et al. (2009); Seacat et al., (2003); and Butenhoff et al. (2012)/Thomford (2002b).

[a] Reviewed in Klaunig et al. (2003); Corton et al. (2014); and Corton et al. (2018).

[b] Doses for 0.029, 0.120, 0.299, and 1.251 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012). Dose for 0.47 corresponds to 5 ppm in feed in Seacat et al. (2003). Dose for 1.56/1.77 mg/kg corresponds to 20 ppm in feed for animals exposed for 14 and 4 weeks, respectively, in Seacat et al. (2003).

[c] Indirect measurement of PPARα induction provided as *Cyp4a1*, *Cyp2b2*, or *ACoA* mRNA expression in Chang et al. (2009), as hepatic palmitoyl-CoA oxidase activity at 4 and 14 weeks in Butenhoff et al. (2012)/Thomford (2002b), and as *Cyp4a1*, *Cyp2b1*, *Cyp2b2*, and *Acox1* gene expression in NTP (2019).

The published in vivo and in vitro literature suggests that PFOS is a relatively weak PPARα agonist compared with other known PPARα agonists such as PFOA (Behr et al., 2020b; Rosen et al., 2013; Wolf et al., 2012; Martin et al., 2007). 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 (NTP, 2019; Dong et al., 2016; Elcombe et al., 2012b; Elcombe et al., 2012a; Chang et al., 2009; Martin et al., 2007). 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 et al., 2010). 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 with responses in mice treated with 3 mg/kg/day Wyeth-14,643 (902 genes WT, 10 genes PPARα-null) or PFOA (879 genes WT, 176 genes PPARα-null) (Rosen et al., 2010), consistent with multiple MOAs for PFOS hepatic effects.

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 et al., 2018). Two short-term exposure in vivo rodent studies reported increased serum TNFα levels after 3–4 weeks of PFOS administration (Su et al., 2019; Han et al., 2018b); TNFα is a pro-inflammatory cytokine that can be released upon activation of Kupffer cells (Corton et al., 2018). In addition to serum TNFα levels, Han et al. (2018b) 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 (Butenhoff et al., 2012; Thomford, 2002b), 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 (Seacat et al., 2003; Thomford, 2002b). 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 (2002b) (i.e., 2–10 mg/kg/day) (NTP, 2019; Han et al., 2018b; Elcombe et al., 2012b; Elcombe et al., 2012a). 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 et al., 2018). 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. (2012a) reported substantially greater palmitoyl-CoA oxidation after 50 ppm Wyeth-14,643 administration in

male Sprague-Dawley rats compared with 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. (2012b) 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. (2012a) 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. (2012b; 2012a). 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 et al., 2016), and short-term PFOS studies in both rats and mice reported increases in apoptosis-related hepatic gene expression and/or protein activity/expression (Han et al., 2018a; Lv et al., 2018; Eke et al., 2017; Wan et al., 2016). 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. (2009b), Abbott et al. (2009), and Rosen et al. (2010) 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. (2017; 2010), 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 necessarily expected to induce cell proliferation or suppress apoptosis of hepatocytes in humans (Corton et al., 2018). Specifically, some have argued 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 et al., 2018; Klaunig et al., 2003). Nonetheless, several studies have reported increased cell proliferation or markers of cell proliferation in vitro in human liver cell lines exposed to PFOS (Louisse et al., 2020; Song et al., 2016; Cui et al., 2015a) (see Section 3.4.1.3). For example, Cui et al. (2015a) 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. (2015a) 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) (Louisse et al., 2020; Song et al., 2016). There was no mention of changes in apoptosis accompanying increased cell proliferation in two of the studies of human hepatocytes (Louisse et al., 2020; Cui et al., 2015a), while Song et al. (2016) reported that genes related to "regulation of apoptosis" were significantly altered, although the direction of the change is not specified. Beggs et al. (2016) 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) 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 et al., 2012).

3.5.4.2.1.2 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 et al., 2018) (Table 3-25, Table 3-26). 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 (NTP, 2019; Rosen et al., 2017; Dong et al., 2016; Rosen et al., 2013; Elcombe et al., 2012b; Elcombe et al., 2012a; Rosen et al., 2010; Chang et al., 2009; Martin et al., 2007). Some studies, such as NTP (2019), report greater activation of CAR with PFOS treatment compared with 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 et al., 2014). However, as outlined above, several studies have reported increased cell proliferation or markers of cell proliferation due to PFOS treatment in human cell lines (Louisse et al., 2020; Song et al., 2016; Cui et al., 2015a). Further study is needed to understand the mechanistic underpinnings of PFOS-induced hepatic cell proliferation and whether it is related to CAR activation.

Table 3-25. Evidence of Key Events Associated With the CAR Mode of Action for Hepatic Tumors[a] in Male Sprague-Dawley Rats Exposed to PFOS

Canonical MOA	Key Event 1: CAR Activation	Key Event 2: Altered Gene Expression	Key Event 3: Increased Hepatic Cell Proliferation	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**CAR Activation**	**Altered Gene Expression**	**Hepatic Cell Proliferation**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
0.024	NR	NR	– (4, 14w)	NR	– (103w)
0.098	NR	NR	– (4, 14w)	NR	– (103w)
0.242	NR	NR	– (4, 14w)	NR	– (103w)
0.312	NR	**↑ (4 w)**	NR	NR	NR
0.625	NR	**↑ (4 w)**	NR	NR	NR
0.984	NR	NR	**↑ (4w) – (14, 53w)**	NR	**↑ (103w)**
1	NR	**↑ (F_1 PND 21)**	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; CAR = constitutive androstane receptor; NR = not reported; w = week(s); GD = gestational day; F_1 = first generation of offspring; PND = postnatal day.

Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from NTP (2019); Chang et al. (2009); and Butenhoff et al. (2012)/Thomford (2002b).

[a] Reviewed in Felter et al. (2018).

[b] Doses for 0.024, 0.098, 0.242, and 0.984 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012).

Table 3-26. Evidence of Key Events Associated With the CAR Mode of Action for Hepatic Tumors[a] in Female Sprague-Dawley Rats Exposed to PFOS

Canonical MOA	Key Event 1: CAR Activation	Key Event 2: Altered Gene Expression	Key Event 3: Increased Hepatic Cell Proliferation	Key Event 4: Preneoplastic Clonal Expansion	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**CAR Activation**	**Altered Gene Expression**	**Hepatic Cell Proliferation**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
0.029	NR	NR	– (4, 14w)	NR	– (103w)
0.120	NR	NR	– (4, 14w)	NR	– (103w)
0.299	NR	NR	– (4, 14w)	NR	– (103w)
0.312	NR	**↑ (4w)**	NR	NR	NR
0.625	NR	**↑ (4w)**	NR	NR	NR
1.251	NR	**↑ (P_0 GD 1–20)**	– (4, 14, 53w)	NR	**↑ (103w)**

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; CAR = constitutive androstane receptor; NR = not reported; w = week(s); P_0 = parental generation; GD = gestational day.

Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.

Data represented in table extracted from NTP (2019); Chang et al. (2009); and Butenhoff et al. (2012)/Thomford (2002b).

[a] Reviewed in Felter et al. (2018)

[b] Doses for 0.029, 0.120, 0.299, and 1.251 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012).

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 et al., 2016). 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 (Behr et al., 2020a; Beggs et al., 2016). Beggs et al. (2016) 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 noncancer hepatic effects including hepatomegaly, steatosis, altered lipid metabolism, and fatty liver disease. Beggs et al. (2016) exposed human primary hepatocytes to 0.01–10 μM PFOS and determined after 48 and 96 hours of 10 μM PFOS, HNF4α protein expression was significantly decreased. Beggs et al. (2016) also observed a decrease in HNF4α protein in the livers of 10-week-old CD-1 mice exposed to 10 mg/kg/day PFOS once daily by oral gavage for 7 days. 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 et al., 2020a).

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), as well as 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.

3.5.4.2.1.3 Cytotoxicity

There is suggestive evidence that PFOS may act through a cytotoxic MOA. Felter et al. (2018) 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 corresponding dose response for cytotoxicity and formation of tumors; and 6) reversibility upon cessation of exposure (Table 3-27,Table 3-28). As discussed above in the genotoxicity section (Section 3.5.4.2.1.4), there is no experimental support that PFOS can induce DNA damage and/or micronuclei formation in liver tissue, which supports the first key event in the cytotoxicity MOA. Quantitative liver histopathology is limited to three studies, however the one available chronic study (Butenhoff et al., 2012) 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) reported higher odds (not necessarily statistically significant) of non-alcoholic steatohepatitis ($p < 0.05$), ballooning, fibrosis, and portal inflammation.

Table 3-27. Evidence of Key Events Associated With the Cytotoxicity Mode of Action for Hepatic Tumors[a] in Male Sprague-Dawley Rats

Canonical MOA	Key Event 1: Cytotoxicity	Key Event 2: Increased Serum Enzymes	Key Event 3: Regenerative Proliferation	Key Event 4: Hyperplasia and/or Preneoplastic Lesions	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**Cytotoxicity**	**Serum Enzymes**	**Regenerative Proliferation**	**Hyperplasia and/or Preneoplastic Lesions**	**Hepatic Tumors**
0.024	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.098	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.242	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.312	– (4w)	– (4w)	NR	– (4w)	NR
0.625	– (4w)	**↑ (4w)**	NR	– (4w)	NR
0.984	**↑ (103w) – (4, 14, 53w)**	**↑ (4, 14, 53w) – (27w)**	**↑ (4w) – (14, 53w)**	**↑ (103w) – (14, 53w)**	**↑ (103w)**

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; w = week(s); NR = not reported.
Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.
Data represented in table extracted from: NTP (2019) and Butenhoff et al. (2012)/Thomford (2002b).
[a] Reviewed in Felter et al. (2018).
[b] Doses for 0.024, 0.098, 0.242, and 0.984 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012).

Table 3-28. Evidence of Key Events Associated With the Cytotoxicity Mode of Action for Hepatic Tumors[a] in Female Sprague-Dawley Rats

Canonical MOA	Key Event 1: Cytotoxicity	Key Event 2: Increased Serum Enzymes	Key Event 3: Regenerative Proliferation	Key Event 4: Hyperplasia and/or Preneoplastic Lesions	Outcome: Hepatic Tumors
Dose (mg/kg/day)[b]	**Cytotoxicity**	**Serum Enzymes**	**Regenerative Proliferation**	**Hyperplasia and/or Preneoplastic Lesions**	**Hepatic Tumors**
0.029	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.120	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.299	– (14, 103w)	– (4, 14, 27, 53w)	– (4, 14w)	– (14, 103w)	– (103w)
0.312	– (4w)	– (4w)	NR	– (4w)	NR
0.625	– (4w)	– (4w)	NR	– (4w)	NR
1.251	**↑ (103w) – (4, 14, 53w)**	– (4, 14, 27, 53w)	– (4, 14, 53w)	**↑ (103w) – (14, 53w)**	**↑ (103w)**

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; w = week(s); NR = not reported.
Cells in bolded text with blue shading indicate that the response direction is concordant with the key event in the published MOA. Cells with NR (not reported) indicate that no data were measured for that particular key event at that dose in the studies reviewed.
Data represented in table extracted from: NTP (2019) and Butenhoff et al. (2012)/Thomford (2002b).
[a] Reviewed in Felter et al. (2018).
[b] Doses for 0.029, 0.120, 0.299, and 1.251 mg/kg/day correspond to 0.5, 2, 5, and 20 ppm in feed, respectively, in Butenhoff et al. (2012).

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 (Jain, 2019; Nian et al., 2019; Salihovic et al., 2018; Gallo et al., 2012; Costa et al., 2009; Olsen et al., 2003). 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 et al., 2022). 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 twofold (100% change relative to control) compared with control (NTP, 2019; Han et al., 2018b; Xing et al., 2016; Yan et al., 2014; Butenhoff et al., 2012; Curran et al., 2008; Seacat et al., 2003). However, these changes in serum enzyme levels were accompanied by histopathological evidence of damage, as outlined above, and coherence is observed in humans.

As highlighted in the PPARα activation section, several studies have reported increased cell proliferation or markers of cell proliferation in human cell lines (Louisse et al., 2020; Song et al., 2016; Cui et al., 2015a), though there is limited quantitative histopathological data to determine the ability of PFOS to induce hepatic hyperplasia. Finally, the available data indicate a corresponding dose response for cytotoxicity and the formation of liver tumors as evidence in Table 3-29 and Table 3-30, 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 3-29. Incidences of Liver Tumor and Nonneoplastic Lesions in Male Sprague-Dawley Rats at 103 Weeks, as Reported by Thomford (2002b)

	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 with 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 3-30. Incidences of Liver Tumor and Nonneoplastic Lesions in Female Sprague-Dawley Rats at 103 Weeks, as Reported by Thomford (2002b)

	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 Adenomas & Carcinomas	0/28**	1/29	1/16	1/31	6/32*
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 with the vehicle control group. Statistical significance for the vehicle control indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; ** $p \leq 0.01$.

3.5.4.2.1.4 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, 2016b). 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 et al., 2017; Wang et al., 2015b). 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 versus 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 (NTP, 2019; Eke and Çelik, 2016; Çelik et al., 2013), though there are similar concerns about whether these responses are attributable to direct genotoxicity of PFOS. For example, NTP (2019) 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) 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) 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 et al., 2015b; Wielsøe et al., 2015; Lu et al., 2012). 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 at levels resulting in cytotoxicity (e.g., hepatotoxicity, bone marrow toxicity) can lead to secondary genotoxicity in target tissues. 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 or key event in PFOS tumor formation.

3.5.4.2.1.5 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 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. 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 (Lv et al., 2018; Wan et al., 2016). 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) (Han et al., 2018a; Lv et al., 2018; Wan et al., 2016; Xing et al., 2016; Liu et al., 2009). Several in vivo exposure studies also noted increases in hepatic ROS and markers of oxidative damage (e.g., MDA) (Han et al., 2018a; Lv et al., 2018; Wan et al., 2016; Xing et al., 2016; Liu et al., 2009). Notably, Han et al. (2018a) 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 et al., 2020). 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 (Zhang et al., 2023; Timmermann et al., 2020; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2017b; Grandjean et al., 2017a; Stein et al., 2016b; Mogensen et al., 2015a; Granum et al.,

2013; Grandjean et al., 2012). Additionally, one *medium* confidence study reported higher odds of total infectious diseases with increasing PFOS serum concentrations (Goudarzi et al., 2017), though it should be noted that studies reporting odds ratios for specific 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 (Zhong et al., 2016; Dong et al., 2009; Zheng et al., 2009; Keil et al., 2008; Peden-Adams et al., 2008). While one short-term study in male mice reported increases in splenic T-helper (CD3 + $CD4^+$) and T-cytotoxic (CD3 + $CD8^+$) lymphocytes (Lv et al., 2015), two gestational studies reported reductions in thymic $CD4^+$ cells in male offspring (Zhong et al., 2016; Keil et al., 2008). 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 (NTP, 2019; Qazi et al., 2009a). 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). 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.

3.5.4.2.2 Mode of Action for Pancreatic Tumors

Additional evidence of the carcinogenicity of PFOS comes from a *high* confidence chronic rodent study identifying pancreatic islet cell carcinomas in male rats (Thomford, 2002b). From a review of the literature, no established MOA was identified for pancreatic islet cell carcinogenicity in animals. Considerable uncertainty remains in the underlying mechanisms of PFOS-induced pancreatic islet tumors.

A recent review of the molecular mechanisms of pancreatic islet cell (i.e., neuroendocrine) tumors indicates pancreatic neuroendocrine tumors primarily originate from aberrant cell proliferation in the endocrine pancreas (Maharjan et al., 2021). However, these tumors can also develop from pluripotent cells of the exocrine pancreas (Maharjan et al., 2021). The human islet is similar to the rodent islet, with similarities in β-cell numbers, islet cell patterns, and blood vessel-islet structure and interactions (Bonner-Weir et al., 2015). Some evidence suggests a role for PPARα and PPARγ in rat and human pancreatic islet cell function (Eibl et al., 2001; Sugden et al., 2001; Dubois et al., 2000; Roduit et al., 2000), though PPARα activation has been argued to be related to pancreatic acinar cell tumors rather than to islet cell tumors (Klaunig et al., 2003). Other studies have shown that PFOS exposure can reduce pancreatic islet cell size and viability and can induce ROS (Qin et al., 2022).

Although an established MOA is currently unknown for this tumor type, the observation of pancreatic islet cell tumors in rodents provides additional evidence for the carcinogenic potential of PFOS.

3.5.4.2.3 Conclusions

Based on the weight of evidence evaluation of the available literature, PFOS has the potential to induce hepatic tumors in humans and rodents via multiple MOAs, most notably via the modulation of nuclear receptors (i.e., PPARα and CAR) and cytotoxicity. There is also limited evidence supporting additional 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; NJDWQI, 2018). No established MOA was identified for pancreatic islet cell carcinogenicity in rats.

As described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), "[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 and pancreatic 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 and pancreatic 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, particularly findings of increased risk of hepatocellular carcinoma, from epidemiological studies.

Several health agencies have reviewed the available mechanistic literature and have come to similar conclusions regarding the multiple potential MOAs for PFOS-induced tumorigenesis. For example, CalEPA's Office of Environmental Health Hazard Assessment recently concluded that PFOS "possess[es] several of the key characteristics of carcinogens, including the ability to induce oxidative stress, inflammation, and modulate receptor-mediated effects. Additionally, there is suggestive evidence that… PFOS [is] genotoxic, thus a genotoxic MOA for cancer remains plausible" (CalEPA, 2021). Zahm et al. (2023, 3982387) also concluded that there is moderate evidence for many potential mechanisms for PFOS-induced toxicity and specifically noted that PFOS can induce epigenetic alterations, immunosuppression, and oxidative stress and cause endocrine- and receptor-mediated effects.

3.5.5 Cancer Classification

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 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, 2005a).

The available evidence indicates that PFOS has carcinogenic potential in one animal model for multiple sites and both sexes, as well as supporting evidence from human studies, consistent with the examples described in the *Guidelines for Carcinogen Risk Assessment* for the *Likely* descriptor. 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 (Butenhoff et al., 2012; Thomford, 2002b). In this study, statistically significant increases in the incidences of hepatocellular adenomas or combined adenomas and carcinomas were observed in male and female rats, respectively. 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 (2002b)/Butenhoff et al. (2012) may be relevant to humans. These findings in rats and their potential human relevance are supported by recent epidemiological studies that have reported associations between PFOS and hepatocellular carcinoma in humans (Cao et al., 2022; Goodrich et al., 2022).

In addition to hepatocellular tumors, Thomford (2002b) reported increased incidences of pancreatic islet cell carcinomas 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. Importantly, site concordance is not always assumed between humans and animal models; agents observed to produce tumors may do so at the same or different sites in humans and animals (U.S. EPA, 2005a). While site concordance was present between human studies of liver cancer and animal studies reporting increased incidence of hepatocellular tumors, evidence of carcinogenicity of PFOS from other cancer sites where concordance between humans and animals is not present is still relevant to the carcinogenicity determination for PFOS. See Table 3-31 below for specific details on how PFOS aligns with the examples supporting the *Likely to Be Carcinogenic to Humans* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Table 3-31. Comparison of the PFOS Carcinogenicity Database With the *Likely* Cancer Descriptor as Outlined in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)

***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." (U.S. EPA, 2005a)	**PFOS data are consistent with this description**. Epidemiological evidence supports a plausible association between PFOS exposure and liver cancer which is consistent with evidence of liver cancer in animals. Epidemiological studies evaluating the association between human exposure to PFOS and other cancers 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	**PFOS data are consistent with this description**. PFOS has tested positive in animal experiments in more than

Likely to Be Carcinogenic to Humans	
route, with or without evidence of carcinogenicity in humans." (U.S. EPA, 2005a)	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." (U.S. EPA, 2005a)	**This description is not applicable to PFOS.**
"A rare animal tumor response in a single experiment that is assumed to be relevant to humans." (U.S. EPA, 2005a)	**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, 2020b).
"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, 2005a)	**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.

Notes: MOA = mode of action.

EPA recognizes that other state and international health agencies have recently classified PFOS as either "possibly carcinogenic to humans" (IARC as reported in Zahm et al. (2023)) or carcinogenic to humans (CalEPA, 2021). As the SAB PFAS Review Panel (U.S. EPA, 2022e) noted, "the criteria used by California EPA, for determination that a chemical is a carcinogen, are not identical to the criteria in the U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*" and, similarly, IARC's classification criteria are not identical to EPA's guidelines (IARC, 2019). Rationale for why PFOS exceeds the *Suggestive Evidence of Carcinogenic Potential* descriptor and does not meet the *Carcinogenic to Humans* descriptor according to EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) is detailed in Section 5.4.

4 Dose-Response Assessment

Considerations in Selecting Studies and Endpoints for Dose-Response Analysis

There is evidence from both human epidemiological and animal toxicological studies that oral perfluorooctane sulfonic acid (PFOS) exposure can result in adverse health effects across a range of health outcomes. In response to recommendations made by the EPA's Science Advisory Board (SAB) and the conclusions presented in the U.S. Environmental Protection Agency's (EPA's) preliminary analysis, the 2021 SAB review draft *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctane Sulfonic Acid (PFOS) (CASRN 1763-23-1) in Drinking Water* (U.S. EPA, 2021b), EPA focused its final toxicity value derivation efforts herein "on those health outcomes that have been concluded to have the strongest evidence" (U.S. EPA, 2022e). Therefore, EPA prioritized health outcomes and endpoints with the strongest overall weight of evidence which were the health outcomes with evidence ***demonstrates*** or evidence ***indicates*** integration judgments based on human, animal, and mechanistic evidence (Sections 3.4 and 3.5) for points of departure (POD) derivation using the systematic review methods described in Section 2 and Appendix A (U.S. EPA, 2024a). For PFOS, the health outcomes with the strongest weight of evidence are cancer (described in Section 4.2) and the noncancer health outcomes of immunological, developmental, cardiovascular (serum lipids), and hepatic effects (described in Section 4.1). For all other health outcomes (e.g., reproductive, endocrine, nervous, hematological, musculoskeletal), the evidence integration summary judgment for the human epidemiological and animal toxicological evidence was ***suggestive*** or ***inadequate*** and these outcomes were not assessed quantitatively. For transparency, health outcomes for which the results were *suggestive* are discussed in the evidence profile tables provided in Appendix C (U.S. EPA, 2024a).

In the previous sections describing the hazard judgment decisions (Sections 3.4 and 3.5), EPA qualitatively considered high, medium, and sometimes *low* confidence studies of PFOS exposure to characterize the weight of evidence for each health outcome. For the quantitative analyses described in the following subsections, EPA focused exclusively on *high* or *medium* confidence human epidemiological and animal toxicological studies for POD derivation, as recommended in Chapter 7.2 of the IRIS Handbook (U.S. EPA, 2022d). While the IRIS Handbook also includes consideration of *low* confidence studies for dose-response analysis under certain circumstances, this EPA assessment did not consider *low* confidence studies because of the relatively large and robust database for PFOS. At this stage, EPA considered additional study attributes to enable extrapolation to relevant exposure levels in humans. These attributes are described in Table 7-2 of the IRIS Handbook and include relevance of the test species, relevance of the studied exposure to human environmental exposures, quality of measurements of exposure and outcomes, and other aspects of study design including specific reconsideration of the potential for bias in the reported association between exposure and outcomes (U.S. EPA, 2022d).

Consideration of these attributes facilitates the transparent selection of studies and data for dose-response modeling and potential RfD or CSF derivation. Studies exhibiting these attributes are expected to provide more accurate human equivalent toxicity values and are therefore preferred in the selection process. Consideration of these attributes in the study selection process are described below for noncancer and cancer endpoints.

4.1 Noncancer

4.1.1 Study and Endpoint Selection

For study and endpoint selection for noncancer health outcomes, the human studies that provided all necessary analytical information (e.g., exposure distribution or variance, dose-response data) for POD derivation, analyzed the outcome of interest in the general population or susceptible population, and demonstrated the dose-response attributes outlined above were preferred. If available, *high* and *medium* confidence studies with exposures levels near the range of typical environmental human exposures, especially exposure levels comparable to human exposure in the United States, were preferred over studies reporting considerably higher exposure levels (e.g., occupational exposure levels). Exposure levels near the typical range of environmental human exposure can facilitate extrapolation to the lower dose range of exposure levels that are relevant to the overall population. When available for a given health outcome, studies with analyses that addressed potential confounding factors affecting exposure concentrations (e.g., addressing temporal variations of PFOS concentrations during pregnancy due to hemodynamics) were also preferred. Additionally, when studies presented overlapping data on the same cohort or study population, various factors were considered to facilitate selection of one study for POD derivation. These factors included the duration of exposure, the length of observation of the study cohort, and the comprehensiveness of the analysis of the cohort in order to capture the most relevant results for dose-response analysis.

The preferred animal toxicological studies consisted of *medium* and *high* confidence studies with exposure durations appropriate for the endpoint of interest (e.g., chronic or subchronic studies vs. short-term studies for chronic effects) or with exposure during sensitive windows of development and with exposure levels near the lower dose range of doses tested across the evidence base. 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 5.3 for a discussion of animal toxicological studies and endpoints selected for POD derivation for this updated assessment compared with those selected for the 2016 PFOS HESD (U.S. EPA, 2016b).

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 3-6, the majority of epidemiological studies assessed endpoints related to serum biomarkers of hepatic injury (12 *medium* confidence studies), while fewer studies reported on liver disease or injury (3 *medium* confidence studies) and other serum markers of liver function (2 *medium* confidence studies). EPA prioritized studies that evaluated endpoints related to serum biomarkers of injury for quantitative analyses because the reported effects on these endpoints were well-represented within the database and were generally consistent across the available *medium* confidence studies. Additionally, 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 et al., 2005). Specifically, all five *medium* confidence studies in general population adults from the updated literature searches reported positive associations between PFOS serum concentrations and ALT, three of which reported

statistically significant responses (Jain, 2019; Jain and Ducatman, 2019c; Nian et al., 2019; Salihovic et al., 2018; Gleason et al., 2015). These more recently published studies provided additional evidence for increased ALT in adults beyond the three *medium* confidence reporting positive associations for ALT from the 2016 PFOS HESD (Yamaguchi et al., 2013; Gallo et al., 2012; Lin et al., 2010). Findings from studies of other liver enzymes, AST and GGT, in adults generally reported a positive association, though less consistently than studies of ALT; therefore, studies of AST and GGT are supportive of the selection of ALT as an endpoint for POD derivation because these results demonstrate coherence across the different liver serum enzyme endpoints.

As mentioned above, 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 (Liu et al., 2014; Boone et al., 2005; U.S. EPA, 2002a). Additionally, evidence from both human epidemiological and animal toxicological studies indicates that increased serum ALT is associated with liver disease (Roth et al., 2021; Kwo et al., 2017; Ioannou et al., 2006b; Ioannou et al., 2006a). Human epidemiological studies have demonstrated that even low magnitude increases in serum ALT can be clinically significant when extrapolated to the overall population (Gilbert and Weiss, 2006). For example, a Scandinavian study in people without any symptoms of liver disease but with relatively small increased serum ALT levels were later diagnosed with liver diseases such as steatosis and chronic hepatitis C (Mathiesen et al., 1999). 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 et al., 2019a).

Numerous studies have also demonstrated an association between elevated ALT and liver-related mortality (reviewed by Kwo et al. (2017)). Furthermore, the American Association for the Study of Liver Diseases (AASLD) recognizes serum ALT as an indicator of overall human health and mortality (Kim et al., 2008). For example, as reported by Kwo et al. (2017), Kim et al. (2004) observed that higher serum ALT concentrations corresponded to an increased risk of liver-related death in Korean men and women; similarly, Ruhl and Everhart (2013, 2009) 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) 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 et al., 2017). Taken together, results of human epidemiological 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 in humans is typically assessed using serum biomarkers of hepatotoxicity (Costello et al., 2022).

Among the available *medium* confidence epidemiological studies reporting alterations in serum ALT in humans, studies of adults in the general population were prioritized over studies in other populations (e.g., occupational) or life stages (e.g. children), as the adult studies provided the most consistent evidence of increases in ALT (see Section 3.4.1.1). Several of these *medium*

confidence studies reporting increases in ALT in adults were excluded from POD derivation for reasons such as combined adolescent and adult populations (Gleason et al., 2015), populations consisting of only elderly adults (Salihovic et al., 2018), use of correlation analyses only (Yamaguchi et al., 2013), and reporting analyses stratified by glomerular filtration without stratifying by exposure level, which were not amenable to modeling (Jain, 2019).

Exclusions of these studies resulted in the consideration of three *medium* confidence studies for POD derivation (Nian et al., 2019; Gallo et al., 2012; Lin et al., 2010) (Table 4-1). These studies exhibited many of the study attributes outlined in Section 4 above and in Appendix A (U.S. EPA, 2024a). For example, Gallo et al. (2012), is the largest study assessing PFOS and ALT in adults which was conducted in over 30,000 individuals from the general population, aged 18-years and older, as part of the C8 Health Project in the United States. Further, Gallo et al. (2012) demonstrated a statistically significant trend in increased ALT across deciles. Two additional studies (Nian et al., 2019; Lin et al., 2010) were considered for POD derivation because they reported associations in general populations in the United States and a Chinese population located near a PFAS manufacturing facility, respectively. Nian et al. (2019) 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 reported significantly increased level of ALT associated with PFOS. Lin et al. (2010) was also considered for POD derivation since it is a large (2,216 men and 1,063 women) nationally representative study in an NHANES adult population and observed increased ALT levels per log-unit increase in PFOS in the models adjusted for age, gender, and race/ethnicity. However, the association no longer remained in the fully adjusted models, or in the models additionally adjusted for PFOA, PFHxS, and PFNA. Additionally, several methodological limitations precluded its use for POD derivation. Limitations include lack of clarity about the base of logarithmic transformation applied to PFOS concentrations in regression models, and the choice to model ALT as an untransformed variable, which is a departure from the lognormality assumed in most of the ALT literature. Therefore, two *medium* confidence epidemiological studies were prioritized for POD derivation (Nian et al., 2019; Gallo et al., 2012) (Table 4-1).

Liver toxicity results reported in animal toxicological studies after PFOS exposure are concordant with the observed increased ALT indicative of hepatic damage observed in epidemiological studies. Specifically, studies in rodents found that oral PFOS treatment resulted in increased liver weight (11/14 *high* and *medium* confidence studies), increased 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 increased ALT observed in human populations. Mechanistic studies in mammals and evidence from in vitro studies and nonmammalian animal models provide additional support for the biological plausibility and human relevance of the PFOA-induced hepatic effects observed in animals. These studies suggest multiple potential MOAs for the observed liver toxicity, including PPARα-dependent and -independent mechanisms of action (MOAs). The observed increases in liver enzymes (e.g., ALT) in rodents are supportive of the hepatic damage confirmed during histopathological examinations in several studies. Taken together, the study results suggest that at least some mechanisms for PFOS-induced hepatic effects are relevant to humans.

For animal toxicological hepatic endpoints, EPA preferred studies reporting quantitative biologically or statistically significant specific measures of severe toxicity (i.e., histopathological lesions related to cell or tissue death or necrosis) with study designs best suited for quantitative analysis (e.g., large sample size, reported effects in the lower dose range). Of the three studies that quantitatively reported incidences of hepatic histopathological alterations, two were excluded because they had relatively small sample sizes (i.e., $n \leq 10$) and used short-term exposure durations (i.e., 28 days) (NTP, 2019; Curran et al., 2008) as compared to Butenhoff et al., (2012). Butenhoff et al. (2012) was a chronic dietary study which 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 study with a large sample size (i.e., $n = 50$) , numerous and relatively low-dose levels, and data examining a suite of endpoints, individual cell necrosis in the liver in females was considered for derivation of PODs (Table 4-1). This endpoint was supported by the observation of non-monotonic increases in single cell necrosis in males from the same study.

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 3-12, the majority of epidemiological studies assessed endpoints related to immunosuppression (1 *high* and 21 *medium* confidence studies) and immune hypersensitivity (1 *high* and 20 *medium* confidence studies), while one study (*medium* confidence) also reported on endpoints related to autoimmune disease. Studies that reported on specific autoimmune diseases were excluded from POD derivation because there were a limited number of studies that assessed the same diseases (e.g., rheumatoid arthritis, celiac disease). Studies that evaluated endpoints related to immune hypersensitivity (e.g., asthma) were also not considered for POD derivation because there were inconsistencies in the direction and precision of effects across gender or age subgroups in the available 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, reported differing results across *medium* and *high* confidence studies and were therefore excluded from further consideration, though they provide qualitative support of an association between PFOS exposure and altered immune function.

Evidence of immunosuppression in children associated with exposure to PFOS reported by epidemiological studies were consistent across studies and endpoints. Specifically, epidemiological studies reported associations between PFOS exposure and reduced humoral immune response to routine childhood immunizations, including lower levels of tetanus and diphtheria (Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012) and rubella (Zhang et al., 2023; Stein et al., 2016b; Granum et al., 2013) antibody titers. Reductions in antibody response were observed at multiple timepoints during childhood (specifically ages between 3-19 years in these studies), for either prenatal or postnatal childhood PFOS exposure levels, and were consistent across studies in children populations from *medium* confidence studies. Therefore, reduced antibody response in children was selected as an endpoint for POD derivation.

Measurement of antigen-specific antibodies following vaccination(s) is a measure of the overall 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, which is a type of immunity that develops when a person's immune system responds to a foreign substance or microorganism, and it has been used as a comprehensive approach to detect immunosuppression across a range of cells and signals (Myers, 2018). 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 specific challenge and is considered an adverse immunological health outcome" (U.S. EPA, 2022e). This is consistent with a review article by Selgrade (2007) 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. (2019; 2017; 2016) and in comments from other subject matter experts on the SAB's PFAS review panel (U.S. EPA, 2022e), the clinical manifestation of a disease after chemical exposure is not required 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 in response to chemical exposure 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, 2022e). 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). Decreases in antibody response, even at smaller magnitudes in individuals, are clinically relevant when extrapolated to the overall population (Gilbert and Weiss, 2006). This response also translates across multiple species, including rodents, and extensive historical data indicate that suppression of antigen-specific antibody responses by exogenous agents is predictive of immunotoxicity.

Studies of developmental exposure to environmental toxicants demonstrate an association with immune suppression (Selgrade, 2007). When immunosuppression occurs during immune system development, the risks of developing infectious diseases and other immunosuppression-linked diseases may increase (Dietert et al., 2010). The immune system continues developing postnatally; because of this, exposures to PFAS and other immunotoxic agents during development may have serious, long-lasting, and irreversible health consequences (Dewitt et al., 2019; Macgillivray and Kollmann, 2014; Selgrade, 2007). Indeed, Hessel et al. (2015) 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 that were assessed. Developmental immunotoxicity as a result of chemical exposure is generally observed at doses lower than required to elicit immunotoxicity in adults (vonderEmbse and DeWitt, 2018). Therefore, developmental immunotoxicity is generally a highly sensitive health outcome, both when considering other types of developmental toxicity and when comparing it to immunotoxicity observed in exposed adults. Luster et al. (2005) similarly noted that the specific immunotoxic endpoint of 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.

One *high* and 10 *medium* confidence studies (Zhang et al., 2023; Shih et al., 2021; Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018; Pilkerton et al., 2018; Grandjean et al., 2017b; Grandjean et al., 2017a; Stein et al., 2016b; Mogensen et al., 2015a; Granum et al., 2013; Grandjean et al., 2012) reported findings on antibody response to tetanus, diphtheria, or rubella in children or adolescents. At least two *medium* confidence studies representing two different populations of children or adolescents reported inverse associations or increased risks of falling below seroprotective levels between each vaccine type and PFOS concentrations. For diphtheria and tetanus, this included five *medium* and one *high* confidence studies on the Faroe Island cohort (Shih et al., 2021; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a; Grandjean et al., 2012) and one *medium* confidence study in Greenlandic children (Timmermann et al., 2021). For rubella, this included one *medium* confidence study in Norwegian children (Granum et al., 2013) and two *medium* confidence studies on partially overlapping sets of children from the United States (Zhang et al., 2023; Stein et al., 2016b). Given the consistency of this response across multiple vaccine types and populations, including children from the United States, EPA considered studies reporting on all three vaccines for POD derivation. Specifically, EPA selected one *medium* confidence study representing each population (i.e., children or adolescents from the United States, Faroe Islands, Norway, and Greenland) for POD derivation.

Five separate studies (Shih et al., 2021; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a; Grandjean et al., 2012) reported on diphtheria and tetanus antibody responses in the same population (i.e., the same individuals) of Faroese children. One study reported on the same Faroese children cohort in a more recent *medium* confidence publication (Budtz-Jørgensen and Grandjean, 2018). Because this most recent *medium* confidence study is the only one of the five studies that provided dose-response data with untransformed PFOA concentrations which are more amenable to BMD modeling, only results from Budtz-Jørgensen and Grandjean (2018) were prioritized for POD derivation and the four other studies conducted in the Faroe Island population were excluded. For rubella, the NHANES populations examined in Zhang et al. (2023), Stein et al. (2016b), and Pilkerton et al. (2018) partially overlapped, and Zhang et al. (2023) was selected for POD derivation as it reported more recent data and a significant inverse response.

In total, four *medium* confidence epidemiologic studies (Zhang et al., 2023; Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018) exhibited many of the study attributes outlined in Section 4 above and in Appendix A (U.S. EPA, 2024a) and were considered for POD derivation (Table 4-1). Budtz-Jørgensen and Grandjean (2018) investigated anti-tetanus and anti-diphtheria responses in Faroese children aged 5–7 and Timmerman et al. (2021) investigated anti-tetanus and anti-diphtheria responses in Greenlandic children aged 7–12. Granum et al. (2013) investigated rubella responses in Norwegian children aged 3 and Zhang et al. (2023) investigated rubella responses in U.S. adolescents.

Immunotoxicity results reported in animal toxicological studies are concordant 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) (two *high* and three *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 concordant with decreased antibody responses seen in human populations. EPA prioritized PFC responses over NK cell activity for POD derivation because several studies (Zhong et al., 2016; Dong et al., 2009; Peden-Adams et al., 2008) reported non-monotonic dose-response curves for NK cell activity, increasing the uncertainty in the dose-response relationship for that endpoint. Of the six studies reporting reductions in PFC response in rodents (Zhong et al., 2016; Dong et al., 2011; Dong et al., 2009; Zheng et al., 2009; Keil et al., 2008; Peden-Adams et al., 2008), one *medium* confidence study (Zhong et al., 2016)was selected for POD derivation because the study tested a relatively low-dose range compared with the other five studies, the response was observed in both males and females, and the effect was measured in pups treated with PFOS in utero, consistent with the sensitive lifestage (i.e., children) identified from human studies (Table 4-1). 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 immunological endpoints. EPA prioritized results from NTP (2019) for POD derivation over the other *high* confidence study (Lv et al., 2015) because it reported consistent effects of PFOS treatment on a suite of endpoints related to immune cellularity which were confirmed by histopathological evidence (if applicable), including increased bone marrow hypocellularity, increased splenic extramedullary hematopoiesis, and reduced leukocytes, neutrophils, and white blood cell counts in male and female rats. The endpoint of splenic extramedullary hematopoiesis was observed in both sexes and was consistent with other *high* and *medium* confidence studies that reported alterations in circulating immune cells, splenic cellularity, and thymic cellularity, both of which increase the confidence in this endpoint (Table 4-1).

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 3-15, the majority of epidemiological studies assessed endpoints related to serum lipids (2 *high*, 28 *medium*, and 12 *mixed*[16] confidence studies) and blood pressure and hypertension (2 *high* and 17 *medium* confidence studies), while some 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 excluded from consideration for POD derivation because there were limited high and medium confidence studies and they reported mixed (i.e., positive and inverse associations) or mostly null results. Endpoints related to blood pressure and hypertension were also excluded from quantitative analyses because there was higher confidence in analytically determined serum lipid levels compared with blood pressure measurements and there was a larger evidence base for serum lipids as compared to blood pressure. However, there was evidence of associations between PFOS exposure and at least one measure of continuous blood pressure in adults and increased risk of hypertension. These results are qualitatively supportive of an association between PFOS and cardiovascular effects in humans.

[16] *Mixed* confidence studies on serum lipids were primarily of medium confidence for total cholesterol and HDL cholesterol, and *low* confidence for LDL cholesterol and triglycerides.

The majority of studies in adults from the general population, including high-exposure communities, reported positive associations between PFOS serum concentrations and serum lipids. Studies in adults were prioritized due to the current understanding that serum lipid changes in children are age-dependent and fluctuate during puberty (Daniels et al., 2008), which may impact the consistency of results from studies in children. Specifically, *medium* confidence epidemiological studies in the general population reported positive associations between PFOS exposure and total cholesterol (TC) (18/23 studies) and low-density lipoprotein (LDL) (13/18 studies). Associations between PFOS and high-density lipoprotein (HDL) or triglycerides in the general population were inconsistent and were therefore excluded from POD derivation. EPA selected TC for quantitative assessments because the association was the most consistently observed in adults, and studies for TC were of higher confidence for outcome measurements compared with LDL. Additionally, the positive associations with TC in these studies were further supported by a recent meta-analysis that included 14 general population studies in adults (U.S. EPA, 2024b) and reported an association between increased cholesterol and increased PFOS exposure.

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 (Lloyd-Jones et al., 2017; Goff et al., 2014; D'Agostino et al., 2008). Additionally, disturbances in cholesterol homeostasis contribute to the pathology of nonalcoholic fatty liver disease (NAFLD) and to accumulation of lipids in hepatocytes (Malhotra et al., 2020). 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. Increases in serum cholesterol, even at smaller magnitudes at the individual level, are clinically relevant when extrapolated to the overall population (Gilbert and Weiss, 2006). This is because, at the population level, even small magnitude increases in serum cholesterol could shift the distribution of serum cholesterol in the overall population relative to the clinical cut-off, leading to an increased number of individuals at risk for cardiovascular disease. The SAB PFAS Panel agreed with this interpretation, stating that "an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to…PFAS cannot be predicted on an individual basis, the increased number of individuals within a population with clinically defined abnormal values is of public health concern" (U.S. EPA, 2022e).

A total of 13 *medium* confidence studies (Canova et al., 2020; Fan et al., 2020; Lin et al., 2020d; Dong et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Liu et al., 2018d; Eriksen et al., 2013; Fitz-Simon et al., 2013; Château-Degat et al., 2010; Nelson et al., 2010; Steenland et al., 2009; Olsen et al., 2003) reported on positive associations between exposure to PFOS and total cholesterol in adults from the general population. One study evaluated occupational adult populations only (Olsen et al., 2003) was not considered as exposure pathways and concentrations in this population did not represent typical exposure scenarios for human environmental exposure. Three studies (Canova et al., 2020; Lin et al., 2020d; Eriksen et al., 2013) were excluded from POD derivation due to narrow age ranges (i.e., 50–65 years of age,

55–75 years of age, and 20–39 years of age, respectively) of the study populations that were less comprehensive than the age groups included by other studies and therefore, may not apply across the general adult population. One study (Jain and Ducatman, 2019b) was excluded form POD derivation because the study reported findings stratified by BMI status but was not stratified by exposure.

Although the positive associations between PFOS and TC were supported by a recent meta-analysis that included 14 general population studies of adults (U.S. EPA, 2024b), EPA did not use the pooled effect from this meta-analysis for POD derivation. This meta-analysis was not comprehensive of the entire database of studies on PFOS and TC because it was performed specifically with the purpose of informing aspects of the Pooled Cohort Atherosclerotic Cardiovascular Disease (ASCVD) model which relies on CVD risk reduction analysis for those ages 40–89 (U.S. EPA, 2024b). The results of another recent meta-analysis on PFOS and serum lipids (Abdullah Soheimi et al., 2021) was excluded from POD derivation because the pooled effects reported combined 11 studies with TC, triglycerides and LDL in multiple populations (i.e., children, adolescents, pregnant women, and adults). As previously noted, serum lipids rise in early childhood and fluctuate in puberty (Daniels et al., 2008), and combining study populations at different lifestages would likely result in unaddressed confounding by age.

Four studies presented overlapping data from NHANES (Fan et al., 2020; Dong et al., 2019; Liu et al., 2018d; Nelson et al., 2010). Of these four, Dong et al. (2019) was selected for POD derivation because this larger study included data from all NHANES cycles between 2003 and 2014, while the other three studies reported results for only one or two cycles within the 2003-2014 range and were therefore not further considered. Similarly, two studies (Fitz-Simon et al., 2013; Steenland et al., 2009) presented data on the C8 Health Project population. Fitz-Simon et al. (2013) was not selected for POD derivation because it was a part of a short-term follow-up and was not as comprehensive as the population examined by Steenland et al. (2009). Likewise, another higher exposure community study (Château-Degat et al., 2010) reported TC changes in approximately 700 Nunavik Inuit adults which was not as comprehensive as Steenland et al. (2009) which investigated over 46,000 adults. Therefore, Steenland et al. (2009) was also selected for POD derivation. Finally, Lin et al. (2019) was also selected for POD derivation because it provided data for a large number of adults (n = 940) in the general U.S. population from the Diabetes Prevention Program (DPP) population, with PFOS levels at baseline comparable to those from NHANES 1999–2000.

In summary, three *medium* confidence epidemiologic studies were considered for POD derivation (Table 4-1) (Dong et al., 2019; Lin et al., 2019; Steenland et al., 2009). These candidate studies offer a variety of PFOS exposure measures across various populations and exhibited many of the study attributes outlined in Section 4 above and in Appendix A (U.S. EPA, 2024a). Dong et al. (2019) investigated the NHANES population (2003–2014), while Steenland et al. (2009) investigated effects in a high-exposure community (the C8 Health Project study population). Lin et al. (2019) collected data from prediabetic adults from the DPP and DPPOS at baseline (1996–1999).

Though results reported in animal toxicological studies support the alterations in lipid metabolism observed in epidemiological studies, there are species differences direction of effect with dose. As a result of these differences, there is some uncertainty about the human relevance of these observed responses in rodents. Additionally, the available mechanistic data do not

increase the understanding about the non-monotonicity of serum lipid levels and decreased serum lipid levels at higher dose levels in rodents (Section 3.4.3.3). Due to the uncertainties regarding human relevance of the animal toxicology studies, EPA did not derive PODs for animal toxicological studies reporting cardiovascular effects, such as altered serum lipid levels.

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 3-17, the majority of epidemiological studies assessed endpoints related to fetal growth restriction (21 *high* and 26 *medium* confidence studies) and gestational duration (10 *high* and 11 *medium* confidence studies), while fewer studies reported on endpoints related to fetal loss (3 *high* and 3 *medium* confidence studies) and birth defects (4 *medium* confidence studies). Evidence for birth defects was limited in that there are only 4 *medium* confidence studies and those studies provided mixed findings. Therefore, birth defects not prioritized for POD derivation. Although half of the available *high* and *medium* confidence studies reported increased incidence of fetal loss (3/6), EPA did not prioritize this endpoint for dose-response analyses as there were a relatively limited number of studies compared with 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.

The majority of the available studies reporting metrics of gestational duration observed increased risk associated with PFOS exposure, including among *high* confidence studies. Seven of the 13 *medium* or *high* confidence studies reported inverse associations for gestational age at birth and 7 of the 11 *medium* or *high* confidence studies reported an association with preterm birth. These findings are supportive of an association between PFOS exposure and adverse developmental effects. There were generally consistent associations with increased risk of preterm birth, particularly from the *high* confidence studies, with several studies reporting statistically significant results. While overall there appears to be consistent associations between PFOS exposure and gestational duration, the database for fetal growth restriction demonstrated consistent associations between PFOS and fetal growth restriction and was also both larger and consisted of more *medium* and *high* confidence studies than gestational duration. Therefore, studies demonstrating fetal growth restriction were prioritized for POD derivation.

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 reported inverse associations (8/12 studies; 6 *high* and 2 *medium* confidence). Endpoints characterizing fetal growth restriction were included for POD derivation multiple studies reported effects on these endpoints, particularly decreased birth weight, and reported generally consistent findings across *high* and *medium* confidence studies. As noted in the Developmental Human Evidence Study Evaluation Considerations (Section 3.4.4.1.2), measures of birth weight were considered higher confidence outcomes compared with other measures of fetal growth restriction such as birth length, head circumference, or ponderal index because birth weight measures are less prone to measurement error (Shinwell and Shlomo, 2003). Studies reporting changes in mean birth weight were more amenable to modeling compared with those reporting changes in standardized (e.g., z-score) birth weight measurements. Standardized measurements depend on sources of standardization

and are harder to interpret and compare across studies. As a result, measures of mean changes in birth weight were considered for quantitative analysis.

Low birth weight (LBW) 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) (U.S. EPA, 2013; JAMA, 2002; McIntire et al., 1999). LBW is widely considered a useful population level public health measure (Vilanova et al., 2019; Cutland et al., 2017; WHO and UNICEF, 2004; Lira et al., 1996) and is on the World Health Organization's (WHO's) global reference list of core health indicators (WHO, 2018a, 2014). Decreases in birthweight, even at smaller magnitudes at the individual level, are clinically relevant when extrapolated to the overall population (Gilbert and Weiss, 2006). This is because, at the population level, even small magnitude decreases in birthweight could shift the distribution of birthweight in the overall population relative to the clinical cut-off, leading to an increased number of individuals at risk for decreased birthweight and subsequent effects related to decreased birthweight. The SAB PFAS Panel agreed with this interpretation, stating that "an increase in the number of subjects with a clinically abnormal value is also expected from the overall change (shift in the distribution curve) in the abnormal direction. While the clinical relevance of exposure to PFOA…cannot be predicted on an individual basis, the increased number of individuals within a population with clinically defined abnormal values is of public health concern" (U.S. EPA, 2022e).

Substantial evidence links LBW to a variety of adverse health outcomes at various stages of life. It has been shown to predict prenatal mortality and morbidity (Cutland et al., 2017; WHO, 2014; U.S. EPA, 2013) and is a leading cause of infant mortality in the United States (CDC, 2021). 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 (U.S. EPA, 2013; Zeleke et al., 2012; Guyatt and Snow, 2004; WHO and UNICEF, 2004; JAMA, 2002). Additionally, low-birth-weight infants evaluated at 18 to 22 months of age demonstrated impaired mental development (Laptook et al., 2005).

LBW is also associated with increased risk for diseases in adulthood, including obesity, diabetes, and cardiovascular disease ((Smith et al., 2016a; Risnes et al., 2011; Gluckman et al., 2008; Ong and Dunger, 2002; Osmond and Barker, 2000), as reported in Yang et al. (2022)). Poor academic performance, cognitive difficulties (Hack et al., 2002; Larroque et al., 2001), and depression (Loret de Mola et al., 2014) in adulthood have also been linked to LBW. These associations between LBW and infant mortality, childhood disease, and adult disease establish LBW as an adverse effect. Considering the established consequences of LBW, as well as the consistency of the database and large number of *medium* and *high* confidence studies reporting mean birth weight and other binary birth weight-related measures, the endpoint of decreased birth weight in infants was selected for POD derivation.

Given the abundance of *high* confidence epidemiological studies evaluating decreases in birth weight, *low* and *medium* confidence studies were excluded from POD derivation. Thus, 15 *high* confidence studies reporting inverse associations between exposure to PFOS and mean birth weight (Gardener et al., 2021; Luo et al., 2021; Yao et al., 2021; Chu et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Bell et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lauritzen et al., 2017; Lind et al., 2017a; Starling et al., 2017; Valvi et al., 2017; Darrow et al.,

2013; Whitworth et al., 2012) were considered for POD derivation. Four studies (Gardener et al., 2021; Xiao et al., 2019; Ashley-Martin et al., 2017; Whitworth et al., 2012) were excluded because they reported sex-stratified results rather than results in both sexes or results for the overall population in terms of standardized measurements (e.g., z-score) only. Analyses utilizing standardized measurements as the dependent variable are internally valid, but this type of analysis estimates a change in birthweight relative to the study population, which would not be generalizable to other populations. Two studies (Luo et al., 2021; Bell et al., 2018) were not considered due to the use of non-preferred exposure characterizations such as infant whole blood samples from a heel stick and postpartum maternal exposure samples, which are prone to exposure misclassification. Three studies (Lauritzen et al., 2017; Lind et al., 2017a; Valvi et al., 2017) were not considered further due to inconsistencies by sex or location with no clear biological explanation for the inconsistency.

As a result of these exclusions, six remaining *high* confidence epidemiologic studies (Yao et al., 2021; Chu et al., 2020; Wikström et al., 2020; Sagiv et al., 2018; Starling et al., 2017; Darrow et al., 2013) met the preferred criteria outlined in Section 4.1.1 and were considered for POD derivation (Table 4-1). The candidate epidemiological studies offer a variety of PFOS exposure measures across different developmental windows (i.e., preconception, fetal, neonatal). All six studies reported their exposure metric in units of ng/mL and reported the β coefficients per ng/mL or ln(ng/mL), along with 95% confidence intervals, estimated from linear regression models. Two of the six studies examined PFOS primarily during trimester one (Sagiv et al., 2018 Wikström, 2020, 6311677), one during trimesters two and three (Starling et al., 2017) and one examined PFOS during trimester three (Yao et al., 2021). One study examined PFOS collected within days of birth (Chu et al., 2020) and another study (Darrow et al., 2013) examined PFOS collected at the time of enrollment in the C8 Health Project. In the latter cohort, two sets of analyses were conducted: one analysis including all births identified from women enrolling in the study and one analysis of only the mother's first prospective birth following enrollment (i.e., only births following blood collected during enrollment). EPA identified the first prospective birth analysis as the analysis to consider for POD derivation due to increased confidence in the temporal relationship between exposure measurement and outcome assessment (i.e., not including mothers with samples collected after pregnancy). The Wikström et al. (2020) study reported on the large Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study cohort with samples collected between 2007 and 2010. Sagiv et al. (2018) reported associations between first trimester PFOS samples collected between 1999–2002 in a Project Viva birth cohort in the United States. Darrow et al. (2013) reported large inverse associations between PFOS collected during C8 Health Project enrollment (2005–2006) in the Mid-Ohio Valley. Chu et al. (2020) reported on associations between maternal PFOS collected within three days of delivery and birth weight in the Chinese Guangzhou Birth Cohort Study (2013). Starling et al. (2017) reported on associations between PFOS collected in later pregnancy (range: 20 to 34 weeks gestational age) in the Healthy Start prospective cohort in Colorado (2009–2014). Yao et al. (2021) reported associations between PFOS measured in maternal blood collected three days prior to delivery and decreased birth weight in the Chinese Laizhou Wan Birth Cohort (2010–2013).

Developmental toxicity results reported in animal toxicological studies are concordant with the observed developmental effects in epidemiological studies. Specifically, studies in rodents found that gestational PFOS exposure resulted in reduced offspring weight (8/14 *medium* confidence

studies) and decreased offspring survival (5/9 *medium* confidence studies). Though limited in number, several other studies also reported consistent effects on placental endpoints, reduced ossification, and developmental delays. Some of these developmental effects seen in the offspring of rodents treated with PFOA (e.g., reduced offspring weight) are consistent with the decreases in birth weight and adverse effects associated with LBW observed in human populations.

Given the large number of adverse effects identified in the animal toxicological database for the developmental health outcome, EPA prioritized only the most sensitive effects (i.e., those observed at lower dose levels and/or higher magnitude) in offspring that were 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 decreased birth weight observed in human infants. The one study reporting altered maternal weight without confounding effects on the offspring (Argus Research Laboratories, 2000) could not be considered for POD derivation 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 not rabbits (see Section 4.1.3). EPA also focused on endpoints for which results from multiple animal toxicological studies corroborated the observed effect, thereby increasing the confidence in that effect. EPA additionally focused on studies with exposure durations lasting through the majority of gestation and/or lactation (i.e., from GD 1 through early postnatal development) rather than those that targeted a specific period of gestation or postnatal development as they were more likely to be sensitive for detection of developmental effects. Multiple animal toxicological studies observed effects at low dose levels and demonstrated a dose-related response in decreased fetal weight, offspring body weight and decreased offspring survival. Therefore, these endpoints were prioritized for dose-response analysis.

Five studies in rats and mice reported decreased pup body weight with PFOS exposure (Xia et al., 2011; Yahia et al., 2008; Luebker et al., 2005b; Luebker et al., 2005a; Lau et al., 2003). For this endpoint, EPA selected studies in rats as the effect was observed more consistently in this species and rats appeared to be more sensitive to pup weight changes than mice. Of the four studies reporting this effect in rats, EPA selected the data presented in the 1- and 2-generation studies by Luebker et al. (2005b) and Luebker et al. (2005a) (F_1 generation only) because the exposure duration spanned prior to mating through lactation, there were more dose groups tested than any of the other available studies, the dosing paradigm encompassed relatively low-dose levels, the authors reported pup weight relative to litter weight, and the effect was observed at multiple time points. Specifically, EPA selected the time points of LD 0 and LD 5 from Luebker et al. (2005b) and PND 1 (F1 only) from Luebker et al. (2005a).

Six studies in mice, rats, and rabbits reported decreased fetal body weight with gestational PFOS exposure (Li et al., 2021a; Wan et al., 2020; Li et al., 2016; Lee et al., 2015; Thibodeaux et al., 2003; Argus Research Laboratories, 2000). While the majority of studies reporting this endpoint did not use an exposure paradigm that encompassed the earliest period of gestation (i.e., GD 1–4), thus increasing the uncertainty about the sensitivity of the data selected for dose-response modeling, EPA modeled this endpoint due to the consistency of the response across species and for comparison to PODs derived for pup weight. EPA selected studies in mice as this species appeared to be more sensitive to fetal weight changes than rats at lower dose levels and as

described above, the PK model used in this assessment is not parameterized for rabbits. Ultimately, Lee et al. (2015) was selected for POD derivation as it reported fetal weight for a relatively greater number of dose groups, incorporated a lower dose level than other studies reporting this effect, and reported more than one dose group with a statistically significant response.

Reduced offspring survival or viability was also observed with developmental PFOS exposure in both rats and mice. Various metrics were used to assess prenatal mortality, including measures of post-implantation loss, stillbirths, abortions, resorptions, and fetal death. Though the response was not entirely consistent between studies, potentially due to study design and differences in the endpoint measurement, reduced prenatal viability was observed in mice, rats, and rabbits and qualitatively supports the observation of reduced pup survival in rats and mice. Given these considerations, reduced fetal survival was not selected for dose-response modeling. Eight studies reporting reduced pup survival; seven in rats and two in mice (Lau et al. (2003) reported on both species). Therefore, EPA considered studies in rats for POD derivation. EPA then selected the metric of pup survival (Chen et al., 2012b; Xia et al., 2011; Grasty et al., 2006; Lau et al., 2003; Thibodeaux et al., 2003) over pup viability (Luebker et al., 2005b; Luebker et al., 2005a) since more studies reported on the former (5 vs. 2). Ultimately, EPA selected pup survival at PND 5 and PND 22 as reported by Lau et al. (2003) for POD derivation because this was a *medium* confidence study that presented data for a greater number of dose groups as compared to the other studies, provided data at multiple time points, incorporated relatively low-dose levels as compared to the other studies, used an exposure duration that encompassed the majority of gestation (GD 2–21), and reported more than one dose group with a statistically significant.

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?	Justification
		Immune Effects		
Reduced Antibody Concentrations for Diphtheria, Tetanus, and Rubella	Budtz-Jørgensen and Grandjean (2018)[a] *Medium* Timmerman et al. (2021) *Medium* Granum et al. (2013) *Medium* Zhang et al. (2023) *Medium*	Human, male and female children or adolescents	Yes	Decreases in antibody responses to pathogens such as diphtheria, tetanus, and rubella were observed at multiple timepoints in childhood and during adolescence, using both prenatal and childhood PFOS exposure levels. Effect was large in magnitude and generally coherent with epidemiological and animal toxicological evidence for other immunosuppressive effects. Effects were observed in multiple populations, including adolescents from the United States.
Decreased PFC Response to SRBC	Zhong et al. (2016) *Medium*	C57BL/6 Mice, F_1 males	Yes	Functional assessment indicative of immunosuppression indicative of immunosuppression. Effect was consistently observed across multiple studies: Peden-Adams et al. (2008), Dong et al. (2009), Zheng et al. (2009), and Keil et al. (2008). Zhong et al. (2016) 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) *High*	Sprague-Dawley Rats, adult 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		
Decreased Birth Weight	Chu et al. (2020) *High* Darrow et al. (2013) *High*	Human, male and female infants	Yes	Evidence for developmental effects is based on consistent inverse effects for FGR including birthweight measures which are the most accurate endpoint examined. 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.

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Derived?	Justification
	Sagiv et al. (2018) *High* Starling et al. (2017) *High* Wikström et al. (2020) *High* Yao et al. (2021) *High*			
Decreased Fetal Body Weight	Lee et al. (2015) *Medium*	CD-1 Mice, F_1 males and females	Yes	Effect was consistently observed across six studies and three species (Li et al., 2021a; Wan et al., 2020; Li et al., 2016; Lee et al., 2015; Thibodeaux et al., 2003; Argus Research Laboratories, 2000) and is coherent with epidemiological evidence of decreased birth weight and evidence of reduced pup weight in rodents. Lee et al. (2015) 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, incorporates a relatively greater number of dose groups, and reported more than one dose group with a statistically significant response compared with other studies reporting this effect, and because mice appear to be a more sensitive model for this endpoint than rats.
Decreased Pup Body Weight (relative to litter)	Luebker et al. (2005b) *Medium* Luebker et al. (2005a)	Sprague-Dawley Rats, F_1 male and female (LD 0 and LD 5 (Luebker et al., 2005b); PND 1 (Luebker et al., 2005a))	Yes	Effect was consistently observed across five studies and two species and is coherent with epidemiological evidence of decreased birth weight and evidence of decreased fetal weight in rodents. Luebker et al. (2005b) and Luebker et al. (2005a) were selected because rats appear to be more sensitive than mice to this endpoint, the studies are designed to be sensitive to this effect (i.e., multigeneration studies testing relatively large numbers of dose groups and low-dose ranges), the studies reported effects as relative to litter and the studies observed effects in multiple generations or multiple time points and in multiple dose groups.
Decreased Pup Survival	Lau et al. (2003) *Medium*	Sprague-Dawley Rats, F_1 male and	Yes	Decreased offspring survival was consistently observed across eight studies and two species and is also supported by reduced fetal survival observed in rodents. Lau et al. (2003) was selected because rats appeared to be more sensitive to this effect than mice and because the study presented data for a greater number of dose groups and at multiple time points compared with

Endpoint	Reference, Confidence	Strain/ Species/Sex	POD Derived?	Justification
		female (PND 5 and PND 22)		the other four studies in rats, incorporated relatively low-dose levels, used an exposure duration that encompassed the majority of gestation (GD 2–21), and reported more than one dose group with a statistically significant response.
Serum Lipid Effects				
Increased Total Cholesterol	Dong et al. (2019) *Medium* Lin et al. (2019) *Medium* Steenland et al. (2009)[b] *Medium*	Human, male and female adults	Yes	Effect was consistent and observed across multiple adult populations including general population adults in NHANES (Dong et al., 2019); from prediabetic adults from the DPP and DPPOS cohort (Lin et al., 2019) and the C8 Health project high-exposure community (Steenland et al., 2009), as well as when study designs excluded individuals prescribed cholesterol medication, minimizing concerns of bias due to medical intervention (Dong et al., 2019; Steenland et al., 2009). Endpoint is supported by associations between PFOS and blood pressure.
Hepatic Effects				
Increased ALT	Gallo et al. (2012) *Medium* Nian et al. (2019) *Medium*	Human (male and female adults)	Yes	Effect was consistent and observed across multiple populations including general population adults (Lin et al., 2010) (NHANES) and high-exposure communities (Gallo et al., 2012) (C8 Health Project); (Nian et al., 2019) (Isomers of C8 Health Project in China).
Increased ALT	Lin et al. (2010) *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) *High*	Sprague-Dawley rats, females	Yes	Effect was supported by a non-monotonic response in males from the same study (Butenhoff et al., 2012). Effect was qualitatively observed in Xing et al. (2016) and Cui et al. (2009), 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); Grandjean et al. (2017a); Grandjean et al. (2017b).

[b] See Section 5.6.3 for discussion on the approach to estimating BMDs from regression coefficients.

4.1.2 Estimation or Selection of Points of Departure for RfD Derivation

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), 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, 2012a) 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 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 1 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 serious effects that can result in irreversible structural or functional changes to the organism, 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, 2012a). 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, 2012a)) 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 1 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, 2012a), the IRIS Handbook (U.S. EPA, 2022d) 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 et al., 2021)). 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 noncancer health outcomes are described in the subsections below and alongside the modeling methods and results provided in Appendix E (U.S. EPA, 2024a). Considerations for BMR selection for cancer endpoints are described in Section 4.24.2 and Appendix E (U.S. EPA, 2024a).

4.1.2.1 Hepatic Effects

For the hepatic endpoint of increased serum ALT in adults associated with PFOS exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk from the biologically

significant adverse serum ALT level (see Table 4-2). As described in detail in Appendix E (U.S. EPA, 2024a), EPA reviewed the available information regarding potential clinical definitions of adversity for the endpoint of elevated ALT. Specifically EPA modeled elevated human ALT using cutoff levels of 42 IU/L for males and 30 IU/L for females (Valenti et al., 2021). These are the most updated clinical consensus cutoffs which post-date the American Association for the Study of Liver Diseases (AASLD) journal of Clinical Liver Disease recommended values of 30 IU/L for males, and 19 IU/L for females (Ducatman et al., 2023; Kasarala and Tillmann, 2016). Valenti et al. (2021, 1036989) determined the values using the same approach at the same center, but using an updated standardized method, a large cohort of apparently healthy blood donors (ages 18-65 years) and showed that the updated cutoffs were able to better predict liver disease.

Because EPA identified a biological basis for BMR selection, EPA used the hybrid approach (see Section 2.3.3.1 of USEPA (2012a)) to estimate the probability of an individual with an adverse serum ALT level as a function of PFOS exposure. This approach effectively dichotomizes the data; therefore, EPA considered BMRs of 1%, 5%, and 10% extra risk for this endpoint. As described in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a 10% BMR is often used to describe quantal data, however, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels, and 1% extra risk is often used as a BMR." EPA considered BMRs of 5% and 10% extra risk. EPA did not select a 1% BMR because it is often used for frank effects and cancer incidence (U.S. EPA, 2012a), neither of which apply to the endpoint of elevated serum ALT.

EPA selected a BMR of 5% extra risk because studies have demonstrated that ALT levels at or slightly above the selected cutoff levels can be associated with more severe liver diseases (Wedemeyer et al., 2010; Mathiesen et al., 1999), increased risk of liver-related mortality (Park et al., 2019a; Ruhl and Everhart, 2009; Kim et al., 2004), and mortality (Lee et al., 2008). Based on the severity of the health effects associated with increased ALT, EPA determined that a BMR of 5% extra risk is warranted (U.S. EPA, 2012a); a 10% extra risk would result in a greater number of individuals, especially those in sensitive subpopulations, experiencing more severe liver diseases such as advanced fibrosis, chronic liver disease, and even liver-related death. Since there is currently a relatively high prevalence of elevated ALT in the general population (14% and 13% of U.S. male and female adults, respectively, aged 20 and older (Valenti et al., 2021)), a small increase in the prevalence of elevated ALT associated with PFOA exposure would likely increase the number of individuals with severe liver-related health effects. This also supports using a more health protective BMR of 5% extra risk (rather than 10%) for POD derivation. EPA presents PODs with a 10% BMR for comparison purposes in Appendix E (U.S. EPA, 2024a), as recommended by agency guidance (U.S. EPA, 2012a).

For the adverse effect of individual cell necrosis observed in livers of adult rats following PFOS exposure, there is currently inadequate available biological or toxicological information to permit determination of an effect-specific minimal biologically significant response level. Therefore, in accordance with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a BMR of 10% extra risk was used because it is considered the standard reporting level for quantal (dichotomous) data and a minimally biologically significant response level (see Table 4-2).

4.1.2.2 Immune Effects

For the developmental immune endpoint of decreased diphtheria, rubella, and tetanus antibody response in children 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, 2012a), EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Because Budtz-Jørgensen and Grandjean (2018), Timmerman et al. (2021), Granum et al. (2013), and Zhang et al. (2023) measured antibody concentrations in childhood and PFOS exposure during gestation or childhood, these are considered developmental studies based on EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), 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 cutoff for the health outcome (U.S. EPA, 2012a). As described in detail in Appendix E (U.S. EPA, 2024a), EPA reviewed the available information regarding potential clinical definitions of adversity for this effect. A blood concentration for tetanus and diphtheria antibodies of 0.1 IU/mL has been cited in the literature as a "protective level" (Grandjean et al., 2017b; Galazka and Kardymowicz, 1989). However, in the *Immunological Basis for Immunization Series* of modules (WHO, 2018b), the WHO argued that assay-specific "protective levels" of tetanus antitoxin may not actually guarantee immunity. Galazka et al. (1993) similarly argued that several factors give rise to variability in the vulnerability of individuals to diphtheria and there is no consensus on what level offers full protection. For rubella, 10 IU/mL has been cited in the literature as a protective level (Skendzel, 1996), however, the geographical variability, lack of consensus, and relatively dated assessment of this cutoff precludes its use as the basis of the BMR (Charlton et al., 2016). As such, EPA determined that there is no clear definition of an adverse effect threshold for the endpoints of reduced tetanus, rubella, or diphtheria antibody concentrations in children or adolescents.

With these two factors in mind, a 0.5 SD was selected as the BMR because: 1) the health outcome is developmental, and 2) there is no accepted definition of an adverse level of change or clinical cutoff 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. EPA also presented BMDL modeling using a BMR equivalent to a 1 SD change, as recommended by agency guidance (U.S. EPA, 2012a).

For the adverse effects of decreased PFC response to SRBC observed in PNW 4 mice and splenic extramedullary hematopoiesis in adult rats following PFOS exposure, there is currently inadequate available biological or toxicological information to permit determination of minimal biologically significant response levels. In accordance with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), 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

For the cardiovascular endpoint of increased serum TC in adults associated with PFOS exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk from the biologically significant adverse serum TC concentration (Dong et al., 2019; Steenland et al., 2009) or a BMR of 0.5 SD (Lin et al., 2019), depending on the data provided by the study (see Table 4-2). As described in detail in Appendix E (U.S. EPA, 2024a), EPA reviewed the available information regarding potential clinical definitions of adversity for this effect and identified the definition of hypercholesterolemia from the American Heart Association (NCHS, 2019) as providing the most recent upper reference limit for clinically adverse serum TC. Specifically, when possible, EPA modeled human cholesterol using a cutoff level of 240 mg/dL for elevated serum total cholesterol (NCHS, 2019).

Because EPA identified a biological basis for BMR selection, EPA used the hybrid approach (see Section 2.3.3.1 of USEPA (2012a)) to estimate the probability of an individual with an adverse TC level as a function of PFOS exposure. This approach effectively dichotomizes the data; therefore, EPA considered BMRs of 1%, 5%, and 10% extra risk for this endpoint. As described in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), a 10% BMR is often used to describe quantal data, however, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels, and 1% extra risk is often used as a BMR." EPA considered BMRs of 5% and 10% extra risk. EPA did not select a 1% BMR because it is often used for frank effects and cancer incidence (U.S. EPA, 2012a), neither of which apply to the effect of elevated serum TC. For Lin (2019), EPA relied on the mean serum TC concentrations reported across PFOS quartiles (i.e., continuous data) provided by the study, and therefore considered a BMR of a change in the mean equal to 0.5 SD or 1 SD from the control mean.

Increased serum cholesterol is associated with changes in incidence of cardiovascular disease events such as myocardial infarction (MI, i.e., heart attack), IS, and cardiovascular mortality occurring in populations without prior CVD events (Lloyd-Jones et al., 2017; Goff et al., 2014; D'Agostino et al., 2008). Based on the severity of the cardiovascular-related health effects associated with increased cholesterol, EPA determined that selection of a BMR of 5% extra risk or 0.5 SD is warranted (U.S. EPA, 2012a); a 10% extra risk or 1SD would result in a greater number of individuals, especially those in sensitive subpopulations, experiencing increased incidence of cardiovascular disease events. Since there is currently a relatively high prevalence of elevated TC in the general population (11.5% of U.S. adults aged 20 and older (NCHS, 2019)), a small increase in the prevalence of elevated TC associated with PFOA exposure would likely increase risk of severe health outcomes, such as cardiovascular-related events. Thus, this supports using a more conservative BMR of 5% extra risk or 0.5 SD for POD derivation. EPA presents PODs with a BMR of 10% extra risk (Dong et al., 2019; Steenland et al., 2009) or 1 SD (Lin et al., 2019) for comparison purposes in Appendix E (U.S. EPA, 2024a), as recommended by agency guidance (U.S. EPA, 2012a).

4.1.2.4 Developmental Effects

For the developmental endpoint of decreased birth weight associated with PFOS exposure, the BMD and the BMDL were estimated using a BMR of 5% extra risk from the biologically significant birth weight deficit (see Table 4-2). As described in Appendix E (U.S. EPA, 2024a), LBW is clinically defined as birth weight less than 2,500 g (approximately 5.8 lbs) and can include but is not exclusive to babies born SGA (birth weight below the 10th percentile for gestational age, sex, and parity) (U.S. EPA, 2013; JAMA, 2002; McIntire et al., 1999).

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure. Low birthweight is associated with increased risk for adverse health effects throughout life (Tian et al., 2019; Reyes and Mañalich, 2005; Hack et al., 1995) and therefore, supports a more stringent BMR below 10% (for dichotomous data) or 1 SD (for continuous data). Because EPA identified a biological basis for BMR selection, EPA used the hybrid approach (see Section 2.3.3.1 of U.S. EPA (2012a)) to estimate the probability of an individual with a birth weight deficit as a function of PFOS exposure. This approach effectively dichotomized the data, resulting in a BMR defined as a 5% increase in the number of infants with birth weights below 2,500 g.

For decreased fetal and pup weights and decreased pup survival observed in animal studies, BMRs of 5% relative deviation and 0.5 SD from the control were employed, respectively (see Table 4-2). As with human data, these BMRs are consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) and the IRIS Handbook (U.S. EPA, 2022d), 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) 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), U.S. EPA (2004), and U.S. EPA (2012b)). 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 SD, and found they were statistically similar (Kavlock et al., 1995).. EPA presented modeling results using a BMR of 0.5 SD for decreased fetal and pup body weight and a BMR of 0.1 SD for the frank effect of decreased pup survival for comparison purposes, as recommended by EPA guidance (U.S. EPA, 2012a) (see Appendix, (U.S. EPA, 2024a)).

Table 4-2. Benchmark Response Levels Selected for BMD Modeling of Health Outcomes

Endpoint	BMR	Rationale
		Immune Effects
Reduced antibody concentrations for diphtheria, rubella, and tetanus in children or adolescents	0.5 SD	Consistent with EPA guidance**.** EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect and selects a 1 or 0.5 SD change in cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome (U.S. EPA, 2012a)

Endpoint	BMR	Rationale
Decreased PFC Response to SRBC (PNW 4)	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 1 SD was used as per EPA guidance (U.S. EPA, 2012a)
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, 2012a)
Developmental Effects		
Decreased Birth Weight in Infants	5% extra risk of exceeding adversity cutoff (hybrid approach)	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a). The use of the hybrid approach results in dichotomization of the data and therefore a 5% BMR was selected (U.S. EPA, 2012a)
Decreased Fetal or Pup Weight	5%	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a)
Decreased Pup Survival	0.5 SD	Consistent with EPA guidance. EPA typically selects a 5% or 0.5 SD benchmark response (BMR) when performing dose-response modeling of data from an endpoint resulting from developmental exposure in consideration of the severity of the effect (U.S. EPA, 2012a)
Cardiovascular Effects		
Increased Cholesterol	5% extra risk of exceeding adversity cutoff (hybrid approach)	Although EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) recommends a BMR based on a 10% extra risk for dichotomous endpoints when biological information is not sufficient to identify the BMR, "for epidemiological data, response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels" (U.S. EPA, 2012a). Because increased TC is not a frank effect but is associated with increased incidence of severe cardiovascular-related effects a 5% extra risk was used as the BMR. The response rate of 5% extra risk limits further increases in the prevalence of this effect.
	0.5 SD	Because increased TC is not a frank effect but is associated with increased incidence of severe cardiovascular-related effects, a 0.5 SD was used as the BMR. A change from the mean of 0.5 SD limits further increases in the prevalence of this effect
Hepatic Effects		
Increased ALT	5% extra risk of exceeding adversity cutoff (hybrid approach)	Although EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) recommends a BMR based on a 10% extra risk for dichotomous endpoints when biological information is not sufficient to identify the BMR, "for epidemiological data,

Endpoint	BMR	Rationale
		response rates of 10% extra risk would often involve upward extrapolation, in which case it is desirable to use lower levels" (U.S. EPA, 2012a). Because increased ALT is not a frank effect but is associated with increased incidence of severe liver-related effects a 5% extra risk was used as the BMR. The response rate of 5% extra risk limits further increases in the prevalence of this effect
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, 2012a)

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 (see Section 3.3.2), EPA chose the Wambaugh et al. (2013) 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 Appendix, (U.S. EPA, 2024a)), 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. The species used in the toxicological studies (i.e., species of interest) were rats, mice, and nonhuman primates; model parameters for these species of interest were available. Good agreement with training and test (out-of-sample) datasets shows that the model performance is good compared with both the data used to identify model parameters and to external data. This was assessed using the mean square log error (MSLE) to compare model predicted concentration values to observed PFOS serum concentrations following single dose exposure to animals. Training set data demonstrated an MSLE of 0.17 for PFOS, respectively. For test set data, the MSLE was 0.38 for PFOS. The general agreement between test and training datasets increases confidence that the model can be used to make accurate predictions of internal dose metrics for the dose magnitudes used in the available toxicology studies. 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 lifestages.

In this case, an oral dosing version of the original model structure introduced by Andersen et al. (2006) 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) 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) 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 lifestage component for predicting developmental study designs. These analyses are further described below. Uncertainties and limitations of the selected modeling approach are described in Section 5.6.1.

4.1.3.1.1 Animal Model Parameters

Pharmacokinetic parameters for different species and strains represented in the Wambaugh et al. (2013) model are presented in Table 4-3.

Table 4-3. PK Parameters from Wambaugh et al. (2013) Meta-Analysis of Literature Data for PFOS

Parameter	Units	CD1 Mouse (F)[a]	CD1 Mouse (M)[a]	Sprague-Dawley Rat (F)[a]	Sprague-Dawley Rat (M)[a]	CynomolgusMonkey (M/F)[a]
Body weight[b] (BW)	kg	0.02	0.02	0.203	0.222	3.42
Cardiac Output[c] (Q_{cc})	L/h/kg$^{0.74}$	8.68	8.68	12.39	12.39	19.8
Absorption rate (k_a)	1/h	1.16 (0.617–42,400)	433.4 (0.51–803.8)	4.65 (3.02–1,980)	0.836 (0.522–1.51)	132 (0.225–72,100)
Central Compartment Volume (V_{cc})	L/kg	0.264 (0.24–0.286)	0.292 (0.268–0.317)	0.535 (0.49–0.581)	0.637 (0.593–0.68)	0.303 (0.289–0.314)
Intercompartment transfer rate (k_{12})	1/h	0.0093 ($2.63 \times e^{-10}$–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] Datasets modeled for the mouse and rat were from Chang et al. (2012) and for the monkey from Seacat et al. (2002) and Chang et al. (2012).
[b] Average body weight for species:individual-specific bodyweights.
[c] Cardiac outputs obtained from Davies and Morris (1993).

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 PFOS HESD (Table 4-4). For rats, the data of Kim et al. (2016) and Huang et al. (2021) 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 et al., 2012) was compared with model simulations. This comparison approach demonstrated agreement with the in vivo data.

Using the Wambaugh et al. (2013) 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) 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).
[b] Information obtained from Huang et al. (2019).

4.1.3.1.3 Life Course Modeling

The Wambaugh et al. (2013) 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.

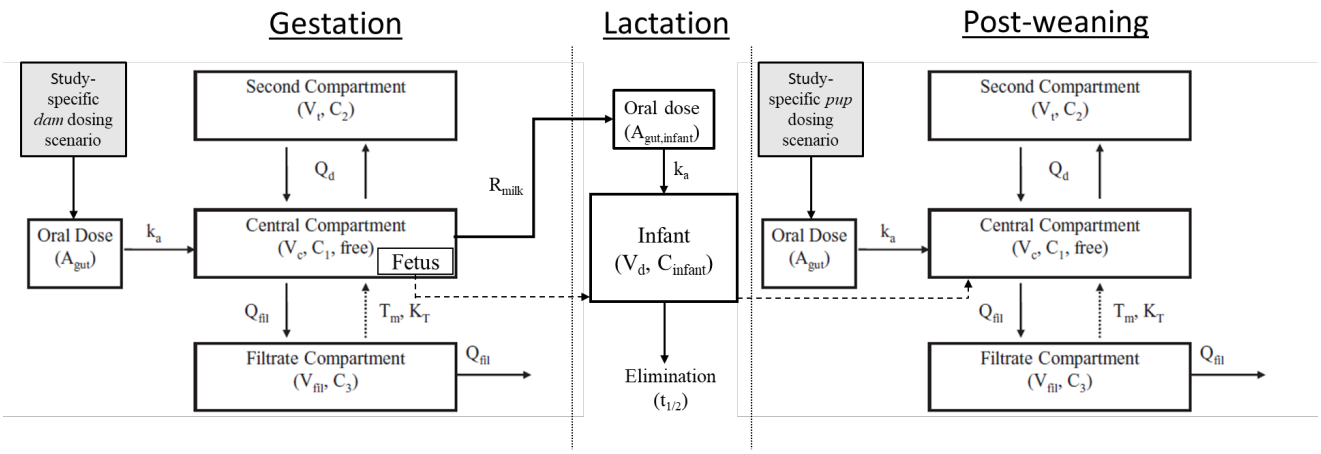


Figure 4-1. Model Structure for Lifestage Modeling

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) 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).
- 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 V_d reported in the literature and Wambaugh et al. (2013) model-predicted V_d following extrapolation to a relatively low infant body weights. Because V_d is assumed to be extracellular water in humans, Goeden et al. (2019) adjusts for lifestage-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). Johanson (1979) 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 et al., 2016; Chang et al., 2012) for the one-compartment model to describe infant toxicokinetics (Table 4-5). Finally, the

Wambaugh et al. (2013) model was not parameterized for a postpartum 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) 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) (derived from Kuklenyik et al. (2004)).
[b] Information obtained from Lau et al. (2003).
[c] Average of male/female half-lives reported in Huang et al. (2019), Kim et al. (2016), and Chang et al. (2012).
[d] Information obtained from Kim et al. (2016).
[e] Assume same P_{milk} as PFOA (lack of mouse data).
[f] Information obtained from Wan et al. (2020).
[g] Information obtained from Chang et al. (2012).
[h] Information obtained from Kapraun et al. (2022) (adapted from Lehmann et al. (2014)).
[i] Information obtained from Kapraun et al. (2022) (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 and lactation study designs in rat dams exposed to 1.6 mg/kg/day PFOS that gave birth to pups who are exposed through gestation and lactation until weaning (Luebker et al., 2005a). For developmental PK simulations, the original Wambaugh et al. (2013) 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.

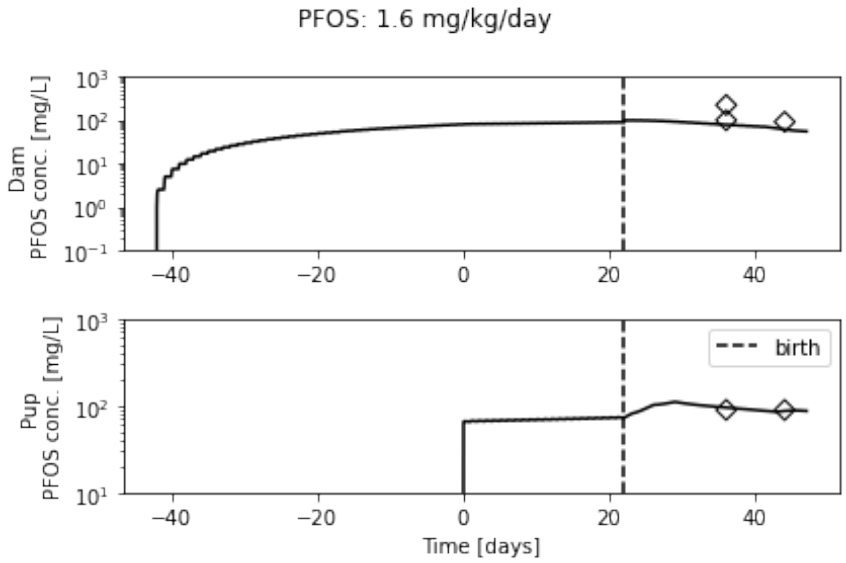


Figure 4-2. Gestation/Lactation Predictions of PFOS in the Rat

Top panel represents predicted dam concentrations with open diamonds (◊) representing the dam concentrations reported in Luebker et al. (2005a). Bottom panel represents predicted pup concentrations with open diamonds (◊) representing the reported pup concentrations in Luebker et al. (2005a) 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 laboratory animal species used in toxicity studies and extrapolate these internal dose POD to humans. Therefore, to evaluate its predictive utility for risk assessment, a number of dose metrics across lifestages 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 lifestages: 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 lifestages 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 $C_{avg\_pup\_gest\_lact}$).

EPA selected the metric of C_{last7} for studies examining noncancer effects using non-developmental exposure paradigms. This metric provides a consistent internal dose for use across disparate chronic and subchronic study designs where steady state may or may not have been reached in the animal following continuous dosing. When the animal has reached steady state, C_{last7} is equal to the steady-state concentration and for non-steady-state study designs, this metric averages the concentration variability over a week's worth of dosing rather than using a single, maximal concentration. This allows for extrapolation to the human model where a steady-state assumption is implemented for adult dose-metric calculations.

For developmental endpoints, the metric of C_{max} is typically used when there is a known MOA related to a threshold effect during a specific window of susceptibility. From the *Guidance for applying quantitative data to develop data-derived extrapolation factors for interspecies and intraspecies extrapolation* (U.S. EPA, 2014), the choice of this metric "depends on whether toxicity is best ascribed to a transient tissue exposure or a cumulative dose to the target tissue." Furthermore, the guidance clarifies that "for chronic effects, in the absence of MOA information to the contrary, it is generally assumed that some integrated cumulative measure of tissue exposure to the active toxicant is the most appropriate dose metric (e.g., AUC)" (U.S. EPA, 2014). Repeat dosing coupled with a lack of a defined MOA for the apical endpoints used for dose-response modeling resulted in EPA excluding C_{max} as an internal dose metric for animal toxicological endpoints. However, EPA provides modeling results using C_{max} for comparison purposes in Appendix E (U.S. EPA, 2024a).

EPA selected the metric of C_{avg} for studies with reproductive or developmental exposure designs encompassing gestation and/or lactation. One factor considered for this selection pertains to the long half-life of PFOA and the degree of accumulation throughout pregnancy and lactation. Because PFOA is not cleared within 24 hours, daily dosing throughout pregnancy/lactation will result in a C_{max} that falls on the final day of pregnancy or lactation or a C_{last7} only representative of the final days of gestation or lactation, even if dosing ceases after birth, due to ongoing lactational exposure. The endpoints in this assessment (decreased fetal or pup weight, decreased pup survival, delayed time to eye opening) do not have established MOAs or known windows of susceptibility and instead are expected to result from sustained internal dose from repeated exposures. If, as anticipated, this window of susceptibly for a given endpoint is not on the final day or the last week of exposure, the C_{max} or C_{last7} will not capture the exposure at the time associated with the adverse effect. A C_{avg} metric is more representative of the exposure throughout the potential window of susceptibility. This selection is also supported by the *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), which state that when pharmacokinetic data are available, as is the case for PFOA, "adjustments may be made to provide an estimate of equal average concentration at the site of action for the human exposure scenario of concern." The selection of C_{avg} for developmental animal studies is therefore consistent with the guidance for humans.

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 body weight 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 et al., 2021). For the human epidemiological and animal toxicological 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). Several alternative models to EPA's updated version of the Verner et al. (2016) 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 et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011) and a developmental PBPK model in rats (Chou and Lin, 2021)), as well as other one-compartment PK models (e.g., Goeden et al. (2019)). Discussion on the justification for selection of the Verner et al. (2016) model as the basis for the pharmacokinetic modeling approach used for PFOS is available in Sections 5.6.2 and 5.7.

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 et al., 2022). 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 (U.S. EPA, 2011b; Kuczmarski et al., 2002). Linear interpolation was used to connect individual timepoints from these two sources to produce a continuous function over time. Body weight during pregnancy was defined based on selected studies of maternal body weight changes during pregnancy (U.S. EPA, 2011b; Portier et al., 2007; Thorsdottir and Birgisdottir, 1998; Carmichael et al., 1997; Dewey et al., 1993). 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 body weight (U.S. EPA, 2011b).

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 Appendix, (U.S. EPA, 2024a)). 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 with the previous EPA assessment of PFOS (U.S. EPA, 2016b). The previous 2016 HESD 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 in children. The current approach explicitly models PFOS exposure to infants during nursing who are undergoing rapid development, including growth, through childhood, and

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 Appendix, (U.S. EPA, 2024a)). 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 Appendix, (U.S. EPA, 2024a)).

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

Notes:
[a] Verner et al. (2016).
[b] Thompson et al. (2010a).
[c] Li et al. (2018b).
[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 Appendix, (U.S. EPA, 2024a)). This is a similar approach to that used by Verner et al. (2016), 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), but also includes studies made available after the publication of that model.

EPA selected a reported half-life value from an exposure to a study population that is demographically representative of the general population, with a clear decrease in exposure at a known time, with a high number of participants and a long follow-up time. Based on these criteria, a half-life of 3.4 years for PFOS was selected (Li et al., 2018b). This value for PFOS comes from a community with contaminated drinking water with serial blood 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 (U.S. EPA, 2024a). Uncertainties related to EPA's selected half-life are discussed in Section 5.6.2.

The updated value for human V_d, 230 mL/kg, was sourced from Thompson et al. (2010a). To estimate the V_d for PFOS, Thompson et al. (2010a) scaled the value they obtained for PFOA by the ratio of V_ds obtained by Andersen et al. (2006) 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 nonhuman

primates (Mordenti et al., 1991). For comparison, the optimized V_d value from oral dosing in monkeys was 220 mL/kg for PFOS (Andersen et al., 2006).

A summary of PFOS V_d values is presented in the Appendix (U.S. EPA, 2024a). Uncertainties related to EPA's selected V_d are discussed in Section 5.6.2.

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 (U.S. EPA, 2024a). 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 with 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) Analysis
Haug et al. (2011)	2577501	0.014	No
Seung-Kyu Kim et al. (2011b)	2919258	0.011	No
Liu et al. (2011)	2919240	0.020	No
Kärrman et al. (2007)	1290903	0.010	No
Cariou et al. (2015)[a]	3859840	0.011	Yes
Sunmi Kim et al. (2011a)[b]	1424975	0.030	Yes
Verner et al. (2016)	3299692	0.014[c]	–
Additional Studies	–	0.016[d]	–

Notes:
Whether studies were included in the analysis of Verner et al. (2016) 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).
[b] Median result as reported by the authors.
[c] Average value of milk:maternal plasma ratio used by Verner et al. (2016).
[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 Appendix, (U.S. EPA, 2024a)). 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 Appendix, (U.S. EPA, 2024a)). 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) and Verner et al. (2016) 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).

4.1.4 Application of Pharmacokinetic Modeling for Animal-Human Extrapolation of PFOS Toxicological Endpoints and Dosimetric Interpretation of Epidemiological Endpoints

Different approaches were taken to estimate POD_{HED}s depending on the species (i.e., human vs. animal model) and lifestage (e.g., developmental, adult). The PODs from epidemiological studies (immune, developmental, hepatic, and serum lipid endpoints) were derived using hybrid or benchmark dose modeling (see Appendix E.1, (U.S. EPA, 2024a)) which provided an internal serum concentration in ng/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 Appendix E.2 (U.S. EPA, 2024a). 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) 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 Appendix E, (U.S. EPA, 2024a)). If BMD modeling did not produce a viable model, a NOAEL or LOAEL approach was used consistent with EPA guidance (U.S. EPA, 2012a). 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 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.

Table 4-8. POD_{HED}s Considered for the Derivation of Candidate RfD Values

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
Immunological Effects						
Decreased serum anti-tetanus antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOS concentrations at age 5 and anti-tetanus antibody serum concentrations at age 7	$BMDL_{0.5\ SD}$	18.5 ng/mL	2.71×10^{-6}	Single- and multi-PFAS models resulted in comparable BMDLs though there was a 55% change in the effect size when controlling for PFOA; selected BMDL was based on a non-significant regression parameter
	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOS concentrations in the mother[c] and anti-tetanus antibody serum concentrations at age 5	$BMDL_{0.5\ SD}$	29.9 ng/mL	5.21×10^{-6}	PFOS concentrations may be influenced by pregnancy hemodynamics; single- and multi-PFAS models resulted in poor quality of model fits; selected BMDL was based on a non-significant regression parameter
	Timmerman et al. (2021) *Medium*	Human, male and female; PFOS concentrations and anti-tetanus antibody concentrations at ages 7–12	$BMDL_{0.5\ SD}$	9.66 ng/mL	1.78×10^{-6}	BMDL based on non-significant regression parameter and resulted in a poor quality of model fit; BMR of 0.5 SD may not be a reasonably good estimate of 5% extra risk
Decreased serum anti-diphtheria antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOS concentrations at age 5 and anti-diphtheria antibody serum concentrations at age 7	$BMDL_{0.5\ SD}$	12.5 ng/mL	1.83×10^{-6}	Single- and multi-PFAS models resulted in comparable BMDLs though there was a 36% change in the effect size when controlling for PFOA; selected BMDL was based on a significant regression parameter

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOS concentrations in the mother[c] and anti-tetanus antibody serum concentrations at age 5	$BMDL_{0.5\ SD}$	20.0 ng/mL	3.48×10^{-6}	PFOS concentrations may be influenced by pregnancy hemodynamics; single- and multi-PFAS models resulted in comparable BMDLs though there was a 22% change in the effect size when controlling for PFOA; selected BMDL was based on a significant regression parameter
	Timmerman et al. (2021) *Medium*	Human, male and female; PFOS concentrations and anti-diphtheria antibody concentrations at ages 7–12	$BMDL_{0.5\ SD}$	5.61 ng/mL	1.03×10^{-6}	BMDL based on model with poor quality of fit; BMDL based on significant regression parameter; BMR of 0.5 SD may not be a reasonably good estimate of 5% extra risk
Decreased serum anti-rubella antibody concentration in children or adolescents	Granum et al. (2013) *Medium*	Human, male and female; PFOS concentrations in the mother at delivery and anti-rubella antibody concentrations at age 3	$BMDL_{0.5\ SD}$	1.6 ng/mL	2.79×10^{-7}	PFOS concentrations may be influenced by pregnancy hemodynamics; BMRs of ½ or 1 SD provide reasonably good estimates of 5% and 10% extra risk; selected BMDL was based on a significant regression parameter
	Zhang et al. (2023) *Medium*	Human, male and female; PFOS concentrations and anti-rubella antibody concentrations at ages 12–19	$BMDL_{0.5\ SD}$	24.3 ng/mL	4.31×10^{-6}	Selected BMDL was based on a significant regression parameter; BMRs of ½ or 1 SD may not be reasonably good estimates of 5% and 10% extra risk
Decreased PFC response to SRBC	Zhong et al. (2016) *Medium*	C57BL/6 Mice, PNW 4 F_1 males	$BMDL_{1\ SD}$, Hill	1.8 mg/L $C_{avg\_pup\_gest\_lact}$	2.88×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and presented most protective

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
						BMDL associated with the effect in a sensitive lifestage; AICs from all models were comparable
Extramedullary Hematopoiesis in the Spleen	NTP (2019) *High*	Sprague-Dawley Rats, female, adults	$BMDL_{10RD}$, Multistage Degree 1	2.27 mg/L $C_{last7,avg}$	2.91×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and presented most protective BMDL; all BMDLs from adequate fitting models were comparable
	NTP (2019) *High*	Sprague-Dawley Rats, male, adults	$BMDL_{10RD}$, Logistic	9.59 mg/L $C_{last7,avg}$	1.23×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Developmental Effects						
Decreased Birth Weight	Chu et al. (2020) *High*	Human, male and female; PFOS serum concentrations in third trimester	$BMDL_{5RD}$, Hybrid	7.3 ng/mL	1.27×10^{-6}	PFOS concentrations may be influenced by pregnancy hemodynamics; selected BMDL based on significant regression parameter
	Sagiv et al. (2018) *High*	Human, male and female; PFOS serum concentrations in first and second trimesters	$BMDL_{5RD}$, Hybrid	41.0 ng/mL	6.00×10^{-6}	Selected BMDL based on non-significant regression parameter
	Starling et al. (2017) *High*	Human, male and female; PFOS serum concentrations in second and third trimesters	$BMDL_{5RD}$, Hybrid	5.7 ng/mL	9.26×10^{-7}	PFOS concentrations may be influenced by pregnancy hemodynamics; selected BMDL based on non-significant regression parameter
	Wikström et al. (2020) *High*	Human, male and female; PFOS serum concentrations in first and second trimesters	$BMDL_{5RD}$, Hybrid	7.7 ng/mL	1.13×10^{-6}	Selected BMDL based on significant regression parameter
	Darrow et al. (2013) *High*	Human, male and female, maternal PFOS serum	$BMDL_{5RD}$, Hybrid	17.4 ng/mL	2.51×10^{-6}	Modeled based on first prospective birth analysis (i.e., PFOS concentrations

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
		concentrations taken at time of enrollment in C8 project[d]				measured prior to pregnancy); selected BMDL based on significant regression parameter
	Yao et al. (2021) *High*	Human, male and female; PFOS serum concentrations in third trimester	$BMDL_{5RD}$, Hybrid	5.0 ng/L	8.70×10^{-7}	PFOS concentrations may be influenced by pregnancy hemodynamics; selected BMDL based on non-significant regression parameter
Decreased Fetal Body Weight	Lee et al. (2015) *Medium*	CD-1 Mice, F_1 males and females (GD 17)	NOAEL[e] (0.5 mg/kg/day)	8.75×10^{-1} mg/L $C_{avg\_pup\_gest}$	3.40×10^{-4}	No models had adequate fit (residuals at BMD or control were greater than 2, or the BMDL was 3x lower than the lowest tested dose); NOAEL approach taken
Decreased Pup Body Weight	Luebker et al. (2005b) *Medium*	Sprague-Dawley Rats, F_1 male and female (LD 1)	$BMDL_{5RD}$, Exponential 3	14.7 mg/L $C_{avg\_pup\_gest}$	5.71×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
	Luebker et al. (2005b) *Medium*	Sprague-Dawley Rats, F_1 male and female (LD 5)	$BMDL_{5RD}$, Polynomial Degree 6	2.30 mg/L $C_{avg\_pup\_gest\_lact}$	3.65×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
	Luebker et al. (2005a) *Medium*	Sprague-Dawley Rats, F_1 male and female (LD 1)	$BMDL_{5RD}$, Exponential 4	11.3 mg/L $C_{avg\_pup\_gest}$	4.39×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Decreased Pup Survival	Lau et al. (2003) *Medium*	Sprague-Dawley Rats, F_1 male and female (PND 5)	NOAEL[e] (1 mg/kg/day)	13.0 mg/L $C_{avg\_pup\_gest\_lact}$	2.06×10^{-3}	No models had adequate fit (for all models, all model control response SD was 1.5x greater than actual response SD, and for most models, the calculated BMD was 3x lower than the lowest administered dose); NOAEL approach taken

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
	Lau et al. (2003) *Medium*	Sprague-Dawley Rats, F_1 male and female (PND 22)	NOAEL[e] (1 mg/kg/day)	17.3 mg/L $C_{avg\_pup\_gest\_lact}$	2.75×10^{-3}	No models had adequate fit (for all models, all model control response SD was 1.5x greater than actual response SD, and for most models, the calculated BMD was 3x lower than the lowest administered dose); NOAEL approach taken
Cardiovascular Effects (Serum Lipids)						
Increased Total Cholesterol	Dong et al. (2019) *Medium*	Human, male and female, age 20-80	$BMDL_{5RD}$, Hybrid	9.34 ng/mL	1.20×10^{-6}	BMDL based on analyses excluding individuals prescribed cholesterol medication and significant regression parameter
	Steenland et al. (2009) *Medium*	Human, male and female, age 18 and older	$BMDL_{5RD}$, Hybrid	9.52 ng/mL	1.22×10^{-6}	BMDL based on analyses excluding individuals prescribed cholesterol medication and significant regression parameter
	Lin et al. (2019) *Medium*	Human, male and female, age 25 and older	$BMDL_{5RD}$, Linear	66.5 ng/mL	8.51×10^{-6}	BMDL based on analyses including individuals prescribed cholesterol medication and non-significant regression parameter
Hepatic Effects						
Elevated ALT	Gallo et al. (2012) *Medium*	Human, female, age 18 and older	$BMDL_{5RD}$, Hybrid	56.8 ng/mL	7.27×10^{-6}	BMDL based on significant regression parameter
	Nian et al. (2019) *Medium*	Human, female, age 22 and older	$BMDL_{5RD}$, Hybrid	15.1 ng/mL	1.94×10^{-6}	BMDL based on significant regression parameter
Increased Individual Cell	Butenhoff et al. (2012)/ Thomford (2002b)[f]	Sprague-Dawley Rats, females, adults	$BMDL_{10RD}$, Log-Logistic	27.0 mg/L $C_{last7,avg}$	3.45×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and

Endpoint	Reference, Confidence	Strain/Species/Sex/Age	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED} (mg/kg/day)	Notes on Modeling
Necrosis in the Liver	*High*					lowest AIC among models with BMD/BMDL ratio < 3

Notes: ALT = alanine aminotransferase; AUC = area under the curve; $BMDL_{0.5\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean response equal to 0.5 SD from the control mean; $BMDL_{1\ SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean response equal to 1 SD 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; $C_{avg\_pup\_gest}$ = average blood concentration during gestation; $C_{last7,avg}$ = average 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 Appendix (U.S. EPA, 2024a) for additional details on BMD modeling.

[b] Supported by Grandjean et al. (2012); Grandjean et al. (2017a); Grandjean et al. (2017b).

[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) 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) and Thomford (2002b) reported the same data.

4.1.4.1 Hepatic Effects

Increased ALT in individuals aged 18 and older (Gallo et al., 2012) or 22 and older (Nian et al., 2019)

The POD for increased ALT in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E.1, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from adults aged 18 years and older (Nian et al., 2019; Gallo et al., 2012), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2) The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (Section 4.1.3.2). 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 was 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, $C_{last7,avg}$ (Butenhoff et al., 2012)

Increased incidence of individual cell necrosis in the liver was observed in female Sprague-Dawley Crl:CD(SD)IGS BR rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The $C_{last7,avg}$ was selected for all non-developmental studies rather than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model (Section 4.1.3.1.3). 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 (Section 4.1.3.2). This calculation was 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-Jørgensen and Grandjean, 2018)

The POD for decreased antibody production at age 7 was derived by quantifying a benchmark dose (see Appendix E.1, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations at age 5, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. 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 body weight, 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 each. The slightly lower HED in males was then selected as it was the most health protective.

Decreased Diphtheria and Tetanus antibody response in vaccinated children at age 5 (Budtz-Jørgensen and Grandjean, 2018)

The POD for decreased antibody production at age 5 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) 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. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). 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 (Timmermann et al., 2021)

The POD for decreased antibody production in children aged 7–12 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations at ages 7–12, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For this, the model was run similarly to the endpoint based on antibodies at age 7 (Budtz-Jørgensen and Grandjean, 2018), 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 different growth curves specific to male and female children were used in the model, the model predicted slightly different (less than 5%) serum concentrations for each sex. The lower HED was then selected as it was the most health protective.

Decreased Rubella antibody response in vaccinated adolescents at ages 12–19 (Zhang et al., 2023)

The POD for decreased antibody production in adolescents aged 12–19 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations at ages 12–19, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). For

this, the model was run similarly to the endpoint based on antibodies at age 7 (Budtz-Jørgensen and Grandjean, 2018), but the model was run until the median age of this cohort at blood collection, 15.5 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 Rubella antibody response in vaccinated children at age 3 (Granum et al., 2013)

The POD for decreased antibody production at age 3 was derived by quantifying a benchmark dose (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother at delivery, which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). 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 at the time of delivery. Reverse dosimetry was performed on model-predicted maternal serum concentration at that time to calculate the POD_{HED}. This metric was independent of the sex of the child in the model.

Decreased plaque forming cell (PFC) response to SRBC, C57BL/6 Mice, PNW 4 F_1 males, $C_{avg\_pup\_gest\_lact}$ (Zhong et al., 2016)

Decreased mean level of PFC response of splenic cells was observed in F_1 male C57BL/6 mice. Using the Wambaugh et al. (2013) model, daily exposure to PFOS through oral gavage was simulated from GD 1–GD 17 using female CD1 mice parameters (C57BL/6 mice parameters are not available for PFOS; Section 4.1.3.1). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). Continuous models were used to fit dose-response data. 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, 2012a) (Section 4.1.2). 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 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. 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 was 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 was 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, $C_{last7,avg}$ (NTP, 2019)

Increased incidence of extramedullary hematopoiesis in the spleen was observed in male and female Sprague-Dawley rats. Using the Wambaugh et al. (2013) model, daily exposure to PFOS through oral gavage was simulated for 28 days using Sprague-Dawley rat parameters (Section 4.1.3.1). The $C_{last7,avg}$ was selected for all non-developmental studies rather than alternate metrics such as C_{max} to provide a consistent internal dose for use across chronic and subchronic study designs where steady state may or may not have been reached and to allow extrapolation to the human PK model (Section 4.1.3.1.3). 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, 2012a) (Section 4.1.2). 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 (Section 4.1.3.2). This calculation was 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 et al., 2019)

The POD for increased TC in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) 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. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (Section 4.1.3.2). 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 was 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 et al., 2009)

The POD for increased TC in adults was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) 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. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). 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 (in mg/kg/day) that would result in a steady-state serum concentration equal to the internal serum POD (Section 4.1.3.2). This calculation was 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 et al., 2019)

The POD for increased TC in adults was derived by quantifying a benchmark dose using BMDS (see Appendix E, (U.S. EPA, 2024a)) from 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 DPP and Outcomes Study (DPPOS), which provided an internal serum concentration POD in mg/L. A BMR of 0.5 SD extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day (Section 4.1.3.2). 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 was 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 et al., 2020)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) 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. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). 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 body weight. 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 the first and second trimesters (Sagiv et al., 2018)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother primarily in the first trimester (median gestational age of 9 weeks), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) 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 et al., 2017)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother in trimesters 2 and 3 (median gestational age of 27 weeks), which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) 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 et al., 2020)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) 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. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). This was performed as described for the Chu et al. (2020) 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 et al., 2021)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) 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. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in Section 4.1.3.2). 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 body weight. 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 et al., 2013)

The POD for decreased birth weight in infants was derived by quantifying a benchmark dose using a hybrid modeling approach (see Appendix E, (U.S. EPA, 2024a)) on the measured PFOS serum concentrations collected from the mother prior to conception, which provided an internal serum concentration POD in mg/L. A BMR of 5% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The internal serum POD was converted to an external dose (POD_{HED}), in mg/kg/day, using the updated Verner model (described in 4.1.3.2). This was performed as described for the Chu et al. (2020) study. In the selected cohort, blood samples were taken from women before conception. Therefore, 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 et al., 2015)

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 a 5% change from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (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 Appendix E, (U.S. EPA, 2024a)). The $C_{avg,pup,gest}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure any time during gestation (Section 4.1.3.1.3). 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 4.1.3.2). For this endpoint, the model was run starting at the birth of the mother, with constant exposure relative to body weight. 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 (LD 5), $C_{avg\_pup\_gest\_lact}$ (Luebker et al., 2005b)

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 a 5% change from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (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 Appendix E, (U.S. EPA,

2024a)). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). 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 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the entire 1-year breastfeeding period Then the model was run through the entire 1-year breastfeeding period because the lactational duration in humans that equates to lactational day 5 in rodents is unknown. Additionally, there is currently no mechanistic information to identify a specific window of susceptibility in lactation for this endpoint. The average serum concentration in the infant through gestation and lactation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Because of different growth curves used for male and female children, the model predicted slightly different serum concentrations for males and females. The lower HED was selected to be more health protective.

Decreased Pup Body Weight, Sprague-Dawley Rats, F_1 male and female (LD 1), $C_{avg\_pup\_gest}$ (Luebker et al., 2005b; Luebker et al., 2005a)

Decreased mean pup body weight relative to the litter at LD 1 (the day of birth) was observed in F_1 male and female Sprague-Dawley rats in 1-generation and 2-generation reproductive studies. Continuous models were used to fit dose-response data. A BMR of a 5% change from the control mean was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (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 Appendix E, (U.S. EPA, 2024a)). The $C_{avg,pup,gest}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure any time during gestation (Section 4.1.3.1.3). 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 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. 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 Survival, Sprague-Dawley Rats, F_1 male and female (PND 5 and 22), $C_{avg\_pup\_gest\_lact}$ (Lau et al., 2003)

Decreased pup survival at PND 5 and PND 22 was observed in F_1 male and female Sprague-Dawley rats. Continuous models were used to fit dose-response data. A BMR of 0.5 SD was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2) and a BMR of a change in the mean equal to 0.1 standard deviations from the control mean was provided for comparison purposes because decreased pup survival is a severe, frank effect (U.S.

EPA, 2012a) (see Appendix E, (U.S. EPA, 2024a)). The $C_{avg,pup,gest\_lact}$ internal dose metric was selected for this model since an average concentration metric is expected to better correlate with this developmental effect that may have resulted from exposure during gestation or lactation (Section 4.1.3.1.3). 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 4.1.3.2). For this, the model was run starting at the birth of the mother, with constant exposure relative to body weight. Pregnancy began at 24.25 years maternal age and birth occurred at 25 years maternal age. The initial concentration in the child was governed by the observed ratio between maternal serum and cord blood at delivery. Then the model was run through the entire 1-year breastfeeding period for both timepoints because the lactational duration in humans that equates to lactational day 5 in rodents is unknown. Additionally, there is currently no mechanistic information to identify a specific window of susceptibility in lactation for this endpoint. The average serum concentration in the infant through gestation and lactation was determined for this scenario and reverse dosimetry was used to calculate the exposure that results in the same value as the POD. Because of different growth curves used for male and female children, the model predicted slightly different serum concentrations for males and females. The lower HED was selected to be more health protective.

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. For epidemiological studies, similar to the discussion of study selection factors in Section 4 and Section 4.1.1, EPA critically considered attributes for each POD_{HED} including timing of endpoint collection or measurement, uncertainties associated with modeling (see Appendix E (U.S. EPA, 2024a) and Table 4-8), and consideration of confounding. For animal toxicological studies, attributes considered included study confidence (i.e., *high* confidence studies were prioritized over *medium* confidence studies), amenability to benchmark dose modeling, study design, sensitive lifestages, and health effects observed after exposure in the lower dose range among the animal toxicological studies. As described in the subsections below, this examination of epidemiological and toxicological studies led to the exclusion of a number of studies from consideration for candidate RfD derivation. Health outcome- and study-specific considerations are discussed in Sections 4.1.5.1 (Hepatic) 4.1.5.2 (Immune) 4.1.5.3 (Cardiovascular), and 4.1.5.4 (Developmental).

Once studies and their corresponding POD_{HED}s were prioritized for candidate RfD derivation, EPA applied uncertainty factors (UFs) according to methods described in EPA's *Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b). Considerations for individual UFs differed between epidemiological and animal toxicological studies and are further described in Section 4.1.5.5. Presentation of the candidate RfDs for each health outcome is provided in Section 4.1.5.6.

4.1.5.1 Hepatic Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Nian et al., 2019; Gallo et al., 2012). EPA considered both studies as they represented the low-dose range of effects across hepatic endpoints and provided data from relatively large populations, including the U.S. population. Additionally, these studies had many study strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias. The two studies reported analyses examining different forms of confounding factors, sensitivity analyses excluding participants with lifestyle characteristics (e.g., excluding smokers, drinkers, medicine takers) impacting outcome assessment (Nian et al., 2019), and non-linear exposure-response relationships (Gallo et al., 2012). Both studies provided the necessary data for modeling.

One *high* confidence animal toxicological study was carried forward for candidate RfD determination (Butenhoff et al., 2012; Thomford, 2002b). This study was prioritized for candidate RfD development because it was determined to be a *high* confidence study, was amenable to BMD modeling, and was the only animal toxicological study with a chronic exposure duration that histopathologically examined the liver of animals treated with PFOS.

4.1.5.2 Immune Effects

Three *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Zhang et al., 2023; Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018). EPA considered all three studies as they represented the low-dose range of effects across immunological endpoints and provided data regarding sensitive populations (i.e., children) across three vaccine types. Although EPA derived POD_{HED}s for two time points reported by Budtz-Jørgensen and Grandjean (2018) (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 potentially increased risk bias due to pregnancy-related hemodynamic effects. Similarly, EPA did not carry forward POD_{HED}s derived from Granum et al. (2013) because PFOS serum concentrations were measured in the mother at the time of delivery and therefore, this study also had potential for increased risk of bias due to pregnancy-related hemodynamic effects. EPA also derived candidate RfDs for both tetanus and diphtheria vaccine responses from Timmerman et al. (2021) for comparison to a second population of children. Zhang et al. (2023) was also selected for candidate RfD derivation because it provided results in adolescents from the U.S. population for a third vaccine type (i.e., rubella). Additionally, the BMDL derived from this study was based on a significant regression parameter. In total, five immunological POD_{HED}s from three epidemiological studies were carried forward for candidate RfD derivation.

Two animal toxicological studies, one *high* and one *medium* confidence, were carried forward for candidate RfD determination (NTP, 2019; Zhong et al., 2016). NTP (2019) 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 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) 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) reported this effect in pups exposed to PFOS during gestation and therefore encompassed a sensitive population that is coherent with the developmental immunotoxicity observed in humans. For these reasons, EPA determined that both of these effects warranted candidate RfD derivation.

4.1.5.3 Cardiovascular Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Dong et al., 2019; Steenland et al., 2009). Of the three studies for which POD_{HED}s were derived, Dong et al. (2019) and Steenland et al. (2009) excluded individuals who were prescribed cholesterol medication, minimizing concerns surrounding confounding due to the medical intervention altering serum total cholesterol levels. This is in contrast to Lin et al. (2019) which did not control for individuals prescribed cholesterol medication and was therefore excluded from further consideration. Modeling of both Dong et al. (2019) and Steenland et al. (2009) resulted in POD_{HED}s with minimal risk of bias, representing both the general population and a high-exposure community, respectively and thus were both considered further for candidate RfD derivation.

4.1.5.4 Developmental Effects

Three *high* confidence epidemiological studies were carried forward for candidate RfD determination for the endpoint of decreased birth weight (Wikström et al., 2020; Sagiv et al., 2018; Darrow et al., 2013). Of the six epidemiological studies for which POD_{HED}s were derived, Darrow et al. (2013), Sagiv et al. (2018), and Wikström et al. (2020) assessed maternal PFOS serum concentrations either prior to conception or primarily in the first trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Although Wikström et al. (2020) collected approximately 4% of samples during early weeks of the second trimester, sensitivity analyses showed no differences when trimester two samples were excluded. Additionally, these studies had many study strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias and reflected two different study populations. Therefore, all three studies were considered further for candidate RfD derivation. The three excluded studies assessed PFOS concentrations in either umbilical cord blood or primarily during the second or third trimesters, increasing the uncertainty associated with the derived POD_{HED}s due to potential pregnancy-related hemodynamic effects, and as a result, were excluded from consideration for candidate RfD derivation (Yao et al., 2021; Chu et al., 2020; Starling et al., 2017).

One *medium* confidence animal toxicological study was carried forward for candidate RfD determination (Luebker et al., 2005b). The endpoint of reduced pup weight at LD 5 from this study was amenable to benchmark dose modeling (i.e., BMD modeling produced viable model fits), unlike the endpoints of decreased fetal weight reported by Lee et al. (2015) and decreased pup survival reported by Lau et al. (2003), which had NOAELs as the basis of the POD_{HED}s. Decreased pup weight at LD 5 was selected over the other time point reported by Luebker et al. (2005b) (i.e., LD 1) and decreased pup weight reported by Luebker et al. (2005a) (also LD 1)

because it was the most protective of the three POD_{HED}s, all of which were derived from BMDLs. The endpoint of decreased pup weight reported by Luebker et al. (2005b) encompassed a sensitive population and was coherent with the observed effect of decreased birth weight in humans and was therefore selected for candidate RfD derivation.

4.1.5.5 Application of Uncertainty Factors

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, 2002b).

Table 4-9. Uncertainty Factors for the Development of the Candidate Chronic RfD Values from Epidemiological Studies (U.S. EPA, 2002b)

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 lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
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 lifestages and populations and allow for a complete characterization of the contaminant's toxicity.
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, lifestage, 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 lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). A UF_S of 1 was also applied to the immune endpoints observed in children and adolescents because exposure is assumed to occur from gestation through childhood, when the response variable was measured. There is uncertainty regarding the critical window of exposure that results in these immune effects in children and adolescents. Therefore, EPA expects that any exposure during this period of development has the potential to impact this response (U.S. EPA, 1991). According to the WHO/International Programme on Chemical Safety (IPCS) *Immunotoxicity Guidance for Risk Assessment*, developmental immunotoxicity is assessed during the prenatal, neonatal, juvenile and adolescent life stages because immune system development occurs throughout these life stages and should be viewed differently in part due to increased susceptibility compared with the immune system of adults from a risk assessment perspective (IPCS, 2012).

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 large number of *medium* and *high* confidence epidemiological studies 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 UF 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 UF 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, 2002b)

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	Value	Justification
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 lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
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 lifestages 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 threefold 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, lifestage, 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 was used as the basis for the POD_{HED} derivation. BMDLs were available for all animal toxicological endpoints and studies advanced for candidate RfD derivation.

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 lifestage when exposure during a time window of development is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991). 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 large number of *medium* and *high* confidence epidemiological studies 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.

In summary, the composite UF that was applied to candidate RfDs derived from all of the epidemiological studies were the same value (UF_C = 10) (Table 4-9). The composite UF that was applied to candidate RfDs derived from animal toxicological studies was either UF_C = 30 or 300 (Table 4-10). In all of these cases, the total uncertainty is well below the maximum recommended UF_C = 3,000 (U.S. EPA, 2002b).

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/Age	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) *Medium*	Human, male and female, PFOS concentrations at age 5 and antibody concentrations at age 7	2.71×10^{-6}	1	10	1	1	1	10	$2.71 \times 10^{-7} = 3 \times 10^{-7}$
	Timmerman et al. (2021) *Medium*	Human, male and female, PFOS and antibody concentrations at age 7–12	1.78×10^{-6}	1	10	1	1	1	10	$1.78 \times 10^{-7} = 2 \times 10^{-7}$
Decreased Serum Anti-Diphtheria Antibody Concentration in Children	Budtz-Jørgensen and Grandjean (2018) *Medium*	Human, male and female, PFOS concentrations at age 5 and antibody concentrations at age 7	1.83×10^{-6}	1	10	1	1	1	10	$1.83 \times 10^{-7} = 2 \times 10^{-7}$
	Timmerman et al. (2021) *Medium*	Human, male and female, PFOS and antibody concentrations at age 7–12	1.03×10^{-6}	1	10	1	1	1	10	$1.03 \times 10^{-7} = 1 \times 10^{-7}$
Decreased Serum Anti-Rubella Antibody Concentration in Adolescents	Budtz-Jørgensen and Grandjean (2018) *Medium*	Human, male and female, PFOS and antibody concentrations at age 12–19	4.31×10^{-6}	1	10	1	1	1	10	$4.31 \times 10^{-7} = 4 \times 10^{-7}$
Decreased Plaque Forming Cell (PFC) Response to SRBC	Zhong et al. (2016) *Medium*	C57BL/6 Mice, PNW 4 F_1 males	2.88×10^{-4}	3	10	1	1	1	30	$9.60 \times 10^{-6} = 1 \times 10^{-5}$
Extramedullary Hematopoiesis in the Spleen	NTP (2019) *High*	Sprague-Dawley rats, female, adults	2.91×10^{-4}	3	10	10	1	1	300	$9.70 \times 10^{-7} = 1 \times 10^{-6}$
Developmental Effects										
Decreased Birth Weight	Sagiv et al. (2018) *High*	Human, male and female, PFOS concentrations in first and second trimesters	6.00×10^{-6}	1	10	1	1	1	10	$6.00 \times 10^{-7} = 6 \times 10^{-7}$

Endpoint	Reference, Confidence	Strain/Species/ Sex/Age	POD_{HED} (mg/kg/day)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_{TOT}	Candidate RfD[a] (mg/kg/day)
	Wikström et al. (2020) *High*	Human, male and female, PFOS concentrations in first and second trimesters	1.13×10^{-6}	1	10	1	1	1	10	$1.13 \times 10^{-7} = 1 \times 10^{-7}$
	Darrow et al. (2013) *High*	Human, male and female, PFOS concentrations at time of enrollment[b]	2.51×10^{-6}	1	10	1	1	1	10	$2.51 \times 10^{-7} = 3 \times 10^{-7}$
Decreased Pup Body Weight	Luebker et al. (2005b) *Medium*	Sprague-Dawley Rats, F_1 male and female (LD 5)	3.65×10^{-4}	3	10	1	1	1	30	$1.22 \times 10^{-5} = 1 \times 10^{-5}$
Cardiovascular Effects										
Increased Serum Total Cholesterol	Dong et al. (2019) *Medium*	Human, male and female, ages 20-80	1.20×10^{-6}	1	10	1	1	1	10	$1.20 \times 10^{-7} = 1 \times 10^{-7}$
	Steenland et al. (2009) *Medium*	Human, male and female, age 18 and older	1.22×10^{-6}	1	10	1	1	1	10	$1.22 \times 10^{-7} = 1 \times 10^{-7}$
Hepatic Effects										
Increased Serum ALT	Gallo et al. (2012) *Medium*	Human, female, age 18 and older	7.27×10^{-6}	1	10	1	1	1	10	$7.27 \times 10^{-7} = 7 \times 10^{-7}$
	Nian et al. (2019) *Medium*	Human, female, at age 22 and older	1.94×10^{-6}	1	10	1	1	1	10	$1.94 \times 10^{-7} = 2 \times 10^{-7}$
Individual Cell Necrosis in the Liver	Butenhoff et al. (2012)/Thomford (2002b)[c] *High*	Sprague-Dawley rats, females, adults	3.45×10^{-3}	3	10	1	1	1	30	$1.15 \times 10^{-4} = 1 \times 10^{-4}$

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] 99% of the pregnancies of participants in Darrow et al. (2013) were within 3 years of the serum PFOS measurement.

[c] Butenhoff et al. (2012) and Thomford (2002b) 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 noncancer 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 or 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). 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).

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 Sections 5.1, 5.6, and 5.7), 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 and sex-specific toxicokinetic differences in rodent species These uncertainties may explain, in part, the higher magnitude of candidate RfDs derived from animal toxicological studies compared to the candidate RfDs derived from epidemiological studies. Moreover, the human epidemiological studies also have greater relevance to human exposure than animal toxicological studies 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, 2022d). 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 5.2 for further comparisons between toxicity values derived from epidemiological and animal toxicological studies.

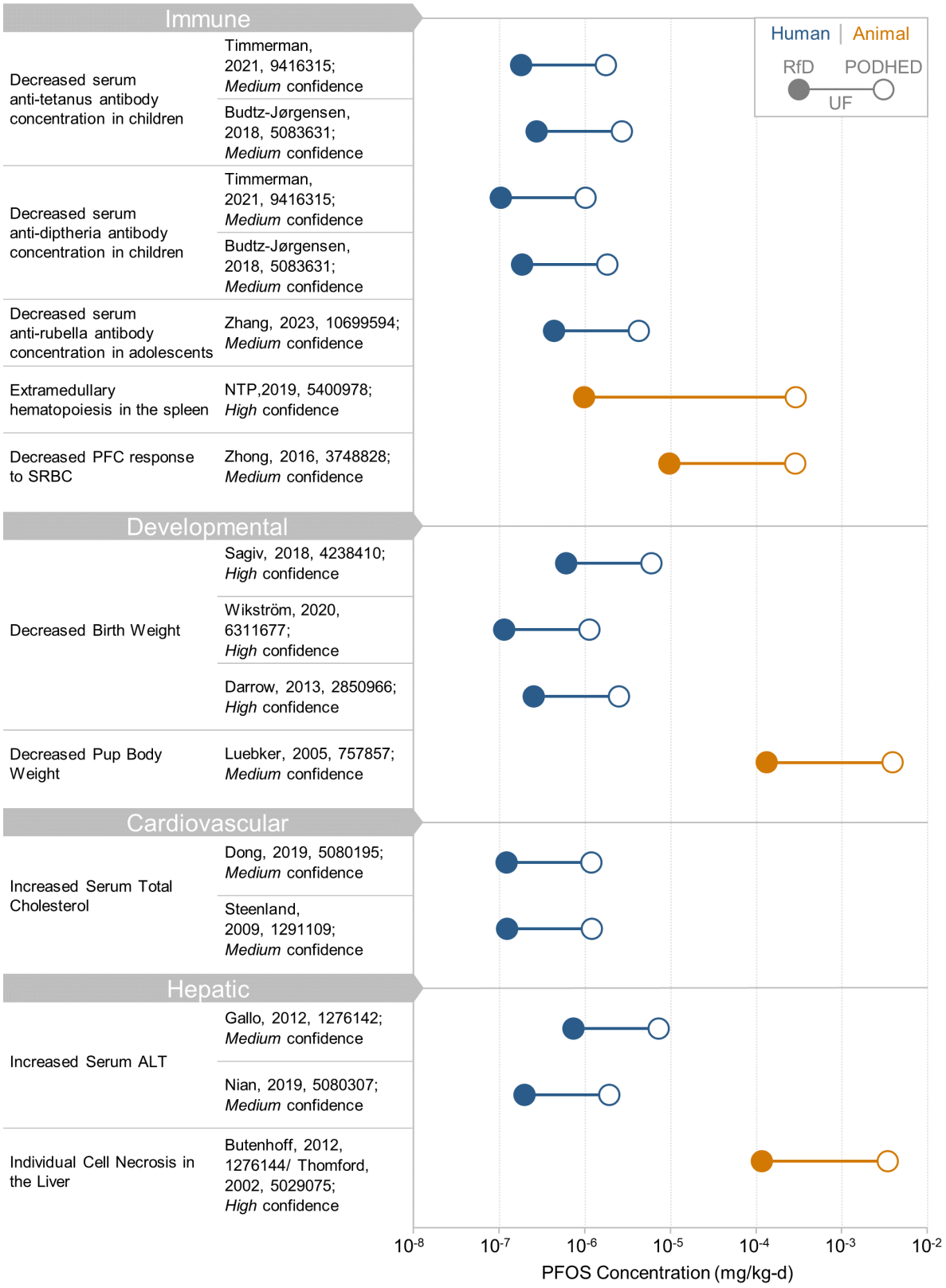


Figure 4-3. Comparison of Candidate RfDs Resulting from the Application of Uncertainty Factors to POD_{HED}s Derived from Epidemiological and Animal Toxicological Studies

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

At least two candidate RfDs were derived from epidemiological studies for each of the four prioritized noncancer health outcomes. EPA considered several factors when selecting health outcome-specific RfDs, including relevance of exposure or population characteristics to the general population, potential confounding factors, and characteristics of the modeled data. Health outcome- and study-specific considerations are discussed in Sections 4.1.6.1.1 (Hepatic), 4.1.6.1.2 (Immune), 4.1.6.1.3 (Cardiovascular), and 4.1.6.1.4 (Developmental), below.

4.1.6.1.1 Hepatic Effects

Two *medium* confidence epidemiological studies were selected for candidate RfD derivation for the endpoint of increased ALT (Nian et al., 2019; Gallo et al., 2012). The larger study of PFOS and ALT in adults (Gallo et al., 2012) was conducted in over 30,000 adults from the C8 Study. The other study (Nian et al., 2019) 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 candidate RfD for increased ALT from Nian et al. (2019) 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 in the population of interest. 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

Candidate RfDs were derived from three *medium* confidence epidemiological studies for the endpoint of decreased antibody production in response to various vaccinations in children (Zhang et al., 2023; Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018). Candidate RfDs derived from Timmerman et al. (2021) were considered lower confidence candidate RfDs than those derived from Budtz-Jørgensen and Grandjean (2018). POD_{HED}s derived from Timmerman et al. (2021) were considered to have increased uncertainty compared with Budtz-Jørgensen and Grandjean (2018) due to two features of the latter study that strengthen the confidence in the POD_{HED}s: 1) the response reported by this study was more precise in that it reached statistical significance, and 2) the analysis considered co-exposures of other PFAS. Therefore, the candidate RfDs from Timmerman et al. (2021) were not considered for selection as the health outcome-specific RfD. Similarly, the candidate RfD derived from Zhang (2023) was also not considered since the analysis did not consider co-occurring PFAS and the resulting health outcome-specific RfD would be less protective.

The RfD for anti-diphtheria responses in 7-year-old Faroese children from Budtz-Jørgensen and Grandjean (2018) was ultimately selected as the basis for the health outcome-specific RfD for immune effects because the POD_{HED} were based on models with adequate quality of fit and

significant regression parameters, the analysis considered co-exposures of other PFAS and indicated minimal potential for confounding in the value of the POD_{HED} due to PFOA, and the response was more consistently observed across the two time points reported in the study between the two vaccine-specific responses reported by Budtz-Jørgensen and Grandjean (2018). 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 selected for candidate RfD derivation for the endpoint of increased total cholesterol (Dong et al., 2019; Steenland et al., 2009). These candidate studies offer a variety of PFOS exposure measures across various populations. Dong et al. (2019) investigated the NHANES population (2003–2014), while Steenland et al. (2009) 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 candidate RfD for increased TC from Dong et al. (2019) 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) 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) resulting in 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) is 1×10^{-7} mg/kg/day (Figure 4-4). Note that the candidate RfDs for the cardiovascular outcome were the same.

4.1.6.1.4 Developmental Effects

Three *high* confidence epidemiological studies were considered for candidate RfD derivation for the endpoint of decreased birth weight (Wikström et al., 2020; Sagiv et al., 2018; Darrow et al., 2013). These candidate studies assessed maternal PFOS serum concentrations before birth (Darrow et al., 2013) or primarily in the first trimester (Wikström et al., 2020; Sagiv et al., 2018) minimizing concerns for bias due to pregnancy-related hemodynamic effects. All three studies were *high* confidence prospective cohort studies with many strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias. Between these three studies, PFOS exposure concentrations observed in Wikström et al. (2020) are more comparable to current exposure levels in the United States and therefore may be more relevant to the general population than the candidate RfD derived from Sagiv et al. (2018) or Darrow et al., (2013). Additionally, the BMDL derived from Wikström et al. (2020) was based on a statistically significant regression parameter. For these reasons, the RfD for decreased birth weight from Wikström et al. (2020) was selected as the basis for the organ-specific RfD for developmental effects. The resulting health outcome-specific RfD is 1×10^{-7} mg/kg/day (Figure 4-4). Note that all three candidate RfDs based on epidemiological studies for the developmental outcome were within one order of magnitude of the selected health outcome-specific RfD.

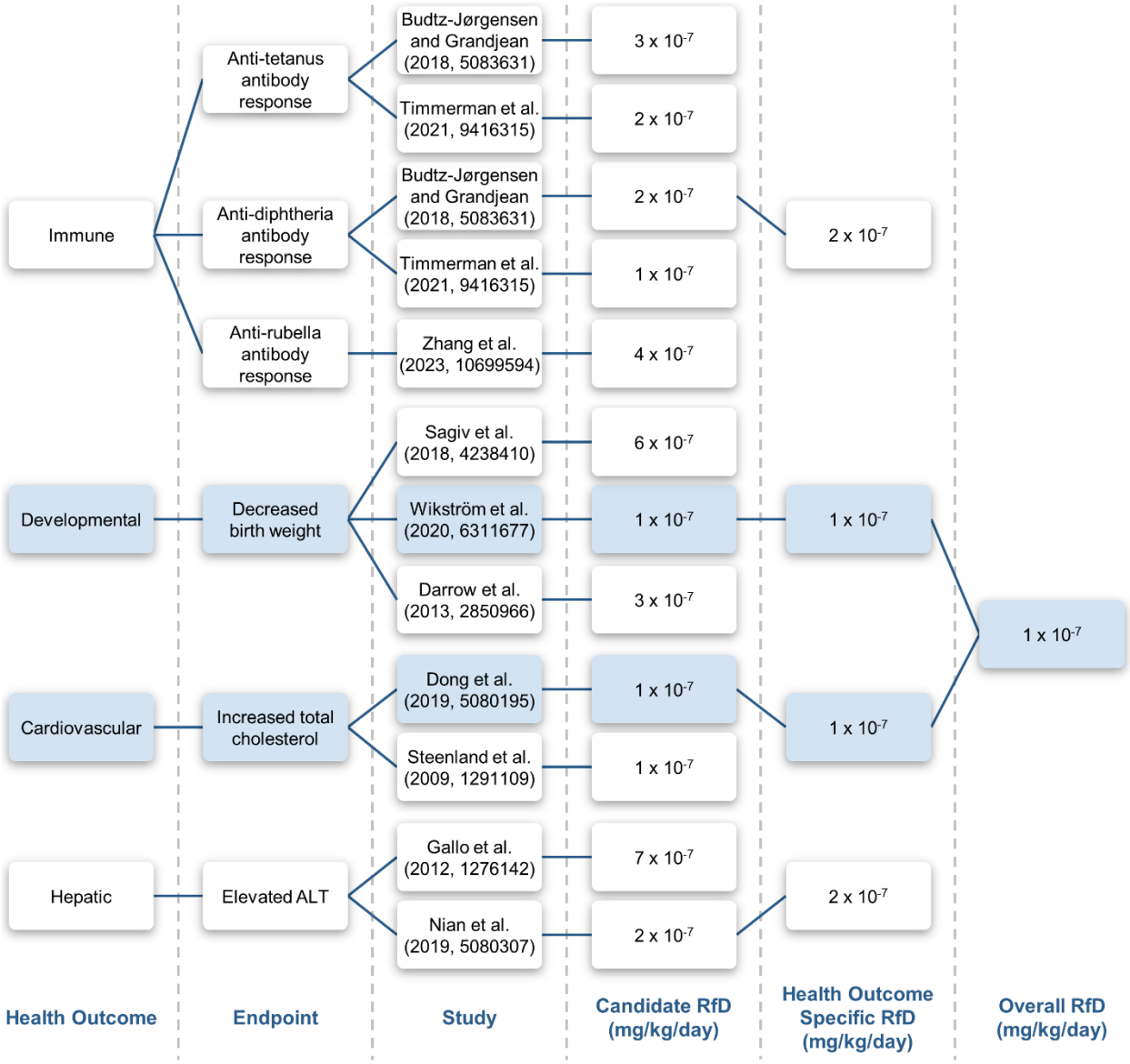


Figure 4-4. Schematic Depicting Selection of the Overall RfD for PFOS

4.1.6.2 Overall Noncancer 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., 1×10^{-7} mg/kg/day). Therefore, EPA has selected an overall RfD for PFOS of 1×10^{-7} mg/kg/day (Figure 4-4). The developmental and cardiovascular RfDs based on endpoints of decreased 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 5.8), 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 *evidence indicates* 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 therefore, the overall RfD is expected to be protective of all other noncancer health effects in humans.

4.2 Cancer

As described in the introduction of Section 3, there is evidence from both epidemiological and animal toxicological studies that oral PFOS exposure may result in adverse health effects across many health outcomes, including cancer (Section 3.5). In Section 3.5.5, EPA concluded that PFOS is *Likely to Be Carcinogenic to Humans* in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Therefore, the quantification of cancer effects was prioritized along with the four noncancer health outcomes that are described in Section 4.1. EPA considered only *high* or *medium* confidence human and animal toxicological studies for CSF derivation.

4.2.1 Study and Endpoint Selection

Human studies selected for CSF derivation reported all necessary analytical information (e.g., exposure distribution or variance) for the outcome of interest (any cancer). If available, *high* and *medium* confidence studies with exposures levels near the range of typical environmental human exposures, especially exposure levels comparable to human exposure in the general population, were preferred over studies reporting considerably higher exposure levels. Exposure levels near the typical range of environmental human exposure can facilitate extrapolation to exposure levels that may be more relevant to the U.S. general population. Additionally, the most recent and comprehensive publication on a single study population was preferred over prior publications on the same or portions of the same population.

Preferred animal toxicological studies consisted of *medium* and *high* confidence studies with chronic exposure durations to capture potential latency of cancer effects. Studies with exposure durations during development (e.g., gestation) were also considered informative for assessing potential early lifestage susceptibility to cancer. Studies encompassing lower dose ranges were also preferred. These types of animal toxicological studies increase the confidence in the CSF relative to other animal toxicological studies because they are based on data with relatively low risk of bias, have sufficient study designs to observe the critical effects, and are associated with less uncertainty related to low-dose and exposure duration extrapolations.

4.2.1.1 Epidemiological Studies

The available epidemiology studies report elevated risk of liver, bladder, kidney, prostate, and breast cancers after chronic PFOS exposure in some studies, though limited evidence for some tumor types (i.e., liver and renal) and mixed results for other tumor types (i.e., bladder, prostate, breast) provide plausible but not definitively causal evidence of a relationship between PFOS

exposure and cancer outcomes from the epidemiological evidence alone. The animal chronic cancer bioassay provides additional support for carcinogenicity with the identification of multi-site tumorigenesis (liver and pancreas) in both male and female rats.

The limited renal or mixed results (breast, bladder, prostate) preclude definitive conclusions about the relationship between PFOS exposure and these cancer outcomes in humans and therefore limits the potential for quantitative assessment of these data. For example, Shearer et al., (2021) is a *medium* confidence study which suggests an association between PFOS and increased kidney cancer. However, it is the only study indicating an association for kidney cancer. Furthermore, the magnitude of the association between PFOS and kidney cancer was lower than that for PFOA and after adjustment for other PFAS, the adjusted OR for the highest quartile was relatively low in magnitude and not statistically significant. For these reasons, Shearer et al., 2021 was not considered for CSF derivation. Additionally, the breast cancer studies provide mixed evidence, with associations between PFOS and breast cancer observed in some studies, but only in specific groups of participants or for certain sub-types of breast cancer. Without plausible evidence for MOAs that inform these responses in specific populations, there is not strong support for quantitative analyses of these studies.

Recently published studies have provided additional evidence of an increased risk of liver cancer with PFOS exposure. Importantly, these data are concordant with the liver tumors observed in the published rodent studies (Butenhoff et al., 2012; Thomford, 2002a), providing cross-stream concordance for liver cancer which strengthens the weight of evidence for this endpoint. Results from publications considered in the 2016 PFOS HESD (U.S. EPA, 2016b), a *low* confidence occupational study (Alexander et al., 2003) and a *medium* confidence general population-based study (Eriksen et al., 2009), investigating associations between liver cancer and PFOS exposure reported non-significant associations, though these studies were considered imprecise (i.e., null results with wide confidence intervals). Recently, statistically significant increased risk of liver cancer has been reported in two additional studies, a *medium* confidence nested case-control study in the U.S. (Goodrich et al., 2022) and a *low* confidence general population study in China (Cao et al., 2022). Given the concordance of tumor site between these studies in humans and the available animal toxicological study, discussed further in Section 4.2.1.2, EPA considered liver cancer reported by Goodrich et al. (2022) for CSF derivation. EPA did not consider Cao et al. (2022) as there were several concerns with this study, including: the potential for selection bias due to lack of information on case recruitment and on source of healthy controls; uncertainties related to outcome assessment due to lack of liver cancer diagnosis detail; and potential for residual confounding because the list of confounders included in PFAS and liver cancer analyses was not provided. These concerns resulted in *low* confidence rating.

Goodrich et al. (2022), is a *medium* confidence study which reported on a small, nested case-control study of adults from the large Multiethnic Cohort (MEC) in California and Hawaii. The study examined incident non-viral hepatocellular carcinoma cases and individually matched controls (Goodrich et al., 2022). EPA identified several factors that also precluded use of Goodrich et al. (2022) from dose-response analyses. First, there was a lack of association observed in continuous analyses of PFOS exposure indicating a lack of dose-response. Thus, the study lacks a precise estimate of the slope needed for POD derivation. Second, the elevated risk in this study was observed only in analyses comparing participants with PFOS concentrations at or above the 85th percentile of PFOS (i.e., 54.9 μg/L). This indicates that only the highest

exposure group demonstrated a response, making 54.9 µg/L PFOS the LOAEL. With only a LOAEL from this dataset, EPA is unable to conduct a low-dose linear extrapolation or derive a CSF. Lastly, the elevated exposure level at which the response was observed in this study is outside the reported PFOS environmental human exposures ranges typical for U.S. and international populations. For example, the mean 90th percentile PFOS serum concentration from the 2017–2018 NHANES cycle was 11.5 µg/L. The small sample size for the study (50 cases and 50 controls) may have limited the study's sensitivity. For these reasons, Goodrich et al. (2022) was not selected for CSF derivation.

4.2.1.2 Animal Toxicological Studies

A single *high* confidence animal chronic cancer bioassay comprises the animal toxicological evidence database for the carcinogenicity of PFOS. This *high* confidence chronic cancer bioassay study, first published as an industry-sponsored report (Thomford, 2002b) and later published as a peer-reviewed journal article (Butenhoff et al., 2012) provides evidence of multisite tumorigenesis in male and female rats.

Hepatocellular tumors were observed in both male and female rats (Butenhoff et al., 2012). In males, there was a statistically significant increase in the incidence of hepatocellular adenomas in the highest dose group tested (20 ppm or approximately 1 mg/kg/day) and a significant trend of increased incidence with increasing PFOS dose. A similar response was observed in females, with the addition of one incidence of hepatocellular carcinoma in a rat from the highest dose group tested (20 ppm or approximately 1.25 mg/kg/day). As these tumors were observed in both sexes with similar sensitivity and since this effect is concordant with the associations between PFOS and liver cancer observed in humans, the endpoints of hepatocellular adenomas in male rats and hepatocellular adenomas or carcinomas in female rats were both selected for candidate CSF derivation.

Increased incidence of pancreatic islet cell tumors were also observed in male rats (Butenhoff et al., 2012). Though there were similar incidences of islet cell adenomas in control and PFOS-treated rats, there was a statistically significant trend of increased incidence of islet cell carcinomas with increasing PFOS dose. EPA additionally selected the incidence of pancreatic islet cell carcinomas in male rats for candidate CSF derivation as this is a malignant tumor and appears to be similar in sensitivity as the hepatocellular tumors observed in male and female rats. EPA also considered incidences of combined islet cell adenomas and carcinomas for quantitative analyses, the modeling for which is presented in Appendix E (U.S. EPA, 2024a) but was not selected for candidate CSF derivation because there was no dose-response relationship observed with the adenomas alone and combining the two tumor types resulted in a slight attenuation of the effect, evidenced by a loss of the statistically significant trend of response.

4.2.2 Candidate CSF Derivation

As described above, EPA did not identify epidemiological studies suitable for CSF derivation. However, EPA derived PODs and candidate CSFs for four endpoints reported by Thomford (2002b)/Butenhoff et al. (2012): 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 3-18, 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. For comparison purposes, EPA presents BMDLs derived using the number of animals in each dose group at the start of the study in Appendix E (U.S. EPA, 2024a). All BMDLs were derived using the BMDS 3.2 program.

Multistage models were used consistent with the longstanding practice of EPA to prefer multistage models to fit tumor dose-response data (U.S. EPA, 2005a) and a BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a). EPA selected the AUC averaged over the study duration (AUC_{avg}), equivalent to the mean serum concentration over the duration of the study, as the dose metric for modeling cancer endpoints. This is consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the IRIS Handbook (U.S. EPA, 2022d), which recommend the cumulative dose received over a lifetime as the measure of exposure to a carcinogen when modeling chronic cancer effects. 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 body weight, 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 Derived From Results Reported by Butenhoff et al. (2012)/Thomford (2002b)[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})	Notes on Modeling
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 $(mg/kg/day)^{-1}$	Model with the lowest AIC was selected as all models had adequate fit and BMDLs were sufficiently close.
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 $(mg/kg/day)^{-1}$	Model with the lowest AIC was selected as all models had adequate fit and BMDLs were sufficiently close.
Combined Hepatocellular Adenomas and Carcinomas	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 $(mg/kg/day)^{-1}$	Model with the lowest AIC was selected as all models had adequate fit and BMDLs were sufficiently close.
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 $(mg/kg/day)^{-1}$	Model with the lowest AIC was selected as all models had adequate fit and BMDLs were sufficiently close.

Notes: $BMDL_{10}$ = benchmark dose level corresponding to the 95% lower confidence limit of a 10% change.
[a] Butenhoff et al. (2012) and Thomford (2002b) reported data from the same experiment.
[b] See Appendix (U.S. EPA, 2024a) for additional details on benchmark dose modeling.

4.2.3 Overall CSF Selection

EPA selected the hepatocellular adenomas and carcinomas in female rats reported by Butenhoff et al. (2012)/Thomford (2002b) as the basis of the overall CSF for PFOS. This endpoint was selected because: 1) there is concordance between the observed hepatocellular tumors in rats with the liver cancer observed in human epidemiological studies; 2) the derived candidate CSF is representative of both malignant and benign tumors; 3) the endpoint is supported by the observation of hepatocellular adenomas in male rats; 4) there was a statistically significant increase in tumor incidence in the highest dose group; and 5) a statistically significant trend of increased incidence with increasing PFOS concentrations across dose groups. The resulting CSF is 39.5 $(mg/kg/day)^{-1}$.

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 lifestage susceptibility to chemical exposure (U.S. EPA, 2005a, b). 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, 2011a).

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 versus adult-only PFOS exposure.

5 Effects Characterization

5.1 Addressing Uncertainties in the Use of Epidemiological Studies for Quantitative Dose-Response Analyses

In the 2016 *Health Effects Support Document for Perfluorooctane Sulfonate* (PFOS) and Drinking Water Health Advisory (U.S. EPA, 2016a, b), the U.S. Environmental Protection Agency (EPA) qualitatively considered epidemiological studies as a supporting line of evidence but did not quantitatively consider them for point-of-departure (POD) derivation, citing the following as reasons to exclude the epidemiological data that were available at that time from quantitative analyses:

- Unexplained 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),
- 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 Sections3.1.1, 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 studies 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 of PFOS for POD derivation in this assessment.

In this assessment EPA has assessed the strength of epidemiological and animal evidence following current agency best practices for systematic review (U.S. EPA, 2022d), a process that was not followed in 2016. By performing an updated assessment using systematic review methods, EPA determined that four noncancer health outcomes and four epidemiological endpoints within these outcomes (i.e., decreased antibody response to vaccination in children, decreased birthweight, increased total cholesterol, and increased alanine aminotransferase (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 *high* and/or *medium* confidence epidemiological studies with low risk of bias in different populations, including general and highly exposed populations. Many of these supporting studies have been published since 2016 and have strengthened the weight of evidence for this assessment.

As described in Section 4.1.1.34.1.3, EPA has improved upon the pharmacokinetic modeling approach used in 2016. Though there are challenges in estimations of human dosimetry from

measured or modeled serum concentrations (see Section 5.6.2), 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 uncertainty factor (UF) accounting for intraspecies variation of 10× 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 of chronic, nondevelopmental effects, is the documented decline 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 Appendix E, (U.S. EPA, 2024a)), 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 United States that occurred in the 1990's (Dong et al., 2019; Nian et al., 2019; Gallo et al., 2012; Steenland et al., 2009). The modeling results show that the benchmark dose lower confidence limit (BMDL) estimates for increased TC derived using the longest range of exposure data (1999-2018) 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. Given 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 consistently selected 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 Appendix E, (U.S. EPA, 2024a)).

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, mixture analysis remains an area of emerging research (Taylor et al., 2016), and there is no scientific consensus yet for the best approach to account for exposure by co-occurring PFAS. Additionally, multipollutant analyses from studies included in this assessment did not provide direct evidence that associations between exposure to PFOS and health effects are confounded by or are fully attributable to confounding by co-occurring PFAS. A detailed discussion of statical approaches for accounting for co-occurring PFAS and results from studies performing multipollutant analysis is provided in Section 5.1.1. 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, 2020b).

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 et al., 2022; D'eon and Mabury, 2011; Vestergren et al., 2008). 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. (Park et al., 2019c; Kato et al., 2014; Nelson et al., 2012; Calafat et al., 2007). EPA acknowledges that in observational epidemiological studies, potential residual confounding may result from complexities related to SES and racial/ethnic disparities. Additional racially and ethnically diverse studies in multiple U.S. communities are needed to fill this important data gap. Appendix D (U.S. EPA, 2024a) 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, the four selected critical effects (i.e., decreased antibody response to vaccination, increased serum ALT, increased TC, and decreased birthweight) are biologically significant effects and/or precursors to disease (e.g., CVD), which, according to agency guidance and methods, both warrant consideration as the basis of RfDs for PFOA (U.S. EPA, 2022d, 2005a, 2002b). EPA's *A Review of the Reference Dose and Reference Concentration Processes*, states that a reference dose (RfD) should be based on an adverse effect or a precursor to an adverse effect (e.g., increased risk of an adverse effect occurring) (U.S. EPA, 2002b). Also, at the individual level, the interpretation and impact of small magnitude changes in endpoints such as increased TC, increased ALT, decreased birth weight, and decreased antibody response to vaccination may be less clear. However, at the population level, even small magnitude changes in these effects will shift the distribution in the overall population and increase the number of individuals at risk for diseases, such as cardiovascular disease and liver disease(Gilbert and Weiss, 2006).

There are challenges associated with quantitative use of epidemiological data for risk assessment (Deener et al., 2018) 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.

5.1.1 Uncertainty Due to Potential Confounding by Co-Occurring PFAS

5.1.1.1 PFAS Co-Exposure Statistical Approaches and Confounding Analysis

A potential source of uncertainty in epidemiologic studies examining associations between a particular PFAS and health outcomes is confounding by other co-occurring PFAS. In studies of PFOS, such confounding may occur if there are other PFAS that are moderately or highly correlated with PFOS, associated with the outcome of interest, and not on the causal pathway between PFOS and the outcome. If the association between co-occurring PFAS and the outcome is in the same direction as the association between PFOS and that outcome, the anticipated direction of bias resulting from not accounting for other PFAS would be away from the null. For an extended review of the uncertainties associated with PFAS co-exposures, see the *Systematic Review Protocol for the PFBA, PFHxA, PFHxS, PFNA, and PFDA (anionic and acid forms) IRIS Assessments* (U.S. EPA, 2020b).

Several statistical methods are currently used to estimate associations while accounting for potential confounding by co-occurring PFAS and other pollutants. One common approach is to include co-occurring PFAS as covariates in regression models. This approach allows for an estimation of the association between PFOS and the outcome of interest, adjusted for other covariates and the copollutants. Another approach is to screen large groups of exposures to identify which ones are most strongly related to the outcome, using methods such as principal components analysis, elastic net regression, and Bayesian kernel machine regression (BKMR). Each of these approaches has strengths and limitations. For example, when PFOS and the copollutants are highly correlated, then multipollutant models could be affected by multicollinearity or result in amplification bias, rather than reduce confounding bias compared with single-pollutant models (Weisskopf et al., 2018). Additionally, accounting for a variable in a multivariable regression model that is not a significant predictor of the response variable reduces the degrees of freedom and effectively dilutes the significance of the other exposure variables that are predictors of the response. The use of screening approaches, while effective at accounting for copollutants, can result in estimates that are not easily interpretable and make it difficult to differentiate the impact and contribution of individual PFAS (Meng et al., 2018). Mixture analysis is an emerging research area (Liu et al., 2022; Taylor et al., 2016), and there is no scientific consensus yet on the best approach for estimating independent effects of PFOS within complex PFAS mixtures.

In this assessment, the risk of bias due to confounding by co-occurring PFAS was considered as part of the study quality evaluation process. To further support the assessment, Section 5.1.1.2 below summarizes evidence from *high* and *medium* confidence studies that included at least one of the approaches described above (hereafter referred to collectively as "multipollutant models") to account for copollutants, in order to assesses the extent to which there may be confounding by other PFAS in studies reporting the associations between PFOS and birth weight.

5.1.1.2 Multipollutant Models of PFOS and Birth Weight

When assessing the associations between PFOS and a health effect of interest (e.g., decreased birth weight), there is concern for potential confounding by other PFAS when there is a strong correlation between the occurrence of PFOS and another PFAS and when the magnitude of the association between the co-exposure and the health effect is large.

To identify co-occurring PFAS with potential for confounding, Table 5-1 shows correlations between PFOS and other PFAS exposures in nine studies evaluating the association between exposure to PFOS and birth weight, each of which included mutually adjusted models. Four of these studies are *medium* confidence (Meng et al., 2018; Woods et al., 2017; Lenters et al., 2016; Robledo et al., 2015) and five are *high* confidence studies (Luo et al., 2021; Shoaff et al., 2018; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017a; Starling et al., 2017). Moderately positive correlations (~0.6) between PFOS and PFOA were consistently observed in six of the seven studies that reported such information. Correlations between PFOS and other commonly examined PFAS, including PFNA (four studies), PFDA (four studies), and PFHxS (five studies), were less consistent than correlations with PFOA, ranging from weak (i.e., 0.0–0.3) to strong (i.e., 0.7–1.0). These results suggest that other PFAS may not consistently co-occur with PFOS.

Table 5-1. Correlation Coefficients Between PFOS and Other PFAS in Mutually Adjusted Studies

Reference	Study Setting	Correlations with PFOS			
		PFOA	PFNA	PFDA	PFHxS
Ashley-Martin et al. (2017)[a] *High*	Canada (10 cities)	0.59	–[b]	–	0.55
Luo et al. (2021)[a] *High*	Guangzhou, China	0.11	0.63	0.68	0.01
Manzano-Salgado et al. (2017a)[c] *High*	Gipuzkoa, Sabadell, and Valencia, Spain	NR	NR	NR	NR
Shoaff et al. (2018)[d] *High*	Cincinnati, Ohio, USA	0.60	–	–	–
Starling et al. (2017)[e] *High*	Colorado, USA	0.68	0.62	0.49	0.65
Lenters et al. (2016)[e] *Medium*	Greenland; Kharkiv, Ukraine; Warsaw, Poland	0.61	0.42	0.78	0.34
Meng et al. (2018)[d] *Medium*	Denmark	0.66	0.48	0.48	0.30
Robledo et al. (2015)[c] *Medium*	Michigan and Texas, USA	NR	NR	NR	NR
Woods et al. (2017)[f] *Medium*	Cincinnati, Ohio, USA	+[g]	+	+	+

Notes: NR = not reported.
Shaded cells indicate analytes for which a correlation with PFOA was not measured or reported.
[a] Pearson correlation of log10-transformed (Ashley-Martin et al., 2017) and ln-transformed (Luo et al., 2021) PFAS values.
[b] Analyte not measured.
[c] Correlation coefficients not reported.
[d] Pearson correlation of PFAS values, unclear if transformed prior to correlation analysis.
[e] Spearman rank correlation of PFAS values.
[f] Correlation coefficient type not specified.
[g] Correlations not reported numerically. Heat map of correlation coefficients (Figure S2, in Woods et al. (2017)) shows positive correlations between PFOS and PFOA, PFNA, PFHxS, and PFDA, ranging from about 0.6 to about 0.1, respectively.

Results from mutually adjusted models are summarized and compared in Table 5-2. The statistical approaches for accounting for PFAS co-exposures varied across the studies. Six studies included at least one additional PFAS as a predictor in ordinary least squares (OLS) regression models (Meng et al., 2018; Shoaff et al., 2018; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017a; Starling et al., 2017; Robledo et al., 2015). Woods et al. (Woods et al., 2017) included multiple PFAS as predictors in a Bayesian hierarchical linear model. Three studies (Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016) used elastic net regression, and one study used BKMR (Luo et al., 2021). The impact of other PFAS adjustment on the association between PFOS and birth weight is evaluated by comparing the magnitude and direction of the effects from the single-PFOS model (when available) to those from mutually adjusted models.

Six studies provided results from both single and multipollutant models (Luo et al., 2021; Meng et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Lenters et al., 2016). Multipollutant models in these six studies included PFOA but varied with respect to other PFAS considered (Table 5-2). Lenters et al. (2016) also adjusted for other types of chemicals (such as phthalates and organochlorides) in addition to several PFAS. Generally, the direction of effect estimates remained the same following adjustment for other PFAS, but precision was reduced. None of the studies that showed birth weight deficits in single-pollutant models reported greater magnitude or more precision of the association following statistical adjustment for other PFAS.

Three studies reported large inverse associations (range: −45 to −83 g) between PFOS and mean birth weight in single-pollutant (i.e., PFOS only) models (Luo et al., 2021; Meng et al., 2018; Lenters et al., 2016). In Luo et al. (2021), the association remained statistically significant in a BKMR model that included 11 other PFAS. In Meng et al. (2018), the association was slightly attenuated (from −45 to −38 g) and no longer statistically significant following adjustment for PFOA. Lenters et al. (2016) observed a nonsignificant inverse association between PFOS and reduced birth weight in single-pollutant models, but PFOS was not selected for inclusion in an elastic net regression model that included other pollutants. Manzano-Salgado et al. (2017a), Shoaff et al. (2018), and Starling (2017) reported null results in single and in multi-PFAS regression models. Additionally, Starling (2017) reported that PFOS was not selected for inclusion in an elastic net regression model. Although found in the minority of studies, the large inverse associations (range: −38 to −109 g) from two multipollutant OLS studies were comparable in magnitude to the single-pollutant models.

Three studies provided results only from multipollutant models (Ashley-Martin et al., 2017; Woods et al., 2017; Robledo et al., 2015), thus making assessment of the impact of copollutants difficult. None of these studies reported statistically significant associations between PFOS and birth weight, and PFOS was not selected for the elastic net regression model in Woods et al. (2017), which reported on the same cohort as Shoaff et al. (2018), that included other endocrine-disrupting chemicals in addition to PFAS.

In summary, in the six studies that included both single and multipollutant models, associations were attenuated to various degrees while others were strengthened following adjustment for other PFAS (Luo et al., 2021; Meng et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017a; Starling et al., 2017; Lenters et al., 2016).Three additional studies presented results from multipollutant models only, making it difficult to determine the extent to which confounding by

other PFAS may have impacted the PFOS-birth weight associations (Ashley-Martin et al., 2017; Woods et al., 2017; Robledo et al., 2015).

Considering all nine studies (8 different cohorts) together, it is challenging to draw conclusions about the extent of confounding by co-occurring PFAS, particularly given differences in modeling approaches, PFAS considered in the adjustment, and exposure contrasts used across studies. Additionally, these studies represented only a small fraction of the total number of studies examining associations between PFOS and birth weight and it is unclear whether their results are generalizable to the broader evidence base. Although it is an important source of uncertainty, there is no evidence in the entirety of the large evidence base that the observed associations between PFOS and birth weight deficits are fully attributable to confounding by co-occurring PFAS.

Similar conclusions can be drawn for other health outcomes. Budtz-Jorgensen (2018) evaluated the possibility of confounding across PFAS in analyses of decreased antibody response. The study reported significant decreases in the antibody response with elevated PFOS exposure, and there was no notable attenuation of the observed effects after estimates were adjusted for PFOA (see Section 3.4.2.1.1.1) (Budtz-Jørgensen and Grandjean, 2018). A limited number of studies performed co-exposure analyses for increased ALT and increased TC in adults. Lin et al. (2010) performed multipollutant modeling for the effects on serum ALT, but multipollutant modeling results for the association between PFOS exposure and ALT was not reported. Fan et al. (2020) examined cross-sectional associations between exposure to PFOS and increased TC in single- and multipollutant models in a sample of adults from NHANES (2012–2014). Exposure to PFOS was associated with significantly elevated TC in the single-pollutant model, but the association was no longer significant in multipollutant analyses. A significantly positive association was also observed for PFAS mixture and TC in WQS regression analyses (Fan et al., 2020).

Overall, there is no evidence that the consistently observed associations between exposures to PFOS and the four priority noncancer health outcomes are confounded or are fully attributable to confounding by co-occurring PFAS.

Table 5-2. Impact of Co-Exposure Adjustment on Estimated Change in Mean Birth Weight (Grams) per Unit Change (ng/mL) in PFOS Levels.

Reference	Single PFAS Model Result (95% CI)[a,b]	Multi-PFAS Model Result (95% CI)[a,b]	Elastic Net Regression Result[b]	Exposure Comparison	Effect of PFAS Adjustment on PFOA Birth Weight Results	PFAS Adjustments
Ashley-Martin et al. (2017) *High*	NR	Girls: 94.31 (−76.30, 264.92) Boys: −11.15 (−174.26, 151.95)	–[c]	log_{10}-unit (ng/mL) increase	–	PFOA, PFHxS
Luo et al. (2021) *High*	−93.34 (−157.92, −28.75)	−109 (−215, −4)[d]	–	Single PFAS model: ln-unit (ng/mL) increase Multi-PFAS model: 75th vs. 25th percentile	Results not directly comparable due to different exposure comparisons, but both models showed large inverse associations	PFOA, PFBA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS, 6:2 Cl-PFESA, 8:2 Cl-PFESA
Manzano-Salgado et al. (2017a) *High*	0.44 (−32.48, 33.36)	18.64 (−26.08, 63.36)	–	log_2-unit (ng/mL) increase	Strengthened (increased birth weight)	PFOA, PFNA, PFHxS
Shoaff et al. (2018) *High*	−0.06 (−0.16, 0.04)[e]	−0.06 (−0.26, 0.15)[e]	–	log_2-unit (ng/mL) increase	No change	PFOA, PFNA, PFHxS
Starling et al. (2017) *High*	−13.8 (−53.8, 26.3)	29.09 (−32.56, 90.75)	N/S	ln-unit (ng/mL) increase	Attenuated/changed direction	PFOA, PFNA, PFDA, PFHxS
Lenters et al. (2016) *Medium*	−68.84 (−152.90, 15.22)	–	N/S	2 SD ln-unit (ng/mL) increase	Attenuated	PFOA, PFNA, PFDA, PFHxS, PFHpA, PFUnDA, PFDoDA
Meng et al. (2018)[f] *Medium*	−45.2 (−76.8, −13.6)	−38.11 (−82.09, 5.88)	–	log_2-unit (ng/mL) increase	Slightly Attenuated	PFOA
Robledo et al. (2015)[g] *Medium*	NR	Girls: 14.16 (−81.83, 110.15) Boys: 37.51 (−73.45, 148.46)	–	1 SD ln-unit (ng/mL) increase	–	PFOA, PFDA, PFNA, PFOSA, Et-PFOSA-AcOH, Me-PFOSA-AcOH
Woods et al. (2017) *Medium*	NR	−9 (−53, 35)[h]	N/S	log_{10}-unit (ng/mL) increase	–	PFOA, PFNA, PFDA, PFHxS

Notes: NR = not reported; N/S = not sufficient.

5.2 Comparisons Between Toxicity Values Derived from Animal Toxicological Studies and Epidemiological Studies

As recommended by the SAB (U.S. EPA, 2022e), 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 4-4), 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). 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 with humans. To address this potential source of uncertainty, EPA utilized a pharmacokinetic (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 with 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 nonmonotonic 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. One example of this is the PPARα response. It is unclear to what extent PPARα influences the responses to PFOS exposure observed in humans, though the rodent PPARα response 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.

5.3 Updated Approach to Animal Toxicological RfD Derivation Compared with 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 Health Effects Support Document (HESD) (U.S. EPA, 2016b) 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 with the 2016 PFOS 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 no-observed-adverse-effect level/lowest-observed-adverse-effect level (NOAEL/LOAEL) approach for all candidate studies and endpoints (U.S. EPA, 2016b). 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 relationship 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, 2022d) 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) 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, 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 a less specific 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).

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 5-3.

Table 5-3. 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 2024 for Candidate RfD Derivation
Immune	
NA	Zhong et al. (2016), *medium* confidence – decreased pup PFC response to SRBC NTP (2019), *high* confidence – extramedullary hematopoiesis in the spleen
Developmental	
Luebker et al. (2005b) *medium* confidence – decreased pup body weight Luebker et al. (2005a), *medium* confidence – decreased pup survival Lau et al. (2003), *medium* confidence – decreased pup survival	Luebker et al. (2005b), *medium* confidence – decreased pup body weight
Hepatic	
Seacat et al. (2003), *medium* confidence – increased ALT (and increased BUN)	Butenhoff et al. (2012)/Thomford (2002b), *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 noncancer 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 PFOS HESD or the current assessment.
[b] Candidate RfDs from the 2016 PFOS HESD that correspond to nonprioritized health outcomes (e.g., nervous) are not presented here.

5.4 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, 2021b). 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, 2016b), which was, in part, because no new animal toxicological studies had been published since publication of the 2016 PFOS HESD and the new epidemiological literature published up until 2021 continued 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" for PFOS and requested that the agency provide an "explicit description of why the available data for PFOS do not meet the EPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005) criterion for the higher designation as 'likely carcinogenic'" (U.S. EPA, 2022e). 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) and Li et al. (2022)), mechanistic data (e.g., Benninghoff et al. (2012)), and conclusions about animal toxicological data in rats (i.e., Butenhoff et al. (2012)). 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, the agency has 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, 2005a) and meet the descriptions for *Likely to Be Carcinogenic to Humans,* as described in Section 3.5.5. This conclusion was based on four independent factors. First, EPA considered the SAB's request that EPA "reevaluate the 2012 Butenhoff study" (U.S. EPA, 2022e). After reviewing the available data, as described in Sections 3.5.2, 3.5.5, and below in this subsection, EPA subsequently agreed with the SAB that the agency's prior "interpretation of the hepatocellular carcinoma data from the Butenhoff (2012b) study in the 2016 PFOS HESD is overly conservative in dismissing the appearance of a dose-response relationship for this endpoint, particularly in females" (U.S. EPA, 2022e). Second, as requested by the SAB, and following agency methodology (U.S. EPA, 2022d), EPA incorporated syntheses of mechanistic literature, which served as the basis of EPA's conclusions that multiple, potentially human-relevant MOAs may contribute to the hepatocellular tumors reported in PFOS toxicological studies of rats (see Section 3.5.4.2). This conclusion aligned with the SAB's comments that "multiple MOAs may be operative" in the reported hepatocellular tumorigenesis and that "the rodent liver tumors caused by PFOS do not appear to be PPAR−α

dependent," (U.S. EPA, 2022e). Third, EPA considered the SAB's comment that there were inconsistencies between EPA's draft conclusions and "the California EPA conclusions based on the same human, animal, and mechanistic evidence presented in the EPA PFOS document," leading the EPA to re-review the CalEPA's *draft Public Health Goals for PFOA and PFOS* technical document (CalEPA, 2021) and identify data indicating the occurrence of tumorigenesis in a second tumor site in male rats (i.e., pancreatic islet cell tumors) (U.S. EPA, 2022e). Fourth, EPA identified new supporting epidemiological literature resulting from the SAB's recommendation that EPA update the literature search prior to finalization of the toxicity assessments for PFOA and PFOS (U.S. EPA, 2022e). This new epidemiological literature included two studies reporting increased risk of hepatocellular carcinoma associated with increased PFOS exposure in humans (Cao et al., 2022; Goodrich et al., 2022), which provided concordant evidence between one of the tumor types and sites observed in the available animal toxicological study. This concordance further supports the potential human relevance of the hepatocellular tumors observed in animal toxicological studies of PFOS.

More specifically, the examples for which the PFOS database exceeds the *Suggestive Evidence* 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, 2005a).

The strongest evidence for the carcinogenicity of PFOS is from one chronic animal bioassay which presents findings surpassing several of these criteria (Butenhoff et al., 2012; Thomford, 2002b). The Thomford/Butenhoff et al. (Butenhoff et al., 2012; 2002b) 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. As described in Section 3.5.4.2, EPA determined that these tumor types are potentially relevant to humans.

In the initial draft of this toxicity assessment published for SAB review (i.e., the *Proposed Approaches* document) (U.S. EPA, 2021b), as well as the 2016 PFOS HESD (U.S. EPA, 2016b), EPA relied upon the tumor incidences provided in Butenhoff et al. (2012), which is the peer-reviewed manuscript of an industry report – Thomford (2002b). Upon further review of the results presented in the Thomford (2002b) report prior to finalization of this assessment, the agency identified two factors that limited previous qualitative and quantitative interpretations of the data: 1) the Butenhoff et al. (2012) study reported combined incidences of neoplastic lesions in the control and high-dose groups (males and females) 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) study did not report incidences for pancreatic islet cell neoplasms. The first factor resulted in statistical dilution 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 conducted a re-analysis that 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. As a result of this reanalysis, EPA agreed with the SAB that the original analysis was "overly conservative in dismissing the appearance of a dose-response relationship for this endpoint, particularly in females" (U.S. EPA, 2022e).

The second factor prevented EPA from previously identifying the statistically significant trend in a second tumor site/type (pancreatic islet cell carcinomas) observed in the chronic cancer bioassay. As a result of identifying the second tumor site/type and updating the conclusions regarding hepatocellular tumors in females, the EPA concluded that PFOS met 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" (emphasis added) (U.S. EPA, 2005a).

Overall, the Thomford/Butenhoff et al. (2012; 2002b) report, along with plausible associations between PFOS exposure and carcinogenicity reported in epidemiological studies, particularly for hepatocellular carcinoma, provide 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). See Table 5-4 below for specific details on how PFOS exceeds the examples supporting the *Suggestive Evidence of Carcinogenic Potential* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

After reviewing the examples of the descriptor *Carcinogenic to Humans*, EPA has determined that at this time, the evidence supporting the carcinogenicity of PFOS does not warrant a descriptor exceeding *Likely to Be Carcinogenic to Humans*. The *Guidelines* indicate that a chemical agent can be deemed *Carcinogenic to Humans* if it meets <u>all</u> the following conditions:

- "there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association, and
- there is extensive evidence of carcinogenicity in animals, and
- the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and
- there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information" (U.S. EPA, 2005a).

As discussed in Section 3.5.5, convincing epidemiological evidence supporting a causal association between human exposure to PFOS and cancer is currently lacking. Additionally, though the available evidence indicates that there are positive associations between PFOS and multiple cancer types, there is uncertainty regarding the identification of carcinogenic modes of

action (MOAs) and associated key precursor events for PFOS in animals. See Table 5-4 below for specific details on how PFOS does not align with the examples supporting the *Carcinogenic to Humans* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Table 5-4. Comparison of the PFOS Carcinogenicity Database with Cancer Descriptors as Outlined in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)

Comparison of Evidence for Suggestive and Carcinogenic Cancer Descriptors	
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" (U.S. EPA, 2005a)	**PFOS data exceed this description**. Observed statistically significant increases in hepatic tumors in rats (adenomas in males and adenomas and carcinomas in females) at the high dose and a statistically significant trend overall in both sexes. Concordant evidence of increased risk of hepatocellular carcinoma from two human epidemiological studies. Observed statistically significant trend of increased incidence of pancreatic islet cell tumors in male rats.
"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." (U.S. EPA, 2005a)	**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)." (U.S. EPA, 2005a)	**PFOS data exceed this description**. The animal 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." (U.S. EPA, 2005a)	**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 carcinomas with increasing dose.
Carcinogenic to Humans	
This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.	**PFOS data are not consistent with this description.** There is evidence of a plausible association between PFOS exposure and cancer in humans, however, the database is limited, there is uncertainty regarding the potential confounding of other PFAS, and there is limited mechanistic information that could contribute to the determination of a causal relationship. The database would benefit from large *high* confidence cohort studies in independent populations.
Or, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when *all* of the following conditions are met:	
There is strong evidence of an association between human exposure and either cancer or the key precursor	**PFOS data are not consistent with this description.** There is evidence of an association between human exposure and cancer, however, there is limited mechanistic

Comparison of Evidence for Suggestive and Carcinogenic Cancer Descriptors	
events of the agent's MOA but not enough for a causal association.	information that could contribute to the determination of a causal relationship.
There is extensive evidence of carcinogenicity in animals.	**PFOS data are not consistent with this description.** Only one chronic cancer bioassay is available for PFOS. The database would benefit from *high* confidence chronic studies in other species and/or strains.
The mode(s) of carcinogenic action and associated key precursor events have been identified in animals.	**PFOS data are not consistent with this description.** A definitive MOA has not been identified for each of the PFOS-induced tumor types identified in rats.
There is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information.	**PFOS data are not consistent with this description.** The animal database does not provide significant clarity on the MOA(s) of PFOS in animals.

Notes: MOA = mode of action.

5.5 Health Outcomes with Evidence Integration Judgments of *Evidence Suggests* Bordering on *Evidence Indicates*

EPA evaluated 16 noncancer health outcomes as part of this assessment. In accordance with recommendations from the SAB (U.S. EPA, 2022e) and the IRIS Handbook (U.S. EPA, 2022d), for both quantitative and qualitative analyses in the final 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* (U.S. EPA, 2021b). However, upon further examination using the protocols for evidence integration outlined in Appendix A (U.S. EPA, 2024a) and Section 2.1.5, EPA concluded that the available epidemiological and animal toxicological evidence did not meet the criteria recommended 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 published since the 2016 PFOS HESD considered for evidence integration for adverse endocrine effects include *high* and *medium* confidence studies, though EPA determined that there was *slight evidence* to suggest human endocrine toxicity, including associations between PFOS exposure and thyroid disease. The available evidence supports the relationship between PFOS exposure and thyroid stimulating hormone (TSH) in children and, to a lesser extent, adults. However, 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 (U.S. EPA, 2024a) 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, 2021d). Additionally, the final IRIS Toxicological Reviews for both PFBA (U.S. EPA, 2022c) and PFHxA (U.S. EPA, 2023) concluded that the available *evidence indicates* that the observed thyroid effects were likely due to PFBA and PFHxA exposure, respectively. Given the similarities across PFAS, these findings support potential associations between PFOS and adverse endocrine effects.

There was also *slight* evidence from epidemiological studies published since the 2016 PFOS HESD 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). Although multiple studies examining associations between PFOS and ADHD were available, only one study reported a significant relationship between PFOS and ADHD (Lenters et al., 2019). There was an indication of a potential relationship between PFOS and autistic behaviors or ASD diagnosis in some studies (Shin et al., 2020; Oulhote et al., 2016; Braun et al., 2014), however 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 (U.S. EPA, 2024a) 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 reviews conducted in February 2022 and 2023. 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.

5.6 Challenges and Uncertainty in Modeling

5.6.1 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) animal internal dosimetry model because it had availability of model parameters across almost all species of interest, agreement with out-of-sample datasets (see Appendix F, (U.S. EPA, 2024a)), 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 Appendix F, (U.S. EPA, 2024a)). 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 Appendix, (U.S. EPA, 2024a)). 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 lifestages with a more detailed description of the life-course modeling in Section 4.1.3.1.3.

The Wambaugh et al. (2013) model fit the selected PFOS developmental study data well, though there are several limitations to using this method to model developmental lifestages. 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 lifestages is a significant improvement over the model used in the 2016 PFOS HESD which did not implement life-course modeling (U.S. EPA, 2016b).

5.6.2 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 et al., 2010b).

In the Verner et al. (2016) 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 with blood in neonates and infants compared with adults (Fernandez et al., 2011). This behavior 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 Appendix B, (U.S. EPA, 2024a)). 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 et al., 2015). 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 with 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. (2010a) 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) 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. (2010a) and did not include additional values derived from human data. This average value shows that the value from Thompson et al. (2010a), which was selected based on the fact that it was derived only from human and nonhuman primate data, is reasonable.

Lastly, the description of breastfeeding in the updated Verner et al. (2016) model relied on a number of assumptions: that infants were exclusively breastfed for 1 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 1 year. This is a relatively long duration of breastfeeding, only 27% of children in the United States are being breastfed at 1 year of age (CDC, 2013). 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 United States are very unlikely to be exclusively breastfed for up to 1 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.

5.6.3 Approach of Estimating a Benchmark Dose from a Regression Coefficient

EPA identified epidemiological studies that reported associations between PFOS exposure and response variables as regression coefficients. Since such a regression coefficient is associated with a change in the biological response variable, it is biologically meaningful and can therefore be used for POD derivation. EPA modeled these regression coefficients using the same approach used to model studies that reported measured response variables. The SAB PFAS Review Panel agreed with this approach, stating, "it would seem straightforward to apply the same methodology to derive the beta-coefficients ("re-expressed," if necessary, in units of per ng/mL) for antibody responses to vaccines and other health-effect-specific endpoints. Such a coefficient could then be used for deriving PODs" (U.S. EPA, 2022e). When modeling regression coefficients that were reported per log-transformed units of exposure, EPA used the SAB's recommended approach and re-expressed the reported β coefficients in units of per ng/mL (see Appendix E, (U.S. EPA, 2024a)). Sensitivity analyses to evaluate the potential impact of re-expression in a hybrid approach when modeling hepatic and serum lipid studies for PFOS showed little impact on BMDLs (see Appendix E, (U.S. EPA, 2024a)).

To evaluate this potential uncertainty in BMDLs derived based on regression coefficients, EPA obtained the measured dose-response data across exposure deciles from Steenland et al. (2009) (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 Appendix E (U.S. EPA, 2024a).

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 threefold difference. The RfD allows for an order of magnitude (10-fold or 1,000%) uncertainty in the estimate. Therefore, EPA is confident in its use of regression coefficients, re-expressed or not, as the basis of POD_{HED}s.

5.7 Human Dosimetry Models: Consideration of Alternate Modeling Approaches

Physiologically based pharmacokinetic (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, for which 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 et al., 2013; Loccisano et al., 2012b, a; Loccisano et al., 2011), an unusual implementation of PFOS plasma binding appeared to introduce a mass balance error. Because of 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 and Lin, 2021). This model used the in vitro extrapolation that was previously developed by Worley et al. (2017b) 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) model.

Because of 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) 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 et al., 2020). 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 (Forsthuber et al., 2020) and active transport (Zhao et al., 2017). 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 two one-compartment approaches identified in the literature for PFOA was the model of Verner et al. (2016, 3299692) and the model developed by the Minnesota Department of Health (MDH model) (Goeden et al., 2019), which was published as a PFOA model, but has been applied to other PFAS, including PFOS (Goeden et al., 2019). 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 between the two models 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 et al., 2012). This decrease occurs due to the relatively rapid increase in body weight during pregnancy (compared with the years preceding pregnancy) and the increase in blood volume that occurs to support fetal growth (Sibai and Frangieh, 1995). 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 body weight. 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 with the adult value. There is no chemical-specific information to suggest that V_d is larger in children compared with adults for PFOS. However, it is generally accepted in pharmaceutical research that hydrophilic chemicals have greater V_d in children (Batchelor and Marriott, 2015), 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 it is 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 Appendix F, (U.S. EPA, 2024a)). 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 root mean squared error compared with the model with constant V_d. Because the model with constant V_d had 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 body weight in young children compared with 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 Appendix F, (U.S. EPA, 2024a)). 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 a maximum contaminant level goal (MCLG) calculated thereafter using lifestage 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 with the model with constant exposure relative to body weight. 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 was a much greater effect on PFOS model results relative to PFOA, but in both cases model performance, as quantified by root mean squared error, was superior with constant exposure compared with exposure based on drinking water consumption. 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 under SDWA. The epidemiological endpoints can be placed into three categories based on the age of the individuals at the time of exposure measurement: 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 based on noncancer effects or for nonlinear carcinogens is an algebraic calculation that incorporates the RfD, RSC, and drinking water intake. The drinking water intake used for this type of MCLG calculation would be chosen based on the target population relevant to the exposure interval used in the critical study and/or timing of exposure measurement and the response variable 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 presented in Appendix F (U.S. EPA, 2024a), EPA determined that the updated Verner model was the most appropriate available model structure for POD_{HED} calculation for PFOS. Specifically, the EPA concluded that the determination that assuming V_d in children equal to the adult values and calculating a RfD assuming a constant dose (mg/kg/day) were appropriate for this assessment.

5.8 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 more severe response than others despite similar PFOS exposure due to increased biological sensitivity, as well as populations exhibiting a more severe response due to higher PFOS exposure and/or exposure to other chemicals or nonchemical stressors. Populations with greater biological sensitivity may include pregnant women and their developing fetuses, children, adolescents, lactating women, the elderly, and people with certain underlying medical conditions (see Section 5.8.1). 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 nonchemical stressors such as communities with environmental justice concerns (see Section 5.8.2).

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.

5.8.1 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 Sections 0 and 3.4.2.1, results of some epidemiological studies indicate an association between PFOS exposure during pregnancy and/or early childhood and adverse outcomes such as decreased birth weight and decreased antibody response to vaccinations. 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 lifestages or exposure windows (e.g., prenatal or early postnatal exposure windows) may be more consequential than others. For example, as described in Appendix C (U.S. EPA, 2024a), Grasty et al. (2005; 2003) 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 mount a relatively low antibody response compared to other children and would therefore represent potentially susceptible populations for PFOS exposure. There are also concerns about declines in vaccination status (Bramer et al., 2020; Smith et al., 2011) 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). As noted by Dietert et al. (2010), the risks of developing infectious diseases may increase if immunosuppression occurs in the developing immune system.

5.8.2 Other Susceptible Populations

As noted in the SAB PFAS review panel's final report (U.S. EPA, 2022e), 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 5.1), 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 Final Per- and Polyfluoroalkyl Substances National Primary Drinking Water Regulation* (U.S. EPA, 2024b)). 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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