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FINAL
Human Health Toxicity Assessment for Perfluorooctanoic Acid (PFOA) and Related Salts

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Acronyms and Abbreviations

3D	Three-dimensional
8-NO2Gua	8-nitroguanine
8-OHdG	8-hydroxydeoxy-guanosine
AASLD	American Association for the Study of Liver Diseases
ABC	ATP Binding Casette
ACG	American College of Gastroenterology
AChE	Acetylcholinesterase
Acot	Acyl-CoA thioesterase
ACOX	Acyl-CoA oxidase
Acsl1	Acyl-CoA synthetase
ADME	Absorption, distribution, metabolism, excretion
AFFF	Aqueous film forming foam
AL	Human-hamster hybrid cells
ALP	Alkaline phosphatase
ALSPAC	Avon Longitudinal Study of Parents and Children
ALT	Alanine aminotransferase
Ap1s1	Adaptor related protein complex 1 subunit sigma 1
APC	Antigen presenting cell
APFO	Ammonium perfluorooctanoate
APOA4	Apolipoprotein A4
apoB	Apolipoprotein B
ApoC-III	Apolipoprotein C-III
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area under the curve
BAFF	B cell activating factor
BBB	Blood brain barrier
Bcl-2	B-cell lymphoma 2
BCRP	Breast cancer resistance protein
BK	Bradykinin
BMD	Benchmark dose
BMD_{10}	Dose corresponding to a 10% change in response
BMDL	Benchmark dose lower limit
$BMDL_{10}$	Dose level corresponding to the 95% lower confidence limit of a 10% change
BMDS	Benchmark Dose Software
BMI	Body mass index
BMR	Benchmark response
BTB	Blood testes barrier
BWT	Birth weight
C3a	Complement 3
C_{last7}	Average concentration over final week of study
CAD	Coronary artery disease
CalEPA	California Environmental Protection Agency
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
$C_{avg,pup,total}$	area under the curve in gestation/lactation added to the area under the curve from diet (post-weaning) divided by two years
CCL	Contaminant Candidate List
CCK	Cholecystokinin
CCK-8	Cell Counting Kit-8
CD	Circular dichronism
CDC	Centers for Disease Control and Prevention
cDNA	complementary DNA
Ces	Carboxylesterases
CETP	Cholesteryl ester transfer protein
C-F	Carbon-fluorine
c-fos	Transcription factor complex
CHD	Coronary heart disease
CHF	Congestive heart failure
CHO	Chinese hamster ovary
CHOP	C/EBP homologous protein
CI	Confidence interval
CIMT	Carotid intima-media thickness test
CL_R	Renal clearance
C_{max}	Maximum blood concentration
$C_{max\_pup\_gest}$	Maximum fetal concentration during gestation
$C_{max\_pup\_lact}$	Maximum fetal concentration during lactation
CNS	Central nervous system
Cpt1a	Carnitine palmitoyltransferase 1a
CS	collagen sandwich
CSF	cancer slope factor
CSM	Cholestyramine
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DCF	2′,7′-dichlorofluorescein
DCF-DA	Dichlorodihydro-fluorescein diacetate
DDE	Dichlorodiphenyl dichloroethane
DMP	3,5-dimethyl pyrazole
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNBC	Danish National Birth Cohort
DNMT	Deoxyribonucleic acid methyltransferases
DNP	Dinitrophenyl
dpf	Days post fertilization
DPP	Diabetes Prevention Program
DPPOS	Diabetes Prevention Program and Outcomes Study
DWI-BW	Body weight-based drinking water intake
E2	Estradiol
eGFR	Estimated glomerular filtration rate
EPA	Environmental Protection Agency
ER	Endoplasmic reticulum
ER-	Estrogen receptor negative
ETC	Electron transport chain
F_1	First generation
F_2	Second generation
Fabp	Fatty acid binding protein
FACS	Fluorescence activated cell sorting
FeNO	Fractional exhaled nitric oxide
FFA	Free fatty acids
FT4	Free thyroxine
FXR	Farnesoid X receptor

GBCA	Genetic and Biomarker Study for Childhood Asthma
GCL	Glutamate-cysteine ligase
GD	Gestation day
GFR	Glomerular filtration rate
GGT	γ-glutamyltransferase
GM	Geometric mean
GO	Gene Ontology
GSH	Glutathione
GSPE	Grape seed proanthocyandidn extract
GSSG	Glutathione disulfide
GST	Glutathione S-transferases
HAT	Histone acetylase
HAWC	Health Assessment Workplace Collaborative
HDAC	Histone deacetylase
HDL	High-density-lipoprotein
HED	Human equivalent dose
HEK-293	Human embryonic kidney
HERO	Health and Environmental Research Online
HESD	Health Effects Support Document
HFC	7-hydroxytrifluoro-methylcoumarin
HFD	High-fat diet
HFMD	Hand, foot, and mouth disease
HFPO	Hexafluoropropylene oxide
Hib	*Haemophilus influenza* type b
HK	High-molecular-weight kininogen
hL-FABP	Human liver fatty acid binding protein
HMOX	Heme oxygenase
HNF	Hepatocyte nuclear factor
HOME	Health Outcomes and Measures of the Environment
HPA	Hypothalamic-pituitary-adrenal
HR	Hazard ratio
HRL	Health reference level
HSA	Human serum albumin
IARC	International Agency for Research on Cancer
IDL	Intermediate-density lipoprotein
IFN	Interferon
Ig	Immunoglobulin
IGF-1	Insulin-like growth factor 1
IHD	Ischemic heart diseases
IHIC	Hepatic immune cell
IL	Inflammatory cytokine
INMA	Spanish Environment and Childhood (Infancia y Medio Ambiente)
IP	Intraperitoneal
IPCS	International Programme on Chemical Safety
IQR	Interquartile range
IRIS	Integrated Risk Information System
IV	Intravenous
k_{12}	Intercompartment transfer rate
k_a	Absorption rate
K_d	Disassociation constant
K_H	Henry's Law Constant
KK	Kallikrein-kinn system
KLH	Keyhole limpet hemocyanin
$K_{mem/w}$	Membrane/water partition coefficients
K_{oc}	Organic carbon-water partitioning coefficient
K_{ow}	Octanol-water partition coefficient
LBW	Low birth weight

LCM	Liver capsular macrophage
LCT	Leydig cell tumors
LD	Lactation day
LDL	Low-density lipoprotein
L-FABP	Liver fatty acid binding protein
LH	Luteinizing hormone
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of detection
Lpl	Lipoprotein lipase
LTRI	Lower respiratory tract infection
LXR	Liver X receptor
LYZ	Lysozyme
M/P	Milk/plasma
MAIT	Mucosal associated invariant T
MCLG	Maximum Contaminant Level Goal
Me-PFOSA-AcOH or MeFOSAA	2-(N-Methyl-perfluorooctane sulfonamido) acetic acid
MDA	Malondialdehyde
MFC	7-methoxy-4-trifluoromethylcoumarin
miRNA or miRs	Microribonucleic acids
MMP	Mitochondrial membrane potential
MMR	Measles, mumps, and rubella
MOA	Mode of action
MOBA	Norwegian Mother, Father, and Child Cohort Study
MRL	Minimum reporting level
mRNA	Messenger ribonucleic acid
MRPs	Multidrug resistance-associated proteins
MS	Multiple sclerosis
MyD	Myeloid differentiation
NAFLD	Non-alcoholic fatty liver disease
NCI	National Cancer Institute
NF- κB	Nuclear factor kappa B
NHANES	National Health and Nutrition Examination Survey
NK	Natural killer
NO	Nitric oxide
NOAEL	No-observed-adverse-effect level
NOD	Nucleotide-binding and oligomerization domain
NOS	Nitric oxide synthase
NP	Niemann-Pick disease
NPDWR	National Primary Drinking Water Regulation
Nrf2	Nuclear factor erythroid 2–related factor 2
NTCP	Sodium-taurocholate cotransporting polypeptide
NTP	National Toxicology Program
OATPs	Organic anion transporting polypeptides
OATs	Organic anion transporters
OCM	Organotypic culture models
OECD	Organisation for Economic Co-operation and Development
OPR	Opioid Receptor
OR	Odds Ratio
ORD	Office of Research and Development
OST	Office of Science and Technology
P_0	Parental generation
p0AL	Mitochondrial deficient cell line
PACT	Pancreatic acinar cell tumors

PAD	Peripheral artery disease
PanIN	Pancreatic intraepithelial neoplasia
PBMC	Peripheral blood mononuclear cells
PBPK	Physiologically-based pharmacokinetic
PC	Partition coefficient
PDCD	Programmed cell death protein
PECO	Populations, Exposures, Comparator, and Outcome
PERK	Protein kinase-like endoplasmic reticulum kinase
PFAA	Perfluoroalkyl acids
PFAS	Per- and polyfluoroalkyl Substances
PFBA	Perfluorobutanoic acid
PFCAs	Perfluoroalkyl carboxylic acids
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane-sulfonate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
PG	Prostaglandin
P_{ion}	Passive anionic permeability
PK	Pharmacokinetic
pKa	Negative base-10 logarithm of acid dissociation constant
PLCO	Prostate, Lung, Colorectal, and Ovarian Screening Trial
P_{milk}	Maternal milk: blood 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
PP2A	Protein phosphatase 2A
PPAR	Peroxisome proliferator activated receptor
PPK	Plasma prekallikrein
ppm	Parts per million
PR-	Progesterone receptor negative
PSA	Prostate-specific antigen
PTB	Preterm birth
PWS	Public water system
PXR	Pregnane X receptor
Q1	Quartile one
Q2	Quartile two
Q3	Quartile three
Q4	Quartile four
QA	Quality assurance
R_0	Baseline risk
r^0_{milk}	Starting milk consumption rate
r^1_{milk}	Week 1 milk consumption rate
r^2_{milk}	Week 2 milk consumption rate
r^3_{milk}	Week 3 milk consumption rate
RARα	Retinoic acid receptor α
RASA3	RAS P21 protein Activator 3
RCC	Renal cell carcinoma
RD	Regular diet
RfD	Reference dose
R_{fm}	Fetus:mother concentration ratio
r^i_{milk}	Milk consumption rate for the i^{th} week of lactation

RNA	Ribonucleic acid
RNS	Reaction nitrogen species
ROS	Reactive oxygen species
RR	Rate ratio
RRBS	Reduced representation bisulfite sequencing
RSC	Relative source contribution
SAB	Science Advisory Board
SBP	Systolic blood pressure
SDWA	Safe Drinking Water Act
SES	Socioeconomic status
SGA	Small for gestational age
SIRT	Sirtuin
slco1d	Solute carrier organic anion transporter
SMR	Standardized mortality ratios
SOD	Superoxide dismutase
SRBC	Sheep red blood cells
SREBP	Sterol regulatory element-binding protein
T1D	Type 1 diabetes
T4	Thyroxine
TC	Total cholesterol
TET	Methylcytosine dioxygenases
tfc	Transcription factor
tgf	Transforming growth factor
TLDA	Taqman low density arrays
TLR	Toll-like receptor
T_{max}	Time to C_{max}
TNF	Tumor necrosis factor
TNP	Trinitrophenyl
TReg	Regulatory T cell
TSCATS	Toxic Substance Control Act Test Submissions
TTEs	Transplacental efficiencies
TTR	Transthyretin
TXB	Thromboxane
UCMR3	Third Unregulated Contaminant Monitoring Rule
UF	Uncertainty factors
UF_A	Interspecies UF
UF_D	Database UF
UF_H	Intraspecies UF
UF_L	LOAEL-to-NOAEL extrapolation UF
UF_S	UF for extrapolation from a subchronic to a chronic exposure duration
UF_C	Composite uncertainty factor
µM	Micromolar
UPR	Unfolded protein response
UV-vis	Ultraviolet-visible
V_d	Volume of distribution
vtg1	Vitellogenin 1
VLDL	Very low-density lipoproteins
Vldlr	Very low-density lipoproteins receptor
WHO	World Health Organization
WoS	Web of Science
WTC	World Trade Center
XBP1	Spliced X box-binding protein 1
ZFL	Zebrafish liver cell line

Executive Summary

The U.S. Environmental Protection Agency (EPA) is issuing final toxicity values for *perfluorooctanoic acid (PFOA), including all isomers and nonmetal salts.* The toxicity assessment for PFOA is a scientific report that describes the evaluation of the available animal toxicity and human epidemiology data in order to characterize 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 PFOA 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 PFOA 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 PFOA, 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 Perfluorooctanoic Acid (PFOA)* (hereafter referred to as the 2016 PFOA HESD) (U.S. EPA, 2016c) and is an update of the SAB review draft, *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water (U.S. EPA, 2021c)*, and the subsequent *Public Comment Draft Toxicity Assessment and Proposed Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2022e).

PFOA and its related salts are members 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 PFOA 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 man-made 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.

Under the EPA's PFOA Stewardship Program, the eight major companies of the perfluoropolymer/fluorotelomer industry agreed to voluntarily reduce facility emissions and product content of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals, longer-chain perfluoroalkyl carboxylic acids (PFCAs) by 95% on a global basis by no later than 2010 and to eliminate these substances in products by 2015 (U.S. EPA, 2021a). However, PFOA 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 PFOA. 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 PFOA systematic review protocol (see Appendix A, (U.S. EPA, 2024a)) was developed prior to the initiation of this assessment and 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 PFOA HESD (U.S. EPA, 2016c), 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, 2021c) 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 assessment 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 PFOA 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 1.1 to 5.2 ng/mL and at administered doses in animals as low as 0.3 to 1.0 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 and diphtheria in children, and evidence of immunosuppression and other types of immunotoxicity in studies of adult animals, including decreased IgM response to sheep red blood cells, 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 lipid 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 offspring survival, decreased fetal and pup weight, delayed time to eye opening, and related pre- and postnatal effects 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 PFOA (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 PFOA are available in Section 4.1.

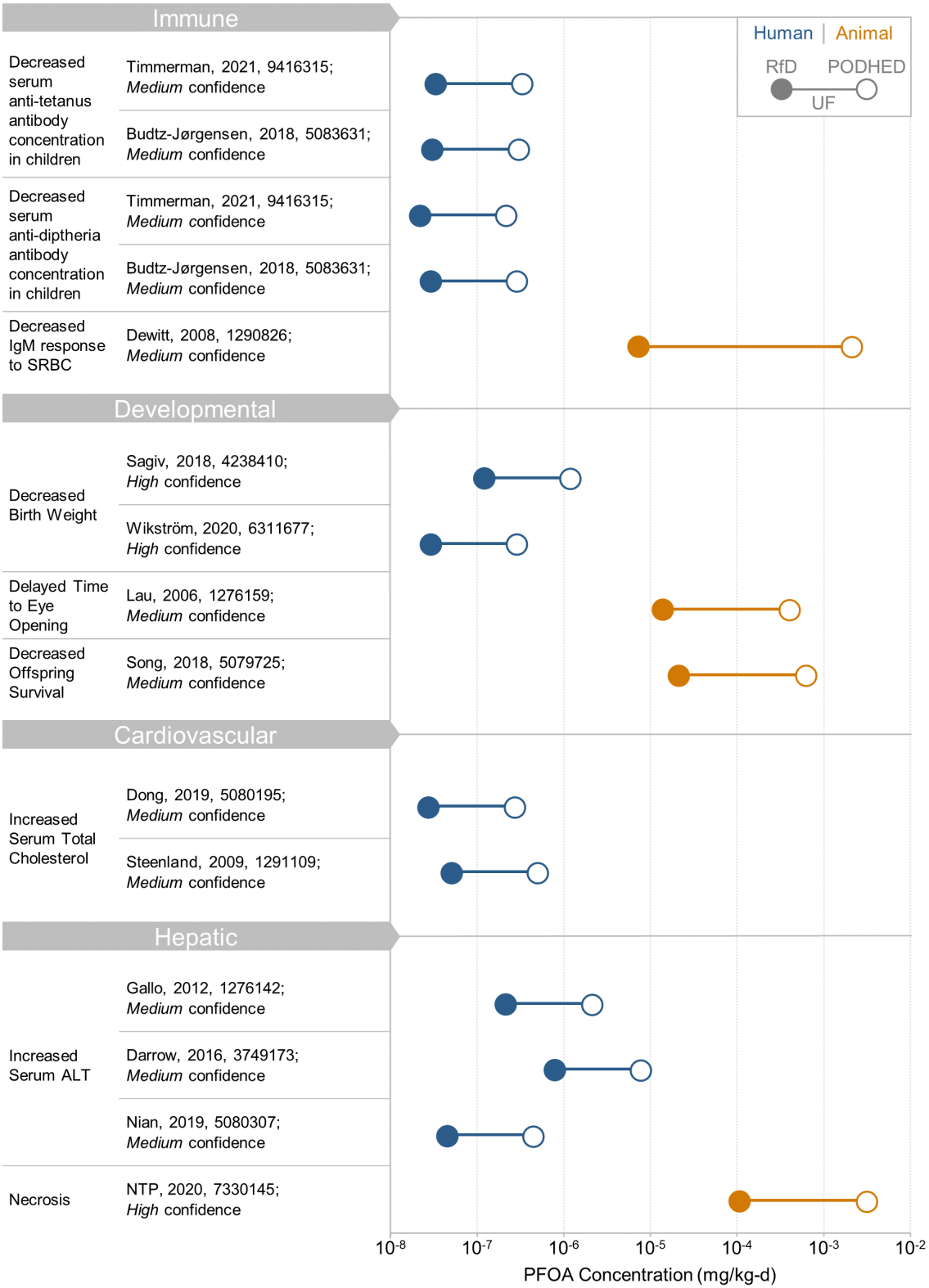


Figure ES-1. Schematic Depicting Candidate RfDs Derived From Epidemiological and Animal Toxicological Studies of PFOA

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

The co-critical effects for the oral RfD of 3 x 10^{-8} mg/kg/day were decreased serum anti-tetanus and anti-diphtheria antibody concentrations in children (Budtz-Jørgensen and Grandjean, 2018), 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 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 PFOA exposure. As two of the co-critical effects identified for PFOA are developmental endpoints and can potentially result from a short-term exposure during critical periods of development, EPA concludes that the overall RfD for PFOA 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 the evidence evaluation across the human epidemiological, animal toxicological, and mechanistic studies and concluded that PFOA is *Likely to Be Carcinogenic to Humans* via the oral route of exposure (see Section 3.5). Epidemiological studies provided evidence of kidney and testicular cancer in humans and some evidence of breast cancer in a study of one susceptible subpopulation. Animal toxicological studies in Sprague-Dawley rats reported Leydig cell tumors (LCT), pancreatic acinar cell tumors (PACT), and hepatocellular tumors after chronic oral exposure. Available mechanistic data suggest that multiple modes of action (MOAs) play a role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in humans and 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 Cancer Slope Factor Derivation

EPA followed agency guidelines for risk assessment in deriving CSFs for PFOA (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). From the studies that met the criteria, EPA derived and considered multiple candidate CSFs from both epidemiological and animal toxicological studies across multiple tissue and organ types (i.e., kidney, liver, pancreas, testes). Candidate CSFs were derived for epidemiological data on renal cell carcinoma (RCC) and kidney cancer using weighted linear regressions to calculate quartile-specific relative kidney cancer risks. Relative risks were then converted to the absolute risk scale, yielding an internal CSF, which represents the excess cancer risk associated with each ng/mL increase in serum PFOA. The internal serum CSF was then divided by the selected clearance value and converted to an external dose CSF. For animal toxicological studies, multistage cancer models were used to predict the doses at which the selected BMR for tumor

incidence would occur. BMDLs for each tumor type (LCTs, hepatocellular adenoma or carcinoma, and pancreatic acinar cell adenoma or adenocarcinoma) served as the PODs, which were then converted to POD_{HED}s by applying the human clearance value. CSFs were then calculated by dividing the selected BMR by the POD_{HED}s for each tumor type.

The oral slope factor of 0.0293 $(ng/kg/day)^{-1}$ for RCC in human males from Shearer et al. (2021) was selected as the basis of the overall CSF for PFOA (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 PFOA, and the available evidence did not report an increased susceptibility to cancer following PFOA exposure during early life. Additional detail on candidate CSF derivation and CSF selection is provided in Table 4-12 and Table 4-13 in Section 4.2.

Final Toxicity Values for PFOA

Table ES-1. Final Toxicity Values for PFOA

Toxicity Value Type	Critical Effect(s)	Study, Confidence	Species, Sex, Age	Toxicity Value[a,b]
Reference Dose	Co-critical effects: decreased serum anti-tetanus and anti-diphtheria antibody concentration in children; decreased birth weight in infants; Increased serum total cholesterol in adults	Budtz-Jørgensen (2018), *Medium*; Wikström et al. (2020), *High*; Dong et al. (2019), *Medium*	Human, male and female, PFOA concentrations at age five years and anti-tetanus antibody serum concentrations at age seven years; human, male and female, PFOA serum concentrations in first and second trimesters; human, male and female, 20–80 years	3×10^{-8} (mg/kg/d)
Cancer Slope Factor	Renal cell carcinoma	Shearer et al. (2021), *Medium*	Human, male and female, 55–74 years	0.0293 (ng/kg/d)−1

Notes:
[a] Reference doses were rounded to one significant figure.
[b] Increase in cancer risk per 1 ng/(kg*d) increase in dose.

1 Background

1.1 Purpose of This Document

The primary purpose of this toxicity assessment for perfluorooctanoic acid (PFOA) is to describe the best available science on the human health effects associated with PFOA exposure and the derivation of toxicity values (i.e., noncancer reference doses (RfDs) and cancer slope factors (CSFs)). The latest health science on PFOA 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 Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2023a), *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021c), and the *Health Effects Support Document for Perfluorooctanoic Acid (PFOA)* (U.S. EPA, 2016c). This final toxicity assessment for PFOA 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 PFOA (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 main 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 PFOA exposure to inform interpretation of findings related to potential health effects in studies of humans and animals, with a focus on five main 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 main 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 PFOA has the potential to be hazardous to humans (Section 3).
- Determine the cancer classification for PFOA 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 PFOA, 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 PFOA, perfluorooctane sulfonic acid (PFOS), 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 5), 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 environmental media (e.g., air, water, ice, sediment) and 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. PFOA belong 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

PFOA is a perfluorinated aliphatic carboxylic acid. It is a fully fluorinated organic synthetic acid that was used in the United States primarily as an aqueous dispersion agent and emulsifier in the manufacture of fluoropolymers and in a variety of water-, oil-, and stain-repellent products (e.g., adhesives, cosmetics, fire-fighting foams, greases and lubricants, paints, polishes) (NLM, 2022b). It can exist in linear- or branched-chain isomeric form. PFOA is a strong acid that is generally present in solution as the perfluorooctanoate anion. Therefore, this assessment applies to all isomers of PFOA, as well as nonmetal salts of PFOA that would be expected to dissociate in aqueous solutions of pH ranging from 4 to 9 (e.g., in the human body).

PFOA is water soluble and mobile in water, with an estimated log organic carbon-water partition coefficient (log K_{oc}) of 2.06 (Zareitalabad et al., 2013). PFOA is stable in environmental media because it is resistant to environmental degradation processes, such as biodegradation, photolysis, and hydrolysis. In water, no natural degradation has been demonstrated, and it dissipates by advection, dispersion, and sorption to particulate matter. PFOA 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 PFOA 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 PFOA are provided in Table 1-1. There is uncertainty in the estimation, measurement, and/or applicability of certain physical/chemical properties of PFOA in drinking water, including the K_{oc} (Nguyen et al., 2020; Li et al., 2018d), 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) and Lange et al. (2006) reported that a value could not be measured because PFOA is expected to form multiple layers in octanol-water mixtures.

For a more detailed discussion of the chemical and physical properties and environmental fate of PFOA, 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

Health Effects Support Document for Perfluorooctanoic Acid (PFOA) (U.S. EPA, 2016c), and the *Draft Aquatic Life Ambient Water Quality Criteria for Perfluorooctanoic Acid (PFOA)* (U.S. EPA, 2022a).

Table 1-1. Chemical and Physical Properties of PFOA

Property	Perfluorooctanoic Acid; Experimental Average	Source
Chemical Abstracts Service Registry Number (CASRN)[a]	335-67-1	NLM (2022a)
Chemical Abstracts Index Name	2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluorooctanoic acid	
Synonyms	PFOA; pentadecafluoro-1-octanoic acid; pentadecafluoro-n-octanoic acid; octanoic acid, pentadecafluoro-; perfluorocaprylic acid; pentadecafluorooctanoic acid; perfluoroheptanecarboxylic acid	EPA CompTox Chemicals Dashboard
Chemical Formula	$C_8HF_{15}O_2$	NLM (2022a)
Molecular Weight	414.069 g/mol	NLM (2022a)
Color/Physical State	White to off-white powder (ammonium salt)	NLM (2022a)
Boiling Point	192°C	NLM (2022a)
Melting Point	54.3°C	NLM (2022a)
Vapor Pressure	0.0316 mm Hg at 19°C 0.017 mm Hg at 20°C	NLM (2022a); ATSDR (2021) (extrapolated)
Henry's Law Constant (K_H)	0.362 Pa-m^3/mol (converts to 3.57E-06 atm-m^3/mol)	ATSDR (2021)
pK_a	1.30, 2.80, −0.5–4.2, 0.5, 0.5	NLM (2022a); ATSDR (2021)
K_{oc}	631 ± 7.9 L/kg (mean ± 1 standard deviation of selected values)	Zareitalabad et al. (2013) (converted from log K_{oc} to K_{oc})
Solubility in Water	2,290 mg/L at 24°C (estimated); 3,300 mg/L at 25°C; 4,340 mg/L at 24.1°C 9,500 mg/L at 25°C; 3,300 mg/L at 25°C	NLM (2022b) ATSDR (2021)

Notes: CASRN = Chemical Abstracts Service Registry Number; K_{oc} = organic carbon-water partitioning coefficient; K_{ow} = octanol-water partition coefficient; pK_a: negative base-10 logarithm of acid dissociation constant.
[a] The CASRN given is for linear PFOA, but the toxicity studies are based on both linear and branched; thus, this assessment applies to all isomers of PFOA.

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. PFOA 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 PFOA declined by >70% between 1999 and 2018, presumably due to 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 PFOA, 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).

Under EPA's PFOA Stewardship Program, the eight major companies of the perfluoropolymer/fluorotelomer industry agreed to voluntarily reduce facility emissions and product content of PFOA, precursor chemicals that can break down to PFOA, and related higher homologue chemicals, including perfluorononanoic acid (PFNA) and longer-chain perfluorocarboxylic acids (PFCAs), by 95% on a global basis by no later than 2010 and to eliminate these substances in products by 2015 (U.S. EPA, 2021a). Manufacturers have since shifted to alternative short-chain PFAS, such as hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt (two "GenX" chemicals). Additionally, other PFAS were found in human blood samples from recent (2011–2016) NHANES surveys (e.g., perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoDA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonate (PFHxS), 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 PFOA, PFOS, 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, PFOA 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). Most of the current, published PFOA occurrence studies have focused on a handful of broad geographic regions in the United States, often targeting sites with known manufacturing or industrial uses of PFAS such as the Great Lakes, the Cape Fear River, and waterbodies near Decatur, Alabama (Cochran, 2015; Konwick et al., 2008; Nakayama et al., 2007; Boulanger et al., 2004; Hansen et al., 2002; 3M Company, 2000). PFOA concentrations in global surface waters range over seven orders of magnitude, generally in pg/L to ng/L concentrations, but sometimes reaching µg/L levels (Jarvis et al., 2021; Zareitalabad et al., 2013).

PFOA concentrations in surface water tend to increase with increasing levels of urbanization. Across the Great Lakes region, PFOA was higher in the downstream lakes (Lake Erie and Lake Ontario), which are more heavily impacted by urbanization, and lower in the upstream lakes (Lakes Superior, Michigan, and Huron), which are located in a relatively rural and forested area (Remucal, 2019). Similarly, Zhang et al. (2016b) found measured surface water PFOA concentrations in urban areas (urban average PFOA concentration = 10.17 ng/L; n = 20) to be more than three times greater than concentrations in rural areas (rural average PFOA concentration = 2.95 ng/L; n = 17) within New Jersey, New York, and Rhode Island. Seasonal variations in PFOA levels in U.S. surface waters remain largely unknown because of a lack of experimental evidence examining alterations in PFOA concentrations across time.

1.4.3 Drinking Water

Ingestion of drinking water is a potentially significant source of exposure to PFOA. Serum PFOA 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 PFOA (U.S. EPA, 2024d, e). Under UCMR 3, 36,972 samples from 4,920 PWSs were analyzed. PFOA was found above the UCMR 3 minimum reporting level (20 ng/L) in 379 samples at 117 systems serving a population of approximately 7.6 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 PFOA in multiple geographic locations. These data also show PFOA 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, PFOA concentrations ranged from 0.21 to 650 ng/L with a range of median concentrations from 1.27 to 5.61 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 PFOA 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 PFOA, between January 2023 and December 2025 using drinking water analytical methods developed by EPA (U.S. EPA, 2021g). The UCMR 5 defined the minimum reporting level at 4 ng/L for PFOA using EPA Method 533, which is lower than the 20 ng/L used in the UCMR 3 with EPA Method 537 (U.S. EPA, 2021g). Therefore, UCMR 5 will be able to provide nationally representative occurrence data for PFOA

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. In this study, PFOA was reported in source water at 76% of systems, at a median concentration of 6.32 ng/L and maximum concentration of 112 ng/L. Similarly, in treated drinking water, PFOA was detected in 76% of systems, with a median concentration of 4.15 ng/L and maximum concentration of 104 ng/L.

1.5 History of EPA's Human Health Assessment of PFOA

EPA developed an HESD for PFOA 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 PFOA 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 Perfluorooctanoic Acid (PFOA)*(U.S. EPA, 2016c) was published in 2016 and described the assessment of cancer and noncancer health effects and the derivation of a CSF and noncancer RfD for PFOA.

EPA initiated an update to the 2016 PFOA HESD in 2021 when the agency made a determination to regulate PFOA with a national primary drinking water regulation (NPDWR) (U.S. EPA, 2021d). The initial update of the 2016 PFOA HESD was the *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021c). 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 for PFOA. 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 PFOA.

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 comments document (U.S. EPA OW, 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 Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2023a). 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 Perfluorooctanoic Acid (PFOA) in Drinking Water* for a 60-day public comment period. This assessment 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 PFOA.

EPA incorporated feedback from public comment into this final 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 Perfluorooctanoic Acid (PFOA) 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 PFOA.

2 Summary of Assessment Methods

This section summarizes the methods used for the systematic review of the health effects literature for all isomers of perfluorooctanoic acid (PFOA), as well as nonmetal salts of PFOA, 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 PFOA 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, 2023b, 2022c). Although the IRIS Handbook was finalized concurrently with the development of this assessment, the revisions in the final IRIS Handbook compared with the draft version do not conflict with the methods used in this assessment. The assessment team concluded that implementing 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 PFOA 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 Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* draft document that was reviewed by the Science Advisory Board (SAB) (U.S. EPA, 2022e, 2021c). 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 Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2023a).

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 PFOA 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 Perfluorooctanoic Acid (PFOA)* (U.S. EPA, 2016c). All of these streams are described in detail below.

For the literature searches, the search strings focused on the chemical name (PFOA 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 PFOA 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 Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021c). The 2016 PFOA HESD contained a summary of all relevant literature identified in searches conducted through 2013. EPA's 2016 PFOA 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 PFOA HESD for the five priority health outcomes. It also includes "key" animal toxicological studies from the 2016 PFOA HESD, which includes studies that were selected in 2016 for dose-response modeling. The details of the studies included from the 2016 PFOA 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 initial 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 PFOA. 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 PFOA (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 PFOA HESD that reported results on one or more of the five priority health outcomes, and key animal toxicological studies from the 2016 PFOA 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. Because of 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 PFOA 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, 2021b). 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 PFOA HESD, as well as mechanistic evidence. The evidence integration provides a summary of the causal interpretations between PFOA 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 PFOA 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 PFOA 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 PFOA 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 PFOA, multiple candidate RfDs were derived within a health outcome as described in Section 4.

For PFOA 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 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 well 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 PFOA 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 PFOA are summarized below.

Step 1: Evaluate the data to identify and characterize endpoints affected by exposure to PFOA. This step involves selecting the relevant studies and adverse effects to be considered for BMD modeling. Once the appropriate data are collected, evaluated for study 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 lifestages are selected for simulation in a mouse, rat, or monkey. Concentrations of PFOA 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 with 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 (UF_c) 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 with 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 a Cancer Slope Factor

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). This process varied slightly depending on whether the CSF was based on an animal toxicological or epidemiological study, as described below.

The first step in the process is using a model 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 (U.S. EPA, 2005a).

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 PFOA, 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.

For epidemiological data, EPA used linear regression between PFOA exposure and cancer relative risk to estimate dose response as well as the generalized least-squares for trend (glst) modeling (Greenland and Longnecker, 1992) using STATA v17.0 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC). The CSF was then calculated as the excess cancer risk associated with each ng/mL increase in serum PFOA. The internal serum CSF was converted to an external dose CSF, which describes the increase in cancer risk per 1 ng/kg-day increase in dose. The internal serum CSF was converted to an external dose CSF, which describes the increase in cancer risk per 1 ng/(kg-day) increase in

dose. This was done by dividing the internal serum CSF by the selected clearance value, which is equivalent to dividing by the change in external exposure that results in a 1 ng/mL increase in serum concentration at steady-state. EPA also considered evaluating the dose-response data using the BMDS; however, categorical data from case-control studies cannot be used with the BMDS since these models are based on cancer risk, and the data needed to calculate risks (i.e., the denominators) were not available. 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 with 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

Once all of the candidate toxicity values were derived, EPA then selected 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 overall toxicity values for noncancer and cancer effects involves the study preferences described above, consideration of overall toxicity, study confidence, and confidence in each value, including the strength of various dose-response analyses and the possibility of

[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.

basing a more robust result on multiple data sets. The values selected as the overall RfD and CSF are discussed in Section 4.

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, 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 epidemiological, 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 yielded 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, 784 were considered to meet population, exposure, comparison, outcome (PECO) eligibility criteria (see Appendix A, (U.S. EPA, 2024a)) and included relevant information on PFOA. The 784 studies that were determined to meet PECO criteria after full-text level screening included 451 epidemiological (human) studies, 40 animal toxicological studies, 15 physiologically based pharmacokinetic (PBPK) studies (2 of which were also relevant epidemiological studies), and 280 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 20 PBPK studies were identified during the toxicokinetic screening for a total of 35 PBPK studies. Details of the literature search and screening process are shown in Figure 3-1.

The 451 epidemiological studies and 40 animal toxicological studies relevant to PFOA 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 35 studies tagged as containing relevant PBPK models relevant to PFOA 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 129 toxicokinetic and 273 mechanistic studies identified as relevant for PFOA 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 Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2023a) included 944 references 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 HESDs and were not included in the toxicity assessments underwent additional review to 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 were already included in the PFOA or PFOS toxicity assessments. Additionally, several references reported information on PFOA or PFOS

and non- priority health outcomes and were therefore not included. 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).

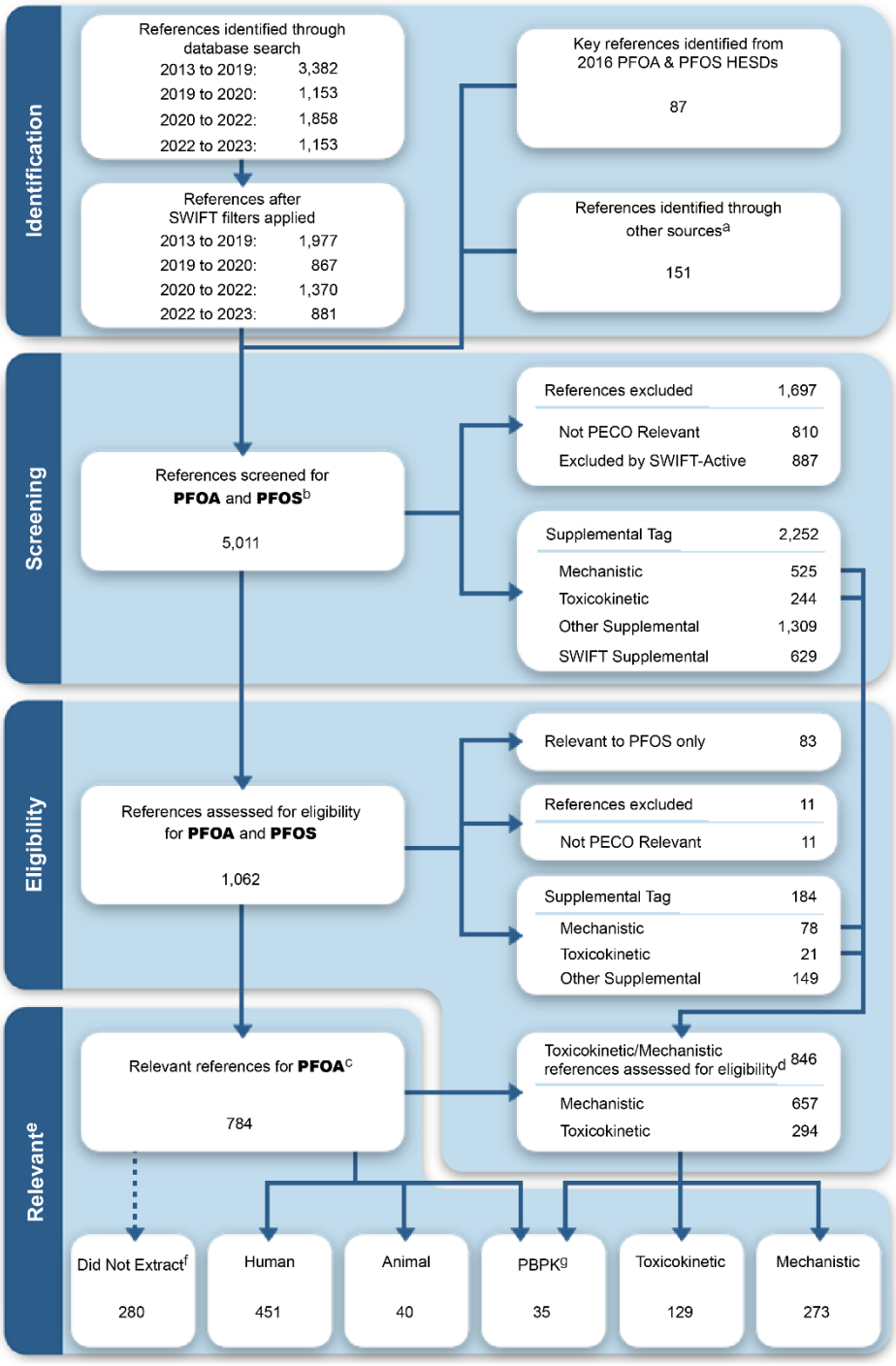


Figure 3-1. Summary of Literature Search and Screening Process for PFOA

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 epidemiological, 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 PFOA.
[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 PFOA.
[f] References tagged to 'Not a priority human health system' include those identified in the 2019 search that overlap with 2016 PFOA HESD references or those identified in 2022 and 2023 searches.
[g] Includes 15 PBPK references (2 of which were also relevant epidemiological references) determined to meet PECO criteria plus an additional 20 PBPK references identified during the toxicokinetic screening.

3.1.1 Results for Epidemiology Studies of PFOA by Health Outcome

Of the 451 epidemiological studies that met the inclusion criteria and underwent extraction, 193 had a cohort study design, 177 had a cross-sectional design, 42 had a case-control design, and 39 had other study designs (e.g., nested case-control). Epidemiological studies were categorized into 18 health outcomes. Most studies reported on the cardiovascular (n = 96), developmental (n = 92), metabolic (n = 78), or immune systems (n = 68). 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	8	6	3	5	22
Cardiovascular	5	24	60	7	96
Dermal	0	1	0	0	1
Developmental	4	61	20	7	92
Endocrine	1	8	18	8	35
Gastrointestinal	1	6	0	0	7
Hematologic	0	0	7	1	8
Hepatic	1	7	20	4	32
Immune	5	35	19	9	68
Metabolic	7	36	30	5	78
Musculoskeletal	0	1	6	2	9
Nervous	3	26	5	3	37
Ocular	0	0	1	0	1
Renal	0	6	18	2	26
Reproductive, Male	0	7	14	1	22
Reproductive, Female	9	24	22	4	59
Respiratory	1	4	1	0	6
Other	0	3	3	0	6
Grand Total	42	193	177	39	451

Figure 3-2. Summary of Epidemiology Studies of PFOA 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 PFOA by Health Outcome

Of the 40 animal toxicological studies that met the inclusion criteria and underwent extraction, most studies had either short-term (n = 16) or developmental (n = 16) study designs and most were conducted in mice (n = 33). The mouse studies had developmental (n = 16), short-term (n = 15), and subchronic (n = 2) study designs. The remaining studies reported results for rats (n = 7) using chronic (n = 3), short-term (n = 2), subchronic (n = 1), or reproductive (n = 1) study designs, or monkeys (n = 1) using a chronic study design. Animal toxicological studies were categorized into 15 health outcomes. Most studies reported results for the hepatic (n = 30), whole-body (n = 25; i.e., systemic effects such as bodyweight), reproductive (n = 19), or developmental (n = 15) 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	Rat	
Cancer	0	0	0	0	0	3	1	0	4
Cardiovascular	2	2	0	0	0	2	3	0	8
Developmental	0	0	0	0	0	1	13	1	15
Endocrine	3	2	0	0	0	3	3	1	11
Gastrointestinal	0	0	0	0	1	2	0	0	3
Hematologic	1	1	0	0	0	1	0	0	3
Hepatic	11	2	2	1	0	3	11	1	30
Immune	5	2	2	0	0	2	2	1	13
Metabolic	0	1	0	0	0	2	3	0	6
Musculoskeletal	1	0	0	0	0	0	0	0	1
Nervous	2	0	0	0	0	1	2	1	6
Renal	1	1	1	0	0	2	1	1	7
Reproductive	3	1	1	1	0	3	9	1	19
Respiratory	0	1	0	0	0	1	0	0	2
Whole Body	10	2	2	1	0	3	7	1	25
Grand Total	15	2	2	1	1	3	16	1	40

Figure 3-3. Summary of Animal Toxicological Studies of PFOA 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 site (https://hawc.epa.gov/assessment/100500248/) displayed as exposure-response arrays, forest plots, and evidence maps. Data extracted from the 451 epidemiological studies are available here. Data extracted from the 40 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 are also available in HAWC.

3.3 Toxicokinetic Synthesis

As described in Section 3.1, EPA identified 129 and 35 studies containing information relevant to the toxicokinetics and PBPK modeling of PFOA, respectively. The results of these studies are described in the subsections below and additional information related to toxicokinetic characteristics of PFOA can be found in Appendix B (U.S. EPA, 2024a).

3.3.1 ADME

PFOA is resistant to metabolic and environmental degradation due to its strong carbon-fluorine bonds. It also is resistant to metabolic biotransformation. Thus, the toxicity and pharmacodynamics of the parent compound (the anion when dissociated in water or the body) are the concern. Because of its impacts on cellular receptors and proteins, PFOA can influence the biotransformation of dietary constituents, intermediate metabolites, and other xenobiotic chemicals by altering enzyme activities and transport kinetics. PFOA is known to activate peroxisome proliferator-activated receptor (PPAR) pathways by increasing transcription of mitochondrial and peroxisomal lipid metabolism, sterol, and bile acid biosynthesis and retinol metabolism genes. Findings of transcriptional activation of many genes in peroxisome proliferator-activated receptor alpha (PPARα)-null mice after PFOA exposure, however, indicate that the effects of PFOA are mediated by other modes of action (MOAs) in addition to PPAR activation and consequent peroxisome proliferation (Wen et al., 2019c; Rosen et al., 2017; U.S. EPA, 2016c; Oshida et al., 2015a; Oshida et al., 2015b). The available data indicate that PFOA exposure can also activate the constitutive androstane receptor (CAR), farnesoid X receptor (FXR), and pregnane X receptor (PXR), and can affect metabolic activities linked to these nuclear receptors (Rosen et al., 2017; U.S. EPA, 2016c; Oshida et al., 2015a; Oshida et al., 2015b). Activation of these receptors resulting from PFOA exposure could in turn impact the toxicokinetics of PFOA itself (Andersen et al., 2008).

PFOA is not readily eliminated from humans and other primates. Toxicokinetic profiles and the underlying mechanism for half-life differences between species and sexes are not completely understood, although many of the differences appear to be related to elimination kinetics and factors that control membrane transport. Thus far, three transport families appear to play a role in PFOA absorption, distribution, and excretion: organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and multidrug resistance-associated proteins (MRPs) (Klaassen and Aleksunes, 2010; Launay-Vacher et al., 2006). These transporters are critical for gastrointestinal absorption, uptake by the tissues, and excretion via bile and the kidney. These transport systems are located at the membrane surfaces of the kidney tubules, intestines, liver, lungs, heart, blood brain barrier (BBB), blood placental barrier, blood testes barrier (BTB), and mammary glands where they function to protect the organs, tissues, and fetus through active removal of foreign compounds (Klaassen and Aleksunes, 2010 Zaïr, 2008, 9641805; Ito and Alcorn, 2003). However, luminal transporters in the kidney may cause reuptake of PFOA from the proximal tubule resulting in decreased excretion from the body (Weaver et al., 2010). This reuptake would lead to PFOA persisting in the body over time. Transporters involved in enterohepatic circulation have also been identified that may facilitate uptake and reuptake of PFOA from the gut (Ruggiero et al., 2021).

There are differences in transporters across species, sexes, and individuals. In addition, more PFOA-specific information is available for the OAT and OATP families than for the MRPs.

These data limitations have hindered the development of PK models for use in predicting effects in humans based on the data from animal toxicological studies.

3.3.1.1 Absorption

PFOA absorption data are available in laboratory animals for oral, inhalation, and dermal exposures, and extensive data are available from humans demonstrating the presence of PFOA in serum (descriptions of available studies are provided in the Appendix, (U.S. EPA, 2024a)). In vitro absorption data indicate that uptake is influenced by pH, temperature, and concentration as well as OATP activity (see Appendix B, (U.S. EPA, 2024a)).

3.3.1.1.1 Cellular Uptake

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

In addition to facilitated transport, there is evidence supporting passive diffusion in cells cultured in vitro (Yang et al., 2009b) and in placenta in vivo (Zhang et al., 2013b). Since PFOA is moderately soluble in aqueous solutions and oleophobic (i.e., minimally soluble in body lipids), movement across interface membranes was thought to be dominated by transporters or mechanisms other than simple diffusion across the lipid bilayer. Recent mechanistic studies, however, support transporter-independent uptake through passive diffusion processes. Ebert et al. (2020) determined membrane/water partition coefficients ($K_{mem/w}$) for PFOA and examined passible permeation into cells by measuring the passive anionic permeability (P_{ion}) through planar lipid bilayers. In this system, the partition coefficients (PCs) were considered high enough to explain observed cellular uptake by passive diffusion in the absence of active uptake processes.

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

3.3.1.1.2 Absorption and Bioavailability in Humans and Animals

In vivo, PFOA is well-absorbed following oral exposure, as evidenced by the presence of PFOA in serum of humans following exposure to contaminated drinking water (Xu et al., 2020c; Worley et al., 2017a). Studies on male rats administered PFOA by gavage using a single or multiple dose regimen estimated dose absorption of at least 92.3% (Cui et al., 2010; Gibson and

Johnson, 1979). In rats, the time to reach the maximum PFOA plasma concentration (T_{max}) following oral exposure is very fast and varies by sex (Dzierlenga et al., 2019a; Kim et al., 2016). For example, the study by Kim and colleagues estimated T_{max} after a single oral dose of 1 mg/kg to be 1.44 hours in female rats versus 2.07 days in males.

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

The available data, although limited, also support PFOA absorption through both inhalation (Hinderliter et al., 2006a) and dermal routes (Fasano et al., 2005; Kennedy, 1985; O'Malley and Ebbins, 1981).

3.3.1.2 Distribution

3.3.1.2.1 PFOA Binding to Blood Fractions and Serum Proteins

Detailed study descriptions of literature regarding the distribution of PFOA in humans and animals are provided in 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. Within the blood cell constituents, PFOA preferentially accumulates in platelets over red blood cells and leukocytes (De Toni et al., 2020). Among different kinds of human blood samples, PFOA 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 PFOA concentrations in plasma, serum, and whole blood were 1.90, 1.60, and 0.93 ng/mL, respectively. These findings suggest that the common practice of multiplying by a factor of 2 to convert the concentrations in whole blood to serum (Ehresman et al., 2007) will not provide accurate estimates for PFOA.

PFOA is distributed within the body by noncovalently binding to plasma proteins. Many studies have investigated PFOA interactions with human serum albumin (HSA) (Gao et al., 2019; Cheng and Ng, 2018; Yue et al., 2016; Zhang et al., 2013a; Macmanus-Spencer et al., 2010; Qin et al., 2010; Salvalaglio et al., 2010; Weiss et al., 2009; Wu et al., 2009; Luebker et al., 2002). In vitro analyses found that plasma proteins can bind 97%–100% of the PFOA in plasma from humans, cynomolgus monkeys, and rats (Kerstner-Wood et al., 2003). HSA is the primary PFOA binding protein in plasma (Han et al., 2003) and intermolecular interactions are mediated through van der Waals forces and hydrogen bonds (Chen et al., 2020; Macmanus-Spencer et al., 2010). Beesoon and Martin (2015) determined that linear PFOA molecules bound more strongly to calf serum albumin than the branched-chain isomers in the order of 4m < 3m < 5m < 6m (iso) < linear. PFOA-mediated conformational changes may interfere with albumin's ability to transport its natural ligands and pharmaceuticals (Wu et al., 2009) such as fatty acids, thyroxine (T4), warfarin, indole, and benzodiazepine.

Binding to albumin and other serum proteins may affect transfer of PFOA from maternal blood to the fetus (Gao et al., 2019). Since there is effectively a competition between PFOA 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 high concentration of cord serum albumin was associated with higher PFOA transfer efficiencies, whereas high maternal serum albumin concentration was associated with reduced transfer efficiency.

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

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

Han et al. (2005) observed a sex-dependent subcellular distribution of PFOA in the liver and kidney of male and female adult rats necropsied 2 hours after oral gavage dosing. The proportion of PFOA in the liver cytosol of female rats was almost twice that of the male rats. They hypothesized that females might have a greater amount than males of an unknown liver cytosolic binding protein with an affinity for perfluorinated acids. In the kidney, the subcellular distribution did not show a sex difference comparable to the one seen for liver; however, the protein-bound fraction in males (42%) was about twice that of females (17%), which differs from the sex differences found for the liver.

In a study of human cells (Zhang et al., 2020a), PFOA preferentially distributed to cytosol followed by nuclei and mitochondria in human colorectal cancer cells, human lung epithelial cells, and human normal liver cells. In liver cells, PFOA binds to the liver fatty acid binding protein (L-FABP) through polar and hydrophobic interactions (Yang et al., 2020a; Zhang et al., 2013a; 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.

PFOA interactions with various protein transporters play a role in the tissue uptake of orally ingested PFOA. The transporters are located at the interface between serum and a variety of tissues (e.g., liver, kidneys, lungs, heart, brain, testes, ovaries, placenta, uterus) (Klaassen and Aleksunes, 2010). The liver is an important uptake site for PFOA. OATPs and MRPs, at least one OAT, and the sodium-taurocholate cotransporting polypeptide (NTCP) – a hepatic bile uptake transporter – have been identified at the boundary of the liver at the portal blood and/or the canalicular membranes within the liver (Kusuhara and Sugiyama, 2009; Zaïr et al., 2008; Kim, 2003). Transporters responsible for PFOA transport across the placenta are not well understood, though preliminary studies examining transporter expression identified OAT4 as a candidate receptor (Kummu et al., 2015). The expression of nine transporter genes was found to

vary at different stages of gestation (Li et al., 2020a), though direct experimental evidence for these transporters in mediating transfer of PFOA to the fetus is lacking.

3.3.1.2.3 Tissue Distribution in Humans and Animals

Evidence from human autopsy and surgical tissues demonstrates that PFOA distributes to a wide range of tissues, organs, and matrices throughout the body. Although blood and liver are major sites of PFOA accumulation (Olsen et al., 2001c), recent findings confirm PFOA accumulation in other tissues and fluids including brain and cerebral spinal fluid (Wang et al., 2018; Fujii et al., 2015; Maestri et al., 2006), major organs including lung and kidney (Maestri et al., 2006), endocrine tissues including the thyroid gland, pituitary gland, and pancreas (Pirali et al., 2009; Maestri et al., 2006), and gonads and follicular fluid (Kang et al., 2020; Maestri et al., 2006). Pérez et al. (2013) measured PFOA levels in autopsy tissue samples (liver, kidney, brain, lung, and bone) collected within 24 hours of death and found PFOA in bone (60.2 ng/g), lung (29.2 ng/g), liver (13.6 ng/g), and kidney (2.0 ng/g), with levels below the limit of detection (LOD) in the brain. Maestri et al. (2006) measured pooled post-mortem tissue samples and found the highest levels in lung (3.8 ng/g), kidney (3.5 ng/g), and liver (3.1 ng/g). It should be noted, however, that autopsy and surgical tissues may not necessarily accurately reflect PFAS tissue distribution in the living body (Cao and Ng, 2021). Several studies examined a limited number of tissues in primates and observed higher levels in serum compared with liver (Butenhoff et al., 2004b; Butenhoff et al., 2002; Griffith and Long, 1980).

Most whole animal toxicological studies that measured PFOA distribution were conducted in rats and mice by oral dosing. Studies in primates measured PFOA in blood and liver following oral administration (Butenhoff et al., 2004b; Butenhoff et al., 2002). PFOA primarily distributes to serum, liver, lungs, and kidney across a range of dosing regimens and durations (NTP, 2020, 2019; Kemper, 2003; Ylinen et al., 1990) in rats and in mice (Guo et al., 2019; Burkemper et al., 2017; Li et al., 2017b; Lou et al., 2009; Lau et al., 2006). Sex-specific differences in PFOA levels were observed in several rat studies. For example, in a 28-day study (NTP, 2019), PFOA plasma concentrations were higher in males than in females across all dose groups even though females were administered a 10-fold higher dose of PFOA, suggesting that female rats excrete PFOA more efficiently than males. Sex-specific differences were less striking in studies conducted in mice compared with rats (Lou et al., 2009; Lau et al., 2006).

Liver PFOA levels are regulated in part by PPARα. In human and rodent hepatocytes, PPARα activation induces expression of genes involved in lipid metabolism and cholesterol homeostasis. PFOS and PFOA structurally resemble fatty acids and are well-established ligands of PPARα in the rat and mouse liver. As PPARα agonists, PFOS and PFOA can induce ß-oxidation of fatty acids, induce fatty acid transport across the mitochondrial membrane, decrease hepatic very low-density lipoprotein (VLDL)-triglyceride and apolipoprotein B (apoB) production, and promote lipolysis of triglyceride-rich plasma lipoproteins (Fragki et al., 2021). The liver can transport PFOA from hepatocytes to bile ducts, which is mediated at least partly by PPARα (Minata et al., 2010). PFOA levels were significantly lower in PPARα-null mice than in wild-type mice exposed to doses of 25 and 50 µmol/kg, supporting a role for PPARα in PFOA clearance in the liver (Minata et al., 2010) but not excluding other factors regulating PFOA levels. It is unclear what role PPARα plays in PFOA clearance in the liver of humans.

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

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

3.3.1.2.4 Distribution During Reproduction and Development

Many recent human studies have quantified the distribution of PFOA from pregnant mothers to their fetuses and from mothers to their infants. Distribution from pregnant mother to fetus has been confirmed by measuring PFOA levels in placenta, cord blood, and amniotic fluid during gestation and at birth. The ratio of PFOA in placenta relative to maternal serum during pregnancy (R_{PM}) ranged from 0.326 to 0.460 (Chen et al., 2017a; Zhang et al., 2013b). Gestational age and PFOA branching characteristics influence transport across the placenta. PFOA concentrations within the placenta increase during gestation from the first to third trimester (Mamsen et al., 2019). Linear PFOA is detected at a higher frequency and at higher concentrations in maternal serum than branched PFOA isomers. However, branched PFOA is more efficiently transported into the placenta than linear PFOA (Cai et al., 2020; Chen et al., 2017a).

Several studies reported a strong positive correlation between maternal and cord serum PFOA levels in humans (Kato et al., 2014; Porpora et al., 2013). The ratio of PFOA in cord serum relative to maternal serum ranged from 0.55 to 1.33 (see Appendix, (U.S. EPA, 2024a)) and generally increased with gestational age (Li et al., 2020a). Factors such as exposure sources, parity, and other maternal demographics are postulated to influence variations in maternal serum PFAS concentrations and cord:maternal serum ratios (Brochot et al., 2019; Kato et al., 2014). Cord:maternal serum ratios represent transplacental efficiencies (TTEs), which exhibit a U-shaped curve with PFAS chain length (Zhang et al., 2013b) and generally increase as the PFAS branching point moves closer to the carboxyl or sulfonate moiety (Zhao et al., 2017a).

Lower levels of PFOA were measured in amniotic fluid compared with the placenta and cord blood (all collected at delivery) (Zhang et al., 2013b). The mean concentration ratio between

amniotic fluid and maternal blood (collected no more than one hour before delivery) was higher for PFOA (0.13) than for PFOS (0.0014). The mean concentration ratio between amniotic fluid and cord blood was higher for PFOA (0.023) than for PFOS (0.0065). Authors attributed the differences in ratios between the two compartments to the solubilities of PFOS and PFOA and their respective protein binding capacities in the two matrices.

PFOA also distributes widely in human 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. PFOA was detected in placenta and fetal liver, extremities, heart, intestines, lungs, connective tissues, spinal cord, and ribs, and concentrations were highest in the placenta and lung. Different patterns of PFOA distribution were observed in fetal tissues depending on fetal age (Mamsen et al., 2019). Fetal tissue:maternal serum ratios of PFAS were calculated by dividing the fetal tissue concentration by the maternal serum concentration. In general, fetal tissue:maternal serum ratios of PFOA increased from the first trimester to the third trimester, except for the liver and heart, which showed the highest fetal tissue:maternal serum ratios in the second trimester compared with the third trimester.

Studies in humans also confirm that the distribution of PFOA from nursing mothers to their infants via breastmilk correlates with duration of breastfeeding (Cariou et al., 2015 Mogensen, 2015, 3859839, Gyllenhammar, 2018, 4778766; 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), the mean maternal serum PFOA concentrations were lower in breastfeeding mothers versus non-breastfeeding mothers. Conversely, breastfed infants had higher mean serum PFOA than infants who were never breastfed. Maternal serum concentrations decreased with each month of breastfeeding (Mogensen et al., 2015b; Mondal et al., 2014). Cariou et al. (2015) reported that PFOA levels in breastmilk were approximately 30-fold lower relative to maternal serum and the ratio between breastmilk and maternal serum PFOA was 0.038 ± 0.013. The authors noted that the transfer rates of PFAS from serum to breastmilk were lower compared with other lipophilic persistent organic pollutants such as polychlorinated biphenyls.

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

3.3.1.2.5 Volume of Distribution in Humans and Animals

In humans, the volume of distribution (V_d) for PFOA has been assigned values between 170 and 200 mL/kg (see Appendix B, (U.S. EPA, 2024a)). V_d values may be influenced by differences in distribution between males and females, between pregnant and nonpregnant females, and across serum, plasma, and whole blood.

V_d estimates derived in mice and rats vary by species, age, sex, and dosing regimen. For example, Dzierlenga et al. (2019a) calculated the apparent volume of central and peripheral

distribution in male and female adult rats after oral dosing. A one-compartment model for males and a two-compartment model for females was used to characterize PFOA levels. Peripheral V_d values were dramatically lower than central V_d values at all doses after oral administration and, interestingly, also after IV administration. While peak tissue levels were reached readily in both males and females, tissue levels in males were steady over the course of several days whereas tissue levels in females dropped quickly, in the span of hours. Further discussion on the V_d for PFOA can be found in Section 5.6.2.

3.3.1.3 Metabolism

Consistent with other peer-reviewed, published reports and reviews (ATSDR, 2021; Pizzurro et al., 2019; U.S. EPA, 2016c), the literature reviewed for this assessment do not provide evidence that PFOA 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 PFOA in humans, cynomolgus monkeys, and rats. Fewer studies measured elimination in mice, hamsters, and rabbits. Available evidence supports urine as the primary route of excretion in most species, though fecal elimination is prominent in rats. In rats, hair is another route of elimination in both males and females. In female humans and animals, elimination pathways include menstruation, pregnancy (cord blood, placenta, amniotic fluid, and fetal tissues) and lactation (breast milk) (see Appendix B, (U.S. EPA, 2024a)). Results of elimination half-life determination studies in mammals suggest that PFOA elimination time is longest in humans (years), intermediate in monkeys (days to weeks), and shortest in rodents (hours to days).

3.3.1.4.1 Urinary and Fecal Excretion

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

Human studies examined PFOA excretion after oral exposure, primarily through the urinary route. The urinary excretion of PFOA in humans is impacted by the isomeric composition of the mixture present in blood and the sex and age of the individual. The half-lives of the branched-chain PFOA isomers are shorter than those for the linear molecule, indicating that renal resorption is less prevalent for the branched-chain isomers (Fu et al., 2016; Zhang et al., 2015).

Fujii et al. (2015) measured PFOA clearance in mice and humans. Male and female FVB/NJcl mice were administered PFOA by IV (0.31 μmol/kg) or gavage (3.13 μmol/kg) and serum concentration data were analyzed using a two-compartment model. Mouse

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

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

In rats, urine PFOA concentrations differed with age, dose, and sex (Hinderliter et al., 2006b). For all rats dosed between 3 and 8 weeks of age, urinary excretion of PFOA was substantially higher in females than in males, and this difference increased with age. Several additional studies in rats found that females excreted much higher levels in urine compared with males and compared with feces (Kim et al., 2016; Cui et al., 2010; Benskin et al., 2009).

3.3.1.4.2 Renal and Enterohepatic Resorption

Several studies have been conducted to elucidate the cause of the sex difference in the elimination of PFOA by rats (Cheng et al., 2006; Hinderliter et al., 2006b; Kudo et al., 2002). Many of the studies have focused on the role of transporters in the kidney tubules, especially the OATs and OATPs located in the proximal portion of the descending tubule (Yang et al., 2010; Nakagawa et al., 2009; Yang et al., 2009b; Nakagawa et al., 2008). The results of in vitro studies were consistent with an in vivo analysis of OATPs gene and protein expression in rat kidneys (Yang et al., 2009b). Organic anion transporters polypeptide 1a1 (OATP1a1) is located on the apical side of proximal tubule cells and is a potential mechanism for renal reabsorption of PFOA in rats. The level of messenger ribonucleic acid (mRNA) of OATP1a1 in male rat kidney is 5–20-fold higher than in female rat kidney and is regulated by sex hormones. Thus, higher expression of OATP1a1 in male rats would favor resorption of PFOA in the glomerular filtrate which is consistent with reduced excretion in males.

Fewer studies have investigated enterohepatic resorption of PFOA. Gastrointestinal elimination of PFOA was reported in a case report of a single human male with high serum levels of perfluorinated chemicals who was treated with a bile acid sequestrant (cholestyramine (CSM)) (Genuis et al., 2010). Before treatment, PFOA was detected in urine (3.72 ng/mL) but not in stool (LOD = 0.5 ng/g) or sweat samples. After treatment with CSM for 1 week, the serum PFOA concentration decreased from 5.9 ng/g to 4.1 ng/g, and stool PFOA levels increased to 0.96 ng/g. This observation suggests that PFOA is excreted in bile and that enterohepatic resorption via intestinal transporters limits the loss of PFOA via feces. Studies in mice (Cheng and Klaassen, 2008a; Maher et al., 2008) suggest that increased expression of MRP3 and MRP4,

coupled with decreased OATP levels, leads to increased biliary excretion of bile acids, bilirubin, and potentially toxic exogenous substances, including PFOA.

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

3.3.1.4.3 Maternal Elimination Through Lactation and Fetal Partitioning

In humans, PFOA can readily pass from mothers to their fetuses during gestation and through breast milk during lactation. In conjunction with elimination through menstruation, discussed in Section 3.3.1.4.4, human females clearly eliminate PFOA through routes not available to males. The total daily elimination of PFOA in pregnant human females was estimated to be 11.4 ng/day, lower than the 30.1 ng/day estimated for PFOS (Zhang and Qin, 2014). Mamsen et al. (2019) estimated a placenta PFOA accumulation rate of 0.11% increase per day during gestation and observed that the magnitude of elimination may be influenced by the sex of the fetus. A human study by Zhang et al. (2013b) observed that the mean levels in the cord blood, placenta, and amniotic fluid were 58%, 47%, and 1.3%, respectively, of those in the mother's blood, demonstrating that cord blood, placenta, and amniotic fluid are additional routes of elimination in pregnant females. Blood loss during childbirth could be another source of excretion. Underscoring the importance of pregnancy as a lifestage when excretion is altered, Zhang et al. (2015) observed that the partitioning ratio of PFOA concentrations between urine and whole blood in pregnant women (0.0011) was lower than the ratios found in nonpregnant women (0.0028). The rate and extent of elimination through these routes are affected by parity (Lee et al., 2017b; Jusko et al., 2016) and may be affected by the increase in blood volume during pregnancy (Pritchard, 1965).

Women can also eliminate PFOA via lactation (Kang et al., 2016a; Thomsen et al., 2010; Tao et al., 2008). Cariou et al. (2015) measured PFOA in maternal serum, cord serum, and breast milk from females with planned Cesarean births. The observed mean ratio of cord serum to maternal serum PFOA was 0.78 in this study. However, the mean ratio between breast milk and maternal serum was 0.038, suggesting transfer from maternal blood to breast milk is lower than transfer from maternal blood to cord blood.

Studies in laboratory animals support elimination through pregnancy and lactation similar to what has been observed in humans. Fujii et al. (2020) used the M/P concentration ratio as a measure of chemical transferability in FVB/NJcl mice. Maternal plasma PFOA concentrations were significantly higher than in milk (M/P ratio was 0.32). The M/P ratios were similar for C8, C9, C12, and C13, arguing against a direct relationship with lipophilicity. Potential roles for binding proteins in breast milk or transporters in breast tissue have not been investigated.

In summary, partitioning to the placenta, amniotic fluid, fetus, and breast milk represent important routes of elimination in humans, and may account for some of the sex differences observed for blood and urinary levels of PFOA by sex and lifestage.

3.3.1.4.4 Other Routes of Elimination

Menstruation may be an important factor in the sex-specific differences observed in PFOA elimination. Zhang et al. (2013c) estimated a menstrual serum PFOA clearance rate of 0.029 mL/day/kg. The link between menstruation and PFOA elimination is based on several observations. First, postmenopausal females and adult males have longer PFOA elimination half-lives than premenopausal adult females (Zhang et al., 2013c). 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. Second, several studies reported on an association between increased serum concentrations of PFOA and PFOS and early menopause (Taylor et al., 2014; Knox et al., 2011). However, a reanalysis of these data (Ruark et al., 2017) suggested that the association between increased serum PFAS and early menopause could be explained by reversed causality, and more specifically, that pharmacokinetic bias could account for the observed association with epidemiological data. Ruark et al. (2017) thus highlight the importance of considering menstrual blood loss as a PFAS elimination pathway. Additional studies may be needed to clarify the significance of menstruation in PFOA elimination.

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

3.3.1.4.5 Half-Life Data

Because there is no evidence that PFOA is metabolized in mammals, half-life determinations are governed by excretion. There have been several studies of half-lives in humans all supporting a long residence time for serum PFOA with estimates measured in years rather than months or weeks (see Appendix B, (U.S. EPA, 2024a)). The calculated PFOA 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 and rodent females relative to males. In the single primate study discussed below, half-lives were shorter in males compared with females.

PFOA half-life values in humans ranged from 0.53 years for a branched PFOA in young adult females (Zhang et al., 2013c) to 22 years in a study of primiparous women in Sweden (Glynn et al., 2012) and varied by geographical region (Gomis et al., 2017) (see Appendix B, (U.S. EPA, 2024a)). Age, lifestage, and sex differences in PFOA half-lives have not been rigorously evaluated, though estimates in males are generally longer than those in females (Li et al., 2018c; Gomis et al., 2017; Fu et al., 2016) and exhibit an age-related increase in adults (Genuis et al., 2014 Zhang, 2013, 3859849). While most studies were conducted in adults and/or adolescents,

one study in newborns (Spliethoff et al., 2008) calculated a half-life for PFOA of 4.4 years. Linear isomers exhibit longer half-lives than branched isomers (Zhang et al., 2013c).

Half-life estimates in humans rely on measured serum and/or urine concentrations. However, relatively few studies calculated PFOA half-lives along with measured intake and serum and urine PFOA concentrations (Xu et al., 2020c; Worley et al., 2017a; Fu et al., 2016; Zhang et al., 2013d) (see Appendix B, (U.S. EPA, 2024a)). PFOA half-life values among these 4 studies varied from 1.7 years in Xu et al. (2020c) to 4.7 years in Fu et al. (2016). These comparisons support principles suggested by the broader literature. First, sex related differences with males exhibiting somewhat longer half-lives compared with females (especially females of reproductive age) may relate, at least in part, to menstruation as a route of elimination (Zhang et al., 2013c). Second, blood and urine concentrations varied by several orders of magnitude across these four studies. While blood and urine PFOA concentrations varied by two orders of magnitude across these studies, half-life estimates were similar, ranging from 1.77 to 4.70 years. This variability in serum and urine concentrations may reflect the role of nonurinary routes of PFOA excretion; the variability in concentrations may also reflect the difficulty in measuring renal resorption. Finally, only two studies estimated PFOA intake in subjects (Xu et al., 2020c; Worley et al., 2017a). The multiple routes of exposure to PFOA and the need to understand historical exposure levels to estimate PFOA intake is an ongoing challenge for many studies that examine PFOA elimination. These factors, as well as age and health status of subjects, likely contribute to the reported variability in PFOA half-life estimates in humans.

In experimental animals, half-life values are reported in days rather than in years. Values in cynomolgus monkeys ranged from 13.6 to 41.7 days (Butenhoff et al., 2004b) and were generally longer than those observed in rodents, but much shorter than values observed in humans. Depending on the experimental conditions, half-lives in rats ranged from 0.03 days in females exposed to a high dose of 40 mg/kg (Dzierlenga et al., 2019a) to 13.4 days in males exposed to a relatively low dose of 0.4 mg/kg (Benskin et al., 2009). Rats exposed by the IV route exhibited shorter half-lives than rats administered the same dose by the oral gavage route (Dzierlenga et al., 2019a; Kim et al., 2016). Similar to humans and mice, half-life estimates were shorter in adult female rats compared with male rats. In contrast, female half-life values exceeded male values in cynomolgus monkeys, suggesting that species-specific factors impact elimination across sexes. Similar to findings in humans, PFOA branched isomers exhibited shorter half-lives compared with linear forms.

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 either 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 PFOA 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 the available data on 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 PFOA ADME is provided below.

3.3.2.1 Classical Compartmental Analysis

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

The most common use for these models in human populations is to predict serum concentrations from estimated exposures. Some examples of this include the work by Shin et al. (2011) who evaluated the exposure predictions from an environmental fate and transport model by comparing the predicted serum PFOA concentrations to observed values and by Avanasi et al. (2016) who extended the work of Shin et al. (2013) by applying a population model to investigate how variability and uncertainty in model parameters affect the prediction of serum concentrations.

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. (2019) 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 PFOA exposure in humans that originates from drinking water.

Other applications are used to better understand the toxicokinetics of PFOA 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 Gomis et al. (2016) who used measurements of serum and exposure, in the form of air concentrations during occupational exposure, to estimate an elimination half-life for PFOA. Those authors were also able to identify the relative contributions of direct occupational exposure to PFOA, indirect occupational exposure to PFOA precursors, and background, non-occupational PFOA exposure. Another example was presented by Worley et al. (2017a) who estimated the half-life of PFOA using exposure predicted from drinking water PFAS concentrations 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 (CL_R) (in urine) and in the whole-body (in serum). One challenge in the estimation of half-life is the problem of estimating exposure to PFOA. Russell et al. (2015) addressed this problem by estimating the amount of bias in elimination half-life that is introduced when the ongoing background exposure is not taken into account, with application to PFOA as an example.

One common modification of the one-compartment model is to perform a "steady-state approximation" (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 PFOA, steady state is a reasonable assumption for adults starting from the age of 25 and above. However, the steady state approximation cannot be applied for ages younger than 21 years of age (EPA defines childhood as <21 years of age; (U.S. EPA, 2021b)) 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 PFOA levels increase until the adult steady-state level is reached. The general acceptability of the steady-state assumption in adults has the caveat that pregnancy or breastfeeding will result in changes in serum concentration and will not be accounted for in the steady-state approximation.

When adopting a steady-state assumption, the rate of change in serum levels over time is zero. It follows that the ratio between exposure to the substance and clearance determines the serum concentration. This is the approach used in the 2016 PFOA HESD to determine the constant exposure associated with a serum concentration (U.S. EPA, 2016c). 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. (2015) who used paired human urine and serum data to estimate the total intake of PFOA 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 PFOA levels and extrapolated that finding to clearance via menstruation.

In animals, three classical PK models for PFOA have been published since the 2016 PFOA HESD. In Dzierlenga et al. (2019a), male Sprague-Dawley rats were dosed with PFOA via oral gavage at 6, 12, and 48 mg/kg, or intravenously at 6 mg/kg. Female Sprague-Dawley rats were dosed with PFOA via oral gavage at 40, 80, 320 mg/kg or intravenously at 40 mg/kg. Following the administration of PFOA, rats were sacrificed from five minutes to 50 days post-dosing for males and from five minutes to 12 days post-dosing in females. Differences in length of study for each sex represent the sex-dependent difference in half-lives for which adult female rats eliminate PFOA more rapidly than adult males. For both sexes, measured plasma concentrations

characterized the biphasic PK curve. From these exposure scenarios, Dzierlenga et al. (2019a) developed a two-compartment model to characterize PK parameters of interest such as the alpha- and beta-phase half-life, central and peripheral compartment volumes, and total PFOA clearance. For each dosing scenario, a single set of PK parameters were fit, making extrapolation to other dosing scenarios difficult. However, the authors demonstrate a significant difference between males and females in beta-phase half-life and overall clearance. This difference in half-life is critical when considering internal dosimetry for a pregnant dam during developmental PK studies.

Fujii et al. (2015) conducted a PK analysis in mice by dosing male and female mice either intravenously with 0.313 μmol/kg or through oval gavage with 3.13 μmol/kg. Following administration of PFOA, blood concentrations were collected through tail veins beginning immediately following dosing up to 24 hours post-dosing. Fujii et al. (2015) used these data to develop a two-compartment model to describe sex-dependent PK in mice. Unfortunately, the follow-up time of 24 hours post-dosing is not long enough to accurately characterize the beta-phase elimination of PFOA, which the authors predicted was 627 days. The small amount of change in PFOA levels within a 24-hour timespan will make the estimated terminal half-life from a two-compartment model unreliable because PFOA will still be in the distribution phase. In addition, the functional form fit for the oral gavage data in Fujii et al. (2015) reflects a one-compartment model with gavage dosing making it not possible to compare the predicted half-lives between the two routes of exposure. While the reported data could be used for characterizing absorption and distribution of PFOA, it cannot be used for characterizing the elimination phase. Additionally, a study with a much longer follow-up time of 80 days post-dosing reported a half-life of 15.6–21.7 days (Lou et al., 2009).

Finally, Gomis et al. (2016) utilized the functional form of a two-compartment model with oral gavage to predict internal dosimetry of PFOA in rats using PK data from Perkins et al. (2004). 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 PFOA concentrations in breast milk (Abdallah et al., 2020). Verner et al. (2016) presented another developmental model to predict PFOA 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 developmental model was developed by Goeden et al. (2019) to evaluate the relationship between drinking water

concentrations and infant serum levels during breastfeeding resulting from gestational and lactational transfer of PFOA that had accumulated within the mother. A distinguishing feature of the Goeden et al. (2019) model is that it incorporates an adjustment for the increased intracellular water in infants and young children compared with adults, under the assumption that PFAS distribution into tissues, quantified by the V_d, will increase proportionally to intracellular water content. This lifestage difference in intracellular water content may explain why the ratio of PFOA in cord blood versus maternal blood at childbirth tends to be less than one. Monroy et al. (2008) reported median cord blood PFOA concentration to be 87% of maternal serum, while the median ratio of fetal tissue to placenta PFOA concentration was found to be generally greater than one (Mamsen et al., 2019). One oversight of this model is that the rate equations take concentration into account, but they do not account for decreases in concentration due to increasing body weight (growth dilution). This is a significant factor for infants who grow quickly.

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

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 one- or two-compartment approaches have been published as tools to estimate internal doses for humans, monkeys, mice, and rats (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 created using the post-dosing plasma data from the Butenhoff et al. (2004b) study in cynomolgus monkeys. In this study, groups of six monkeys (three per sex per group) were dosed for 26 weeks with 0, 3, 10, or 20 mg/kg PFOA (and also a high dose of 30 mg/kg PFOA for only the first 12 days) and followed for more than 160 days after dosing. Metabolism cages were used for overnight urine collection. Since urine specimens could only account for overnight PFOA excretion, total volume and total PFOA were extrapolated to 24-hour values based on the excretion rate (volume per hour) for the volume collected and the hours of collection.

The Andersen et al. (2006) model was based on the hypothesis that saturable resorption capacity in the kidney would possibly account for the unique half-life properties of PFOA across species and sexes. The model structure was derived from a published model for glucose resorption from the glomerular filtrate via transporters on the apical surface of renal tubule epithelial cells (Andersen et al., 2006).

The renal-resorption model includes a central compartment that receives the chemical from the oral dose and a filtrate compartment for the glomerular filtrate from which resorption with

transfer to the central compartment can occur. Transfer from the filtrate compartment to the central compartment decreases the rate of excretion. The resorption in the model was saturable, meaning that there was proportionally less resorption and greater excretion at high serum PFOA concentrations than at low concentrations. In addition to decreased renal excretion due to the renal resorption, excretion is also reduced in the model by implementing a constant proportion of PFOA 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 of cynomolgus monkeys (Butenhoff et al., 2004b) 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 optimized to fit the data for plasma and urine at lower concentrations and then applied for the 20 mg/kg/day dose, which was assumed to represent a concentration at which renal resorption was saturated. On the basis of the data for the dose of 20 mg/kg/day, the model was able to predict the decline in plasma levels after the cessation of dosing. The predictions were adequate for one of the three modeled monkeys; for the other two monkeys, the model predicted higher serum concentrations than were observed. That discrepancy between model prediction and observations could have occurred because the model did not allow for efflux of PFOA into the glomerular filtrate through transporters on the basolateral surface of the tubular cells. The authors also observed that three of the monkeys had faster CL_R of PFOA than the other three monkeys, indicating interindividual variability in clearance.

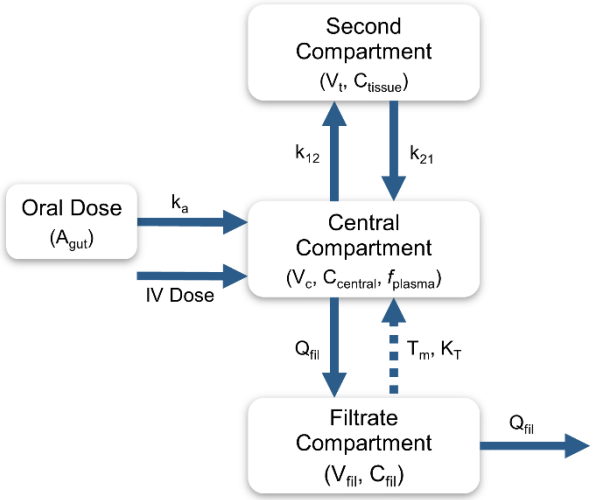


Figure 3-4. Schematic for a Physiologically Motivated Renal-Resorption PK Model for PFOA

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 PFOA (U.S. EPA, 2016c). 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 PFOA 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 PFOA 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 versus total) was estimated in place of establishing a rate of transfer from the tissue to serum, but the rate of transfer from serum to tissue was also estimated from the data. A nonhierarchical model for parameter values was also assumed. Under this assumption, a single numeric value represents all individuals of the same species, sex, and strain. This sex assumption was applied to male and female rats to determine sex-specific parameters because of established sex-specific toxicokinetic differences. Conversely, monkeys and mice were only grouped by species and strain with only female parameters available for mice and male/female monkey data pooled together for a single set of parameters. 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 PFOA PK studies. The monkey PK data were derived from Butenhoff et al. (2004b), and the data for the rats (M/F) were from Kemper et al. (2003). Two strains of female mice were analyzed separately, with CD1 information derived from Lou et al. (2009) and C57BL/6 information derived from DeWitt et al. (2008). 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 is 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 the amount and concentration of the substance can be predicted in specific tissues, in addition to blood. This can be valuable for certain endpoints for which it is expected that a tissue concentration would better reflect the relevant dosimetry compared with blood concentration.

The first PBPK model developed for PFOA was reported in a series of publications by Loccisano et al., which together describe the PK of PFOA in rats, monkeys, and humans, in both adult and developmental (for rat and human) scenarios (Loccisano 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 PFOA 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 PFOA 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 PFOA levels, and Dzierlenga et al. (2020b; 2020c) who applied a model of thyroid disease (Dzierlenga et al., 2019b) to describe changes in PFOA urinary 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 PFOA with gestation and lactation lifestages to describe development and predicted maternal, infant, and breastmilk concentrations over a variety of scenarios including the prediction of maternal levels across multiple pregnancies.

One of the major challenges in the parameterization of PBPK models for PFOA is the estimation of the chemical-dependent parameters such as those involved in protein binding and 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 PFOA. Cheng et al. (2017) went farther in their use of in vitro data and used measurements of PFOA interactions with binding proteins, as well the measured rates of several transporters, to parameterize a rat PBPK model.

No new animal PBPK models for PFOA have been published since the 2016 PFOA HESD (U.S. EPA, 2016c). See the 2016 PFOA HESD (U.S. EPA, 2016c) for a more in-depth review of PFOA PBPK models.

3.4 Noncancer Health Effects Evidence Synthesis and Integration

3.4.1 Hepatic

EPA identified 33 epidemiological studies (reported in 39 publications)[5,6] and 31 animal toxicological studies that investigated the association between PFOA and hepatic effects. Of the epidemiological studies, 21 were classified as *medium* confidence, 8 as *low* confidence, 1 as *mixed* (*medium/low*) confidence, and 9 were considered *uninformative* (Section 3.4.1.1). Of the 31 animal toxicological studies, 5 were classified as *high* confidence, 22 as *medium* confidence, 2 as *low* confidence, and 2 were considered *mixed* (*medium/uninformative* and *medium/low/uninformative*) (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 epidemiology and animal toxicological studies are considered qualitatively in this section (e.g., to inform the weight of the evidence for hazard assessment), 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 PFOA 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). Elevated liver serum biomarkers are frequently an indication of liver injury, though not as specific as structural or functional analyses such as histology findings and liver disease.

There are 13 epidemiological studies (14 publications)[6] from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA exposure and hepatic effects, and study quality evaluations are shown in Figure 3-5. Emmett et al. (2006) and Jain et al. (2014) were rated as *uninformative* and will not be further discussed. Nine out of the 12 remaining studies were rated as *medium* quality and all investigated changes in serum liver enzymes. Results from studies summarized in the 2016 PFOA 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 used 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).

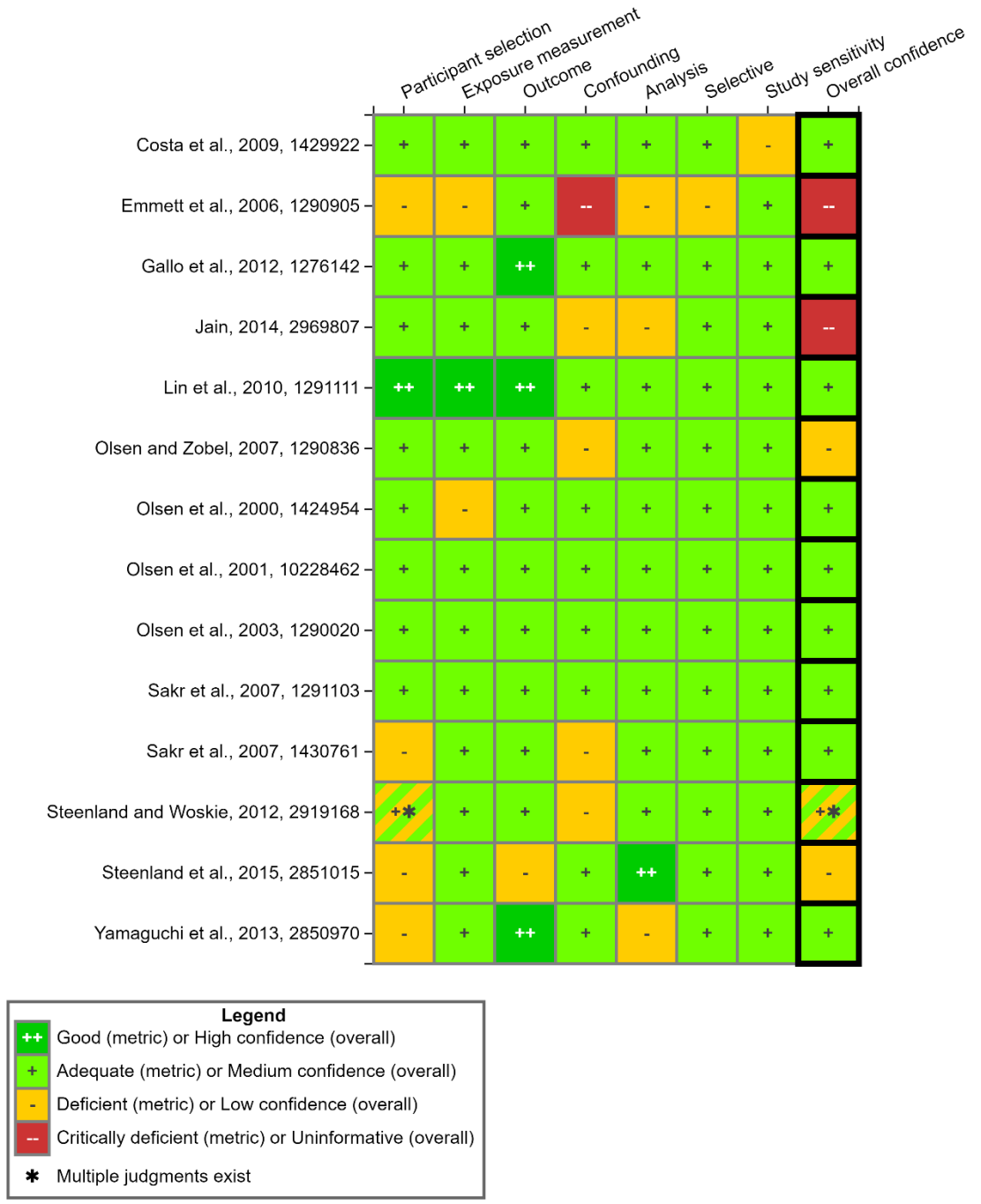


Figure 3-5. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Hepatic Effects Published Before 2016 (References in the 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Lin et al. (2010) is a *medium* confidence study that examined 2,216 adults in the NHANES study (1999–2000, and 2003–2004) and observed that higher serum concentrations of PFOA were associated with abnormal liver enzymes increases in the U.S. general population. For each

increase in log-PFOA, the serum ALT and GGT concentrations (U/L) increased by 1.86 units (95% CI: 1.24, 2.48), and 0.08 units (95% CI: 0.05, 0.11), respectively (Lin et al., 2010). Importantly, when PFOS, PFHxS, and PFNA were simultaneously added in the fully adjusted regression models, the associations remained and were slightly larger; one unit increase in serum log-PFOA concentration was associated with a 2.19 unit (95% CI: 1.4, 2.98) increase in serum ALT concentration (U/L), and a 0.15 unit (95% CI: 0.11, 0.19) increase in serum log-GGT concentration (U/L). Another *medium* confidence cross-sectional study (Yamaguchi et al., 2013) conducted in Japan reported a positive correlation between PFOA and ALT.

A *medium* confidence study in a highly exposed community provides further support for the positive association between PFOA exposure and ALT findings in the U.S. general population. One of the largest studies of PFOA exposure and ALT in adults, Gallo et al. (2012), evaluated 47,092 adults from the C8 Health Project living in communities in Ohio and West Virginia impacted by a manufacturing-related PFOA-contaminated drinking water supply. Natural-log transformed serum PFOA concentrations were associated with ln-ALT in linear regression models (regression coefficient: 0.022; 95% CI: 0.018, 0.025) and with elevated ALT in logistic regression models across deciles of PFOA (OR = 1.10; 95% CI: 1.07, 1.13). The evidence of an association between PFOA and GGT or bilirubin was less consistent. The level of bilirubin increased with increasing PFOA at low PFOA concentrations and decreased with increasing PFOA levels at higher PFOA concentrations, producing an inverse roughly U-shaped curve of the relationship between PFOA and bilirubin.

Several *medium* confidence cross-sectional occupational studies reported that higher concentrations of PFOA were associated with higher liver enzyme levels, such as ALT, AST, GGT, and total bilirubin (Costa et al., 2009; Sakr et al., 2007a; Sakr et al., 2007b). However, other *medium* confidence cross-sectional occupational studies in PFOA production workers reported mostly null findings, with some positive associations with ALT in specific locations or specific years (Olsen and Zobel, 2007; Olsen et al., 2003; Olsen et al., 2001a; Olsen et al., 2000).

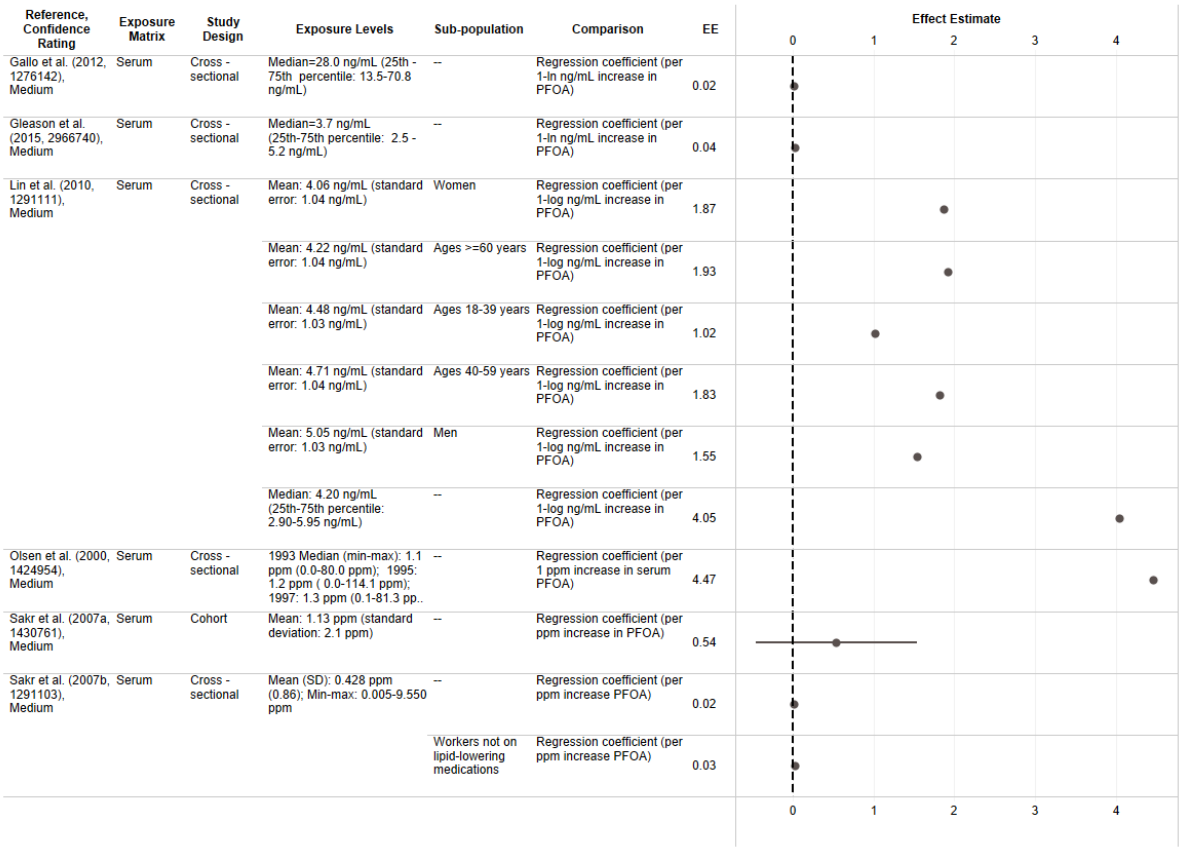


Figure 3-6. Overall ALT Levels from 2016 PFOA HESD Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

The associations with ALT indicate the potential for PFOA to affect liver function; however, studies of functional hepatic endpoints were limited to two studies in an occupational cohort. The first study was a *low* confidence study that observed no association between PFOA and hepatitis or fatty liver disease; however, there was a positive association with non-hepatitis liver disease with a 10-year lag time (Steenland et al., 2015). A *medium* confidence cohort mortality study of workers exposed to PFOA at a DuPont chemical plant in West Virginia observed no association between PFOA exposure levels and nonmalignant chronic liver disease deaths (Steenland and Woskie, 2012).

In conclusion, the majority of the *medium* confidence studies support an association between PFOA exposure and increases in serum ALT in multiple populations, including occupational and highly exposed communities as well as the general population (see Figure 3-6). Multiple studies demonstrated statistically significant increases in ALT (Yamaguchi et al., 2013; Gallo et al., 2012; Lin et al., 2010; Olsen et al., 2000) or elevated ALT (Gallo et al., 2012) after PFOA exposure. Increases were also observed for AST and GGT, though less consistently across the available studies.

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

Reference, confidence	Study Design	Population	ALT[a]	AST[a]	GGT[a]	ALP[a]	Liver Disease[b]	Serum Protein[a]	Albumin[a]
Costa, 2009, 1429922 *Medium*	Cross-sectional	Occupational	↑↑	↑	↑↑	↑↑	NA	↓	↓
Gallo, 2012, 1276142 *Medium*	Cross-sectional	Adults	↑↑	NA	↑↑	NA	NA	NA	NA
Lin, 2010, 1291111 *Medium*	Cohort	Adults	↑↑	NA	↑↑	NA	NA	NA	NA
Olsen and Zobel, 2007, 1290836 *Low*	Cross-sectional	Occupational	↑↑	↓	↑↑	↑↑	NA	NA	NA
Olsen, 2003, 1290020 *Medium*	Cross-sectional	Occupational	↑↑	–	↑	NA	NA	NA	NA
Olsen, 2001, 10228462 *Medium*	Cohort	Occupational	↑	↑	↓	↑	NA	NA	NA
Olsen, 2000, 1424954 *Medium*	Cross-sectional	Occupational	↑↑	NA	NA	NA	NA	NA	NA
Sakr, 2007, 1291103 *Medium*	Cross-sectional	Occupational	↑	↑	↑↑	NA	NA	NA	NA
Sakr, 2007, 1430761 *Medium*	Cohort	Occupational	↑	↑↑	↑	NA	NA	NA	NA
Steenland and Woskie, 2012, 2919168 *Mixed c*	Cohort	Occupational	NA	NA	NA	NA	–	NA	NA
Steenland, 2015, 2851015 *Low*	Cohort	Occupational	NA	NA	NA	NA	↑	NA	NA
Yamaguchi, 2013, 2850970 *Medium*	Cross-sectional	Adults and adolescents	↑↑	↑↑	↑	NA	NA	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.

Emmett et al., 2006, 1290905 was not included in the table due to their *uninformative* overall study confidence ratings.

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).

[c] Steenland and Woskie, 2012, 2919168 was rated *medium* confidence for comparisons with the DuPont referent group and *low* confidence for comparisons with the U.S. population.

3.4.1.1.2 Study Quality Evaluation Results for the Relevant Epidemiology Studies Identified from the Updated Literature Review

There are 20 epidemiological studies (25 publications)[7] that were identified from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and hepatic effects. Study quality evaluations for these 25 publications are shown in Figure 3-7 and Figure 3-8. Of these 25 publications, 12 were classified as *medium* confidence, 6 as *low* confidence, and 7 were considered *uninformative*.

The following informative studies examined liver enzymes in adults: two cross-sectional studies (Nian et al., 2019; Wang et al., 2012); 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 cohort with retrospective exposure assessment (Darrow et al., 2016); one prospective cohort (Salihovic et al., 2018); one open-label controlled trial (Convertino et al., 2018); and one occupational cohort (Olsen et al., 2012). Most of these studies were in general population adults, but some assessed specific populations such as the elderly (Salihovic et al., 2018) and fluorochemical plant workers (Olsen et al., 2012; Wang et al., 2012). In addition, one occupational cohort (Girardi and Merler, 2019) and three cross-sectional studies (Liu et al., 2018b; Darrow et al., 2016; Rantakokko et al., 2015) examined functional liver endpoints in adults (histology, liver disease, hepatic fat mass). In children and adolescents, four studies were available, including one cohort (Mora et al., 2018) and three cross-sectional studies (Jin et al., 2020; Attanasio, 2019; Khalil et al., 2018), with one examining histology endpoints (Jin et al., 2020).

All of the studies of adults and children in the general population, except for Darrow et al. (2016), and one of the two occupational cohorts (Olsen et al., 2012) measured exposure to PFOA using biomarkers in blood. Darrow et al. (2016) modeled exposure based on residential history, drinking water sources, and water consumption rates. The other occupational cohort study estimated PFOA exposure based on job duties (Girardi and Merler, 2019). 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), use of PFAS as the dependent variable (Jain, 2020a), or in cases for which the independent variable is a genetic variant and thus not affected by PFAS exposure (Fan et al., 2014).

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix D, (U.S. EPA, 2024a)). For endpoints with fewer studies (e.g., AST serum levels, functional assays), the evidence synthesis below included details on any *low* confidence studies available in addition to *high* and *medium* confidence studies. Studies considered *uninformative* were not considered further in the evidence synthesis.

[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); and Gleason et al. (2015) all used NHANES data from overlapping years.

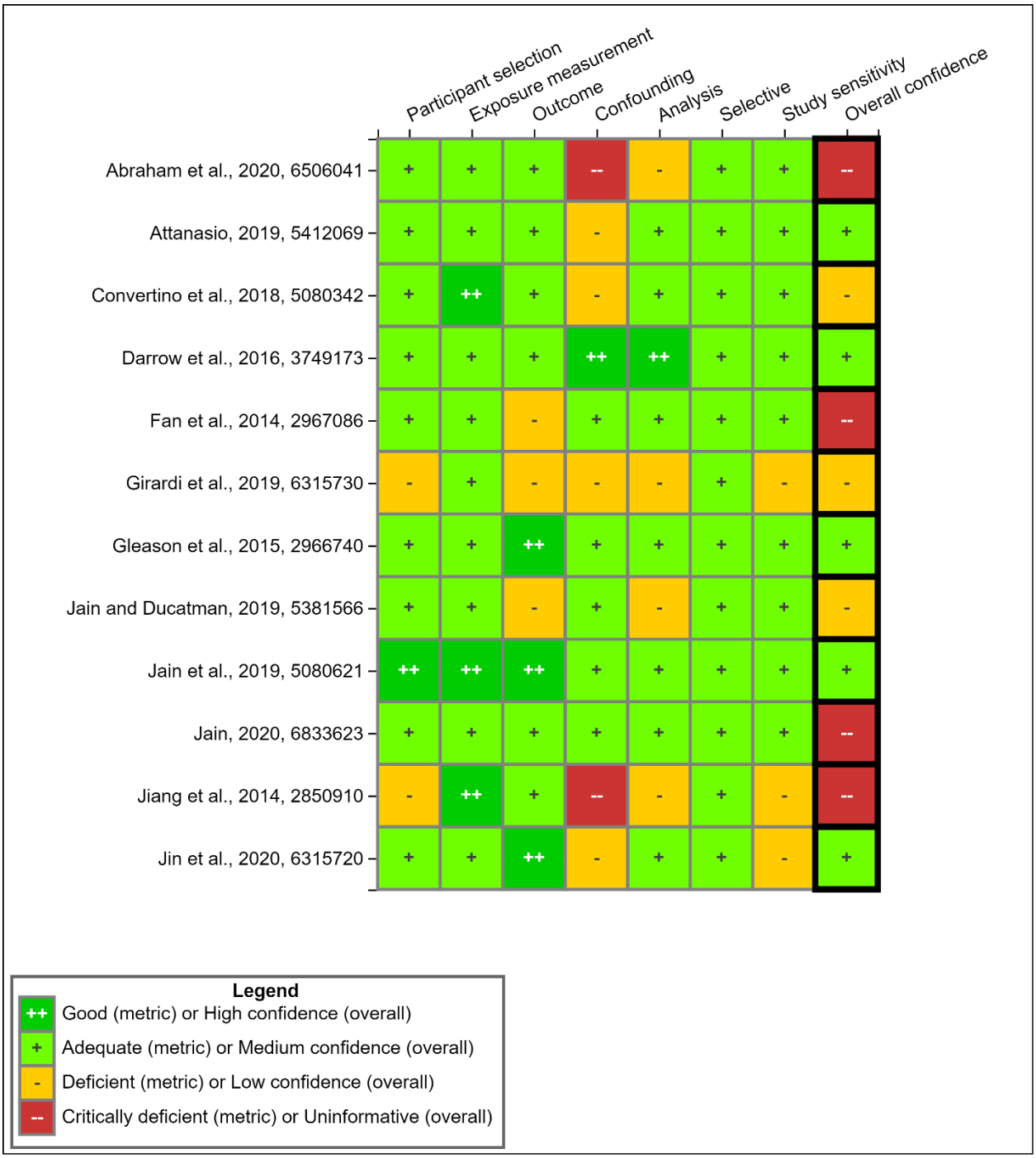


Figure 3-7. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Hepatic Effects[a]

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 et al. (2018d); Gleason et al. (2015) all use NHANES data from overlapping years.

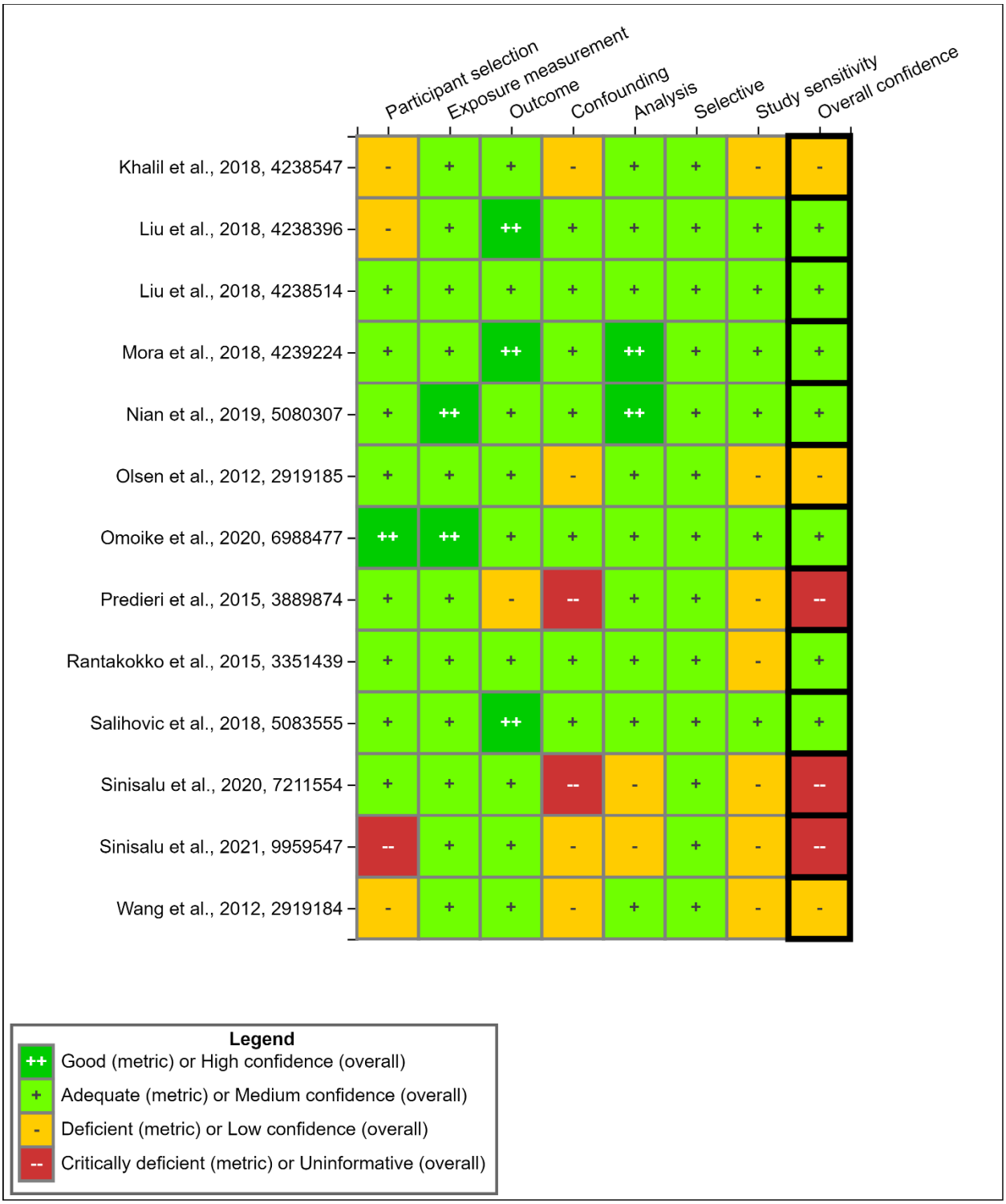


Figure 3-8. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Hepatic Effects (Continued)[a]

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 et al. (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 studies that examined ALT are presented in the Appendix (U.S. EPA, 2024a). As shown in Figure 3-9 and Figure 3-10, of the available informative studies that measured ALT in adults, statistically significant positive associations between ALT and PFOA (i.e., increased ALT as a continuous measure with higher PFOA exposure levels) were observed in all of the *medium* confidence studies, which consisted of one cross-sectional study (Nian et al., 2019), two cohort studies (Salihovic et al., 2018; Darrow et al., 2016), and two NHANES publications (Jain, 2019; Gleason et al., 2015).

In addition, an exposure-response gradient was observed in the single study that examined quintiles of exposure (Darrow et al., 2016). This study additionally examined elevated ALT as a dichotomous outcome and reported an OR of 1.16 (95% CI: 1.02, 1.33) in the highest versus lowest quintiles of exposure (Figure 3-9). The positive associations in Jain (2019) were observed only in certain sub-groups (e.g., by renal function (i.e., glomerular filtration stage), obesity status) and according to no clear pattern across sub-groups (NHANES 2003–2014), but in Gleason et al. (2015), the positive association was observed in the entire study population (NHANES 2007–2010). Results of the *low* confidence studies of ALT in adults are presented in Appendix D (U.S. EPA, 2024a) and not described further in this section because there are numerous *medium* confidence studies describing ALT measures in adults that were included in the 2016 PFOA HESD or identified in the updated literature search.

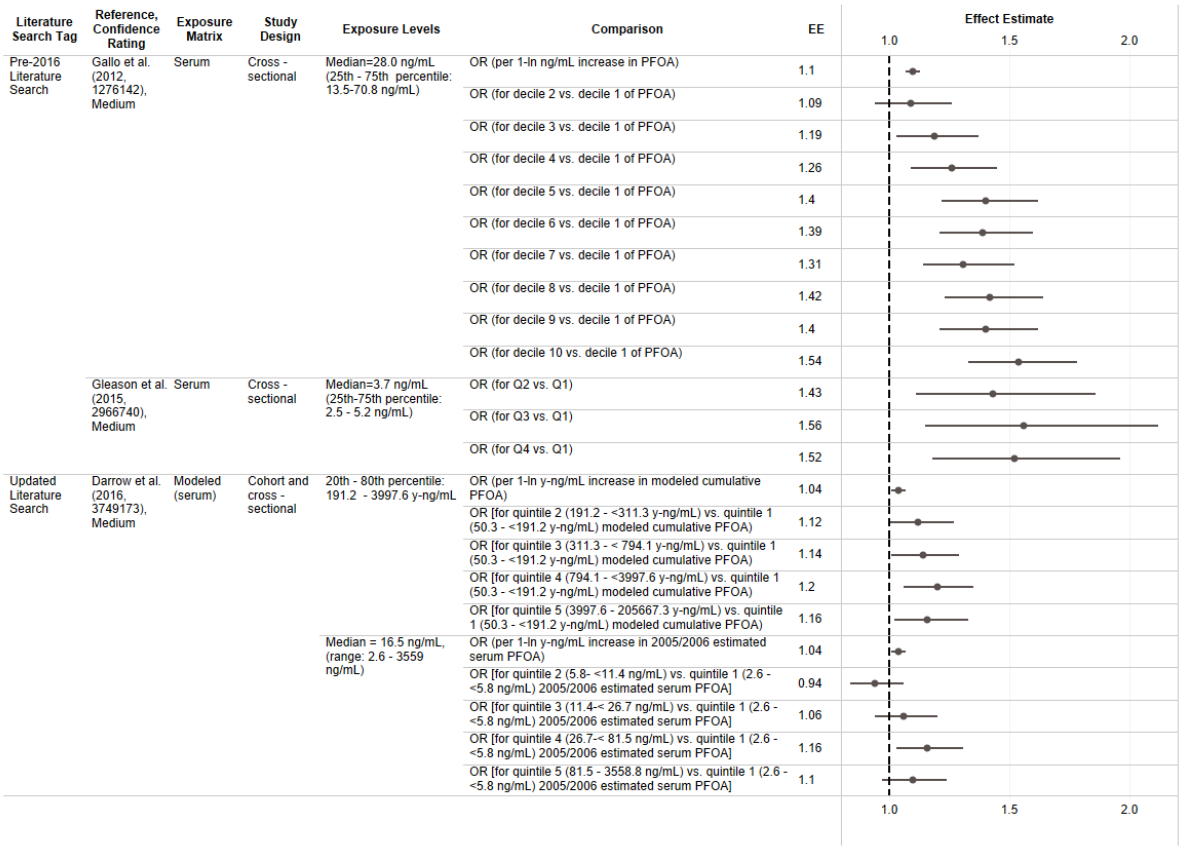


Figure 3-9. Odds of Elevated ALT Levels from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

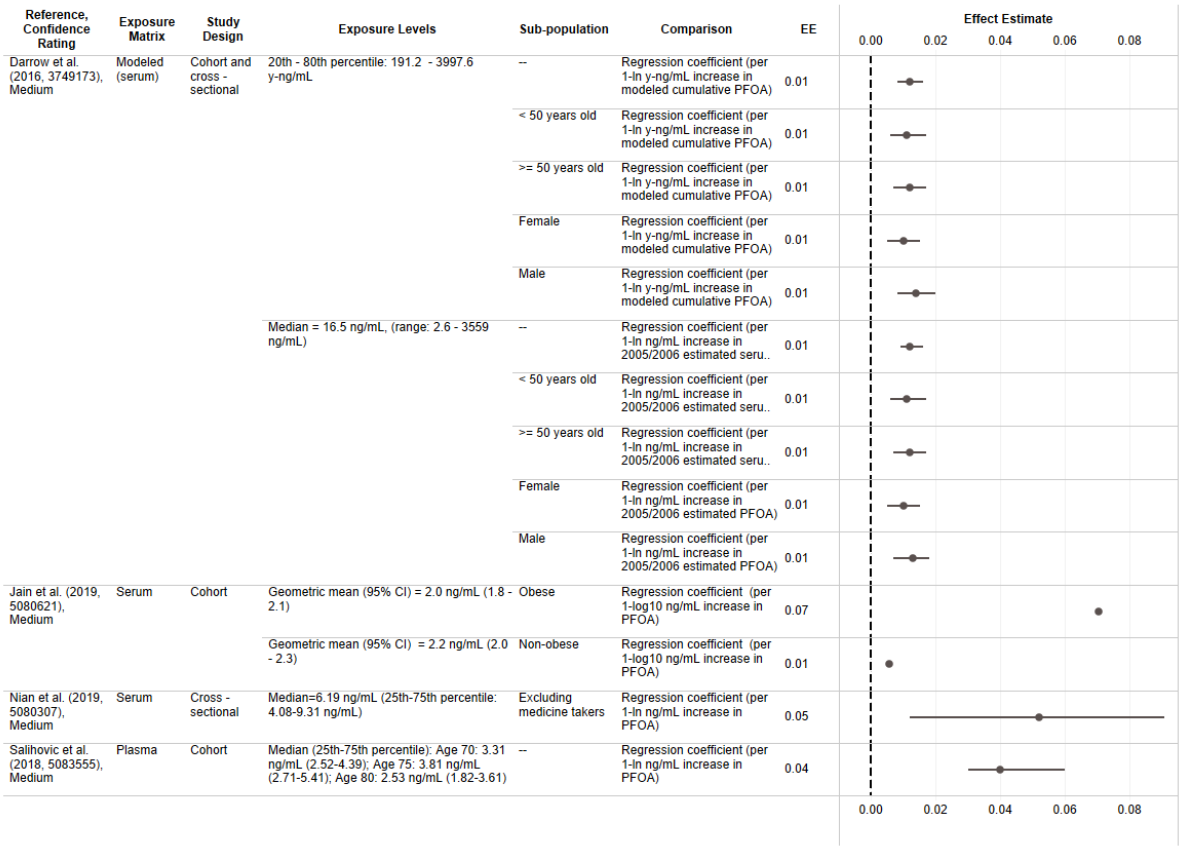


Figure 3-10. ALT Levels from *Medium* Confidence Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

In children and adolescents, positive associations were observed in girls (with exposure-response gradient across quartiles) in the *medium* confidence study by Attanasio et al. (2019) and in the *low* confidence study of obese children (Khalil et al., 2018). However, inverse associations were observed in boys in Attanasio et al. (2019) and 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.

The studies that examined AST are presented in Appendix D (U.S. EPA, 2024a). In adults in the general population, positive associations were observed in the two *medium* confidence studies (Jain, 2019; Nian et al., 2019). In the two *low* confidence studies of fluorochemical plant workers (Olsen et al., 2012; Wang et al., 2012), no associations were observed. In children including adolescents, the *medium* confidence study (Attanasio, 2019) reported a positive association in girls but an inverse association in boys. In the *low* confidence study (Khalil et al., 2018), the direction of association was inverse, but the result was extremely imprecise. For the other liver enzymes (bilirubin, GGT), results were generally consistent with those of ALT and AST, with the exception that inverse associations for bilirubin were observed in some studies (Salihovic et al., 2018; Darrow et al., 2016).

For functional measures of liver injury, two *medium* confidence studies (one in adults and one in children including adolescents) examined histology endpoints. Both studies examined lobular inflammation. Rantakokko et al. (2015) reported that higher PFOA exposure levels were associated with extremely reduced odds of lobular inflammation (OR = 0.02, $p < 0.05$), whereas Jin et al. (2020) reported the opposite direction of association, though the results in the latter study were nonmonotonic and not statistically significant. Jin et al. (2020) additionally reported lower odds of ballooning and portal inflammation, but higher odds of steatosis (association nonmonotonic) and nonalcoholic steatohepatitis. Three additional studies examined some form of liver disease. In a *medium* confidence study, Darrow et al. (2016) reported no increases in any liver disease or specifically enlarged liver, fatty liver, or cirrhosis. In contrast, in a *low* confidence study, Girardi and Merler (2019) reported that workers at a PFAS production plant had higher mortality from liver cancer or cirrhosis when compared with regional mortality statistics and a control group of nonchemical workers ($p < 0.05$ for some comparisons). Lastly, a second *low* confidence study by Liu et al. (2018b) examined hepatic fat mass and found no correlation with PFOA exposure.

3.4.1.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 12 animal toxicological studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and 19 studies identified from recent systematic literature searches and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and hepatic effects. Study quality evaluations for these 31 studies are shown in Figure 3-11 and Figure 3-12.

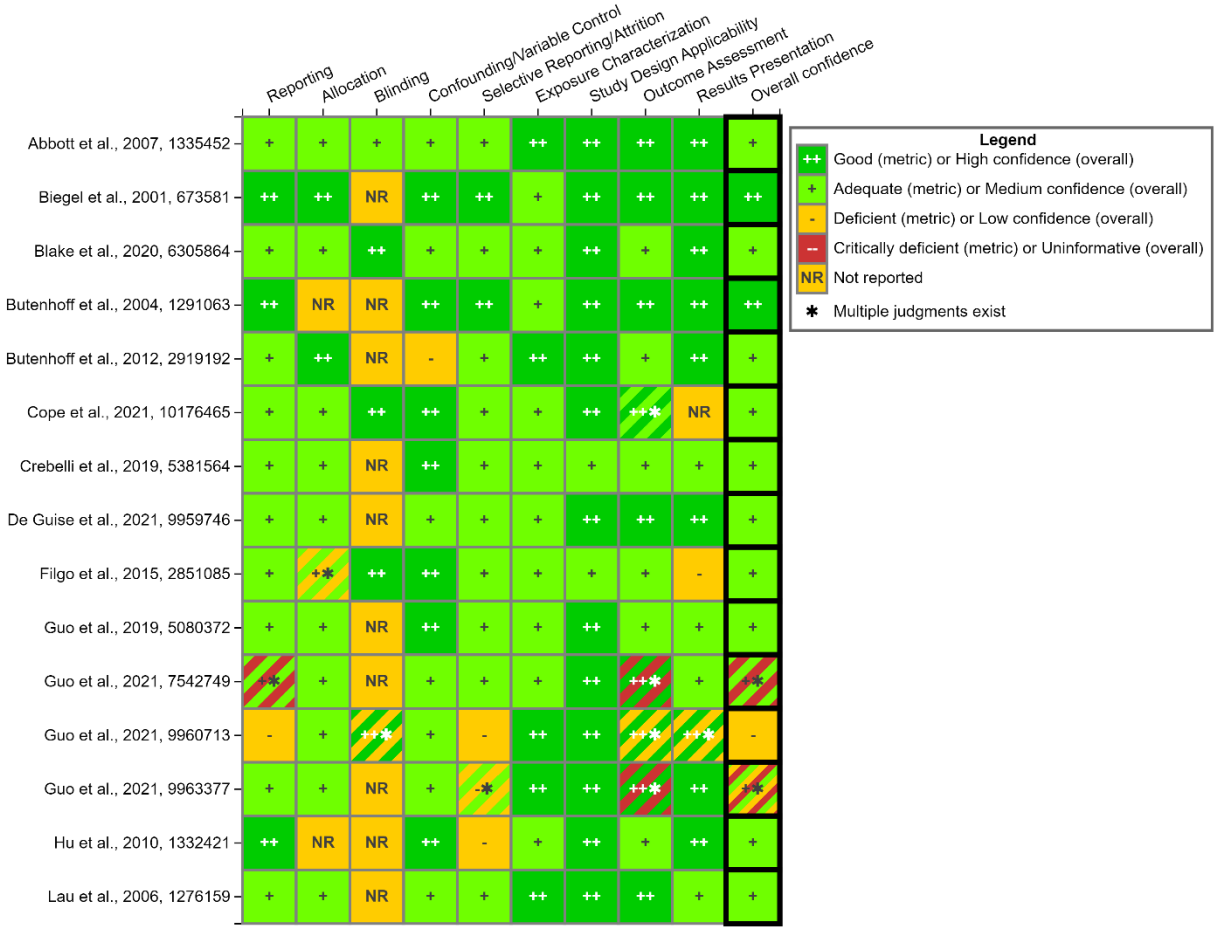


Figure 3-11. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Hepatic Effects

Interactive figure and additional study details available on HAWC.

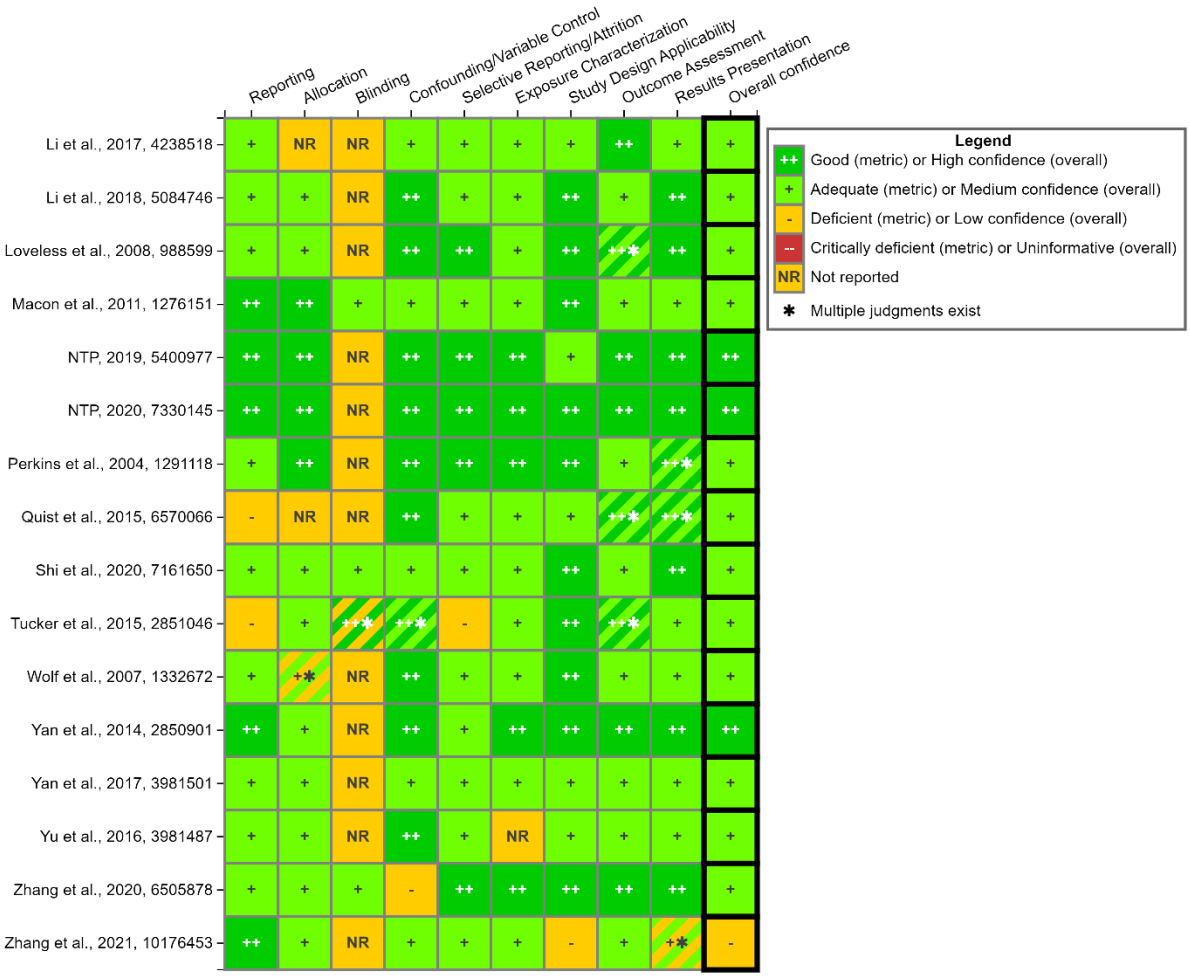


Figure 3-12. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Hepatic Effects (Continued)

Interactive figure and additional study details available on HAWC.

Hepatic effects (e.g., increased absolute and relative liver weight, altered clinical parameters indicating potential liver injury, and histopathological alterations of liver tissue) were observed in male and female mice, rats, and monkeys after oral PFOA exposures of different durations. Data from numerous studies provide evidence confirming that the liver is a target of PFOA toxicity.

3.4.1.2.1 Liver Weight

Generally, increases in absolute and/or relative liver weight were observed in all available PFOA animal toxicological studies, regardless of species, sex, lifestage, and exposure paradigm (Figure 3-13 and Figure 3-14). Significant increases in absolute and relative liver weight were reported at doses as low as 0.05 mg/kg/day and 0.31 mg/kg/day, respectively (Li et al., 2017b; Yan et al.,

2014), and were often observed at the lowest dose administered in each study. In male mice, significant increases in absolute and/or relative liver weights were observed at doses ranging from 0.31 to 30 mg/kg/day after 4–5 weeks of exposure (Guo et al., 2021a; Shi et al., 2020; Crebelli et al., 2019; Guo et al., 2019; Li et al., 2017b; Yu et al., 2016; Yan et al., 2014; Minata et al., 2010; Loveless et al., 2008). Similarly, significant increases in absolute and relative liver weights were reported in male rat short-term/subchronic studies at doses of 0.625–30 mg/kg/day (NTP, 2019; Cui et al., 2009; Loveless et al., 2008; Perkins et al., 2004). Two subchronic dietary studies in adult male rats with exposures lasting 13–16 weeks reported significantly increased absolute and relative liver weights at doses as low as 1 mg/kg/day (NTP, 2020; Perkins et al., 2004). In one chronic study in male Crl:CD BR (CD) rats, relative liver weight was significantly increased after 15 months of exposure to 13.6 mg/kg/day via the diet (Biegel et al., 2001). Similar results were observed at the 1-year interim sacrifice of a 2-year dietary study in male Sprague-Dawley rats exposed to 14.2 mg/kg/day PFOA, but the effect was not statistically significant at the 2-year timepoint (Butenhoff et al., 2012). Male cynomolgus monkeys orally administered PFOA capsules daily for 26 weeks also had significantly increased absolute liver weights at doses ≥3 mg/kg/day, though the increase in relative liver weight was only statistically significant in the highest dose group (30/20 mg/kg/day) (Butenhoff et al., 2002).

Several systemic toxicity studies evaluating liver weight in female mice and rats after short-term, subchronic, or chronic PFOA exposures are also available (De Guise and Levin, 2021; NTP, 2020; Zhang et al., 2020b; NTP, 2019; Li et al., 2017b; Butenhoff et al., 2012). Two 28-day studies in female mice reported significant increases in absolute liver weight at doses ranging from 0.05 to 5 mg/kg/day (relative liver weight not reported) (Zhang et al., 2020b; Li et al., 2017b). A third 28-day study in female B6C3F1 mice reported significant increases in absolute and relative liver weights at both doses tested (1.88 and 7.5 mg/kg/day) (De Guise and Levin, 2021). NTP (2019) conducted a 28-day gavage study in female Sprague-Dawley rats and reported significant increases in both absolute and relative liver weights at doses ≥25 mg/kg/day. In a chronic feeding study (see study design details in Section 3.4.4.2.1.2), NTP (2020) reported significant increases in absolute and relative liver weight in female Sprague-Dawley rats after 16 weeks of exposure to 63.4 but not 18.2 mg/kg/day PFOA. A 2-year feeding study in female Sprague-Dawley rats similarly found no significant difference in absolute or relative liver weight at doses of 1.6 or 16.1 mg/kg/day PFOA (Butenhoff et al., 2012).

There are also multiple reproductive and developmental toxicity studies that report maternal and/or offspring liver weight in rodents after gestational PFOA exposures. Blake et al. (2020) reported significant increases in absolute and relative liver weights in CD-1 mouse dams exposed to PFOA at doses of 1 or 5 mg/kg/day from GD 1.5 to GD 11.5 or GD 1.5 to GD 17.5. Yahia et al. (2010) similarly reported significant increases in maternal ICR mouse absolute liver weights at doses ≥5 mg/kg/day and relative liver weights at doses ≥1 mg/kg/day. Quist et al. (2015) exposed pregnant CD-1 mice to PFOA from GD 1 to GD 17. At PND 21, significantly increased relative liver weights in offspring were observed as low as 0.3 mg/kg/day. In a 2-generation reproductive toxicity study in Sprague-Dawley rats (Butenhoff et al., 2004a), P_0 dams dosed with 1, 3, 10, or 30 mg/kg/day PFOA at least 70 days prior to mating through lactation did not show consistent alterations in absolute or relative liver weights at the time of sacrifice on PND 22. However, significantly increased absolute and relative liver weights were observed in P_0 males and male F_1 offspring starting at the lowest dose of 1 mg/kg/day, whereas no statistically significant differences in absolute or relative liver weights were reported for female F_1 offspring.

Several other developmental toxicity studies reported significantly increased maternal, fetal, and/or pup liver weights associated with gestational PFOA exposure, but the authors did not further examine tissue or serum samples for hepatic effects (Cope et al., 2021; Li et al., 2018a; Tucker et al., 2014; Macon et al., 2011; White et al., 2011; White et al., 2009; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006). For example, White et al. (2011) orally dosed pregnant CD-1 mice with 0, 1, or 5 mg/kg/day PFOA from GD 1 to GD 17. F_1 offspring liver-to-body weight ratios were significantly increased at 1 mg/kg/day on PND 22 and at 5 mg/kg/day on PND 22 and PND 42. Macon et al. (2011) exposed pregnant CD-1 mice to PFOA from GD 1 to GD 17 (full gestation) or GD 10 to GD 17 (late gestation). At PND 7, significantly increased absolute and relative liver weights in offspring were observed as low as 0.3 mg/kg/day after full-gestation exposure; significantly increased absolute and relative liver weights were also observed at the high dose of 1 mg/kg/day PFOA after late-gestation exposure (PND 4 and PND 7; relative liver weights were also significantly increased at PND 14). Wolf et al. (2007) reported that offspring of pregnant CD-1 mice orally dosed with 0 and 5 mg/kg/day on GD 7–GD 17, GD 10–GD 17, GD 13–GD 17, and GD 15–GD 17 or with 20 mg/kg/day on GD 15–GD 17 had significantly increased liver-to-body weight ratios at PND 22. White et al. (2009) reported that offspring of CD-1 mice exposed to 5 mg/kg/day PFOA during gestation or during gestation plus lactation had significantly increased liver-to-body weight ratios on PND 1. Inconsistent results were observed on PND 22 and PND 128 in male and female CD-1 mice gestationally exposed to 0.1 and 1 mg/kg/day PFOA from GD 1.5 to GD 17.5 and then given either a high- or low-fat diet starting on PND 22 (Cope et al., 2021). Specifically, increased relative liver weights were observed at PND 22 for both males and females exposed to 1 mg/kg/day (statistically significant in males only), but not at PND 128 (Cope et al., 2021). One study reported no significant change in relative liver weights, which were only measured on PND 48 in the female offspring of C57BL/6N mouse dams exposed to 0.5 or 1 mg/kg/day PFOA in drinking water from GD 6 to GD 17 (Hu et al., 2010).

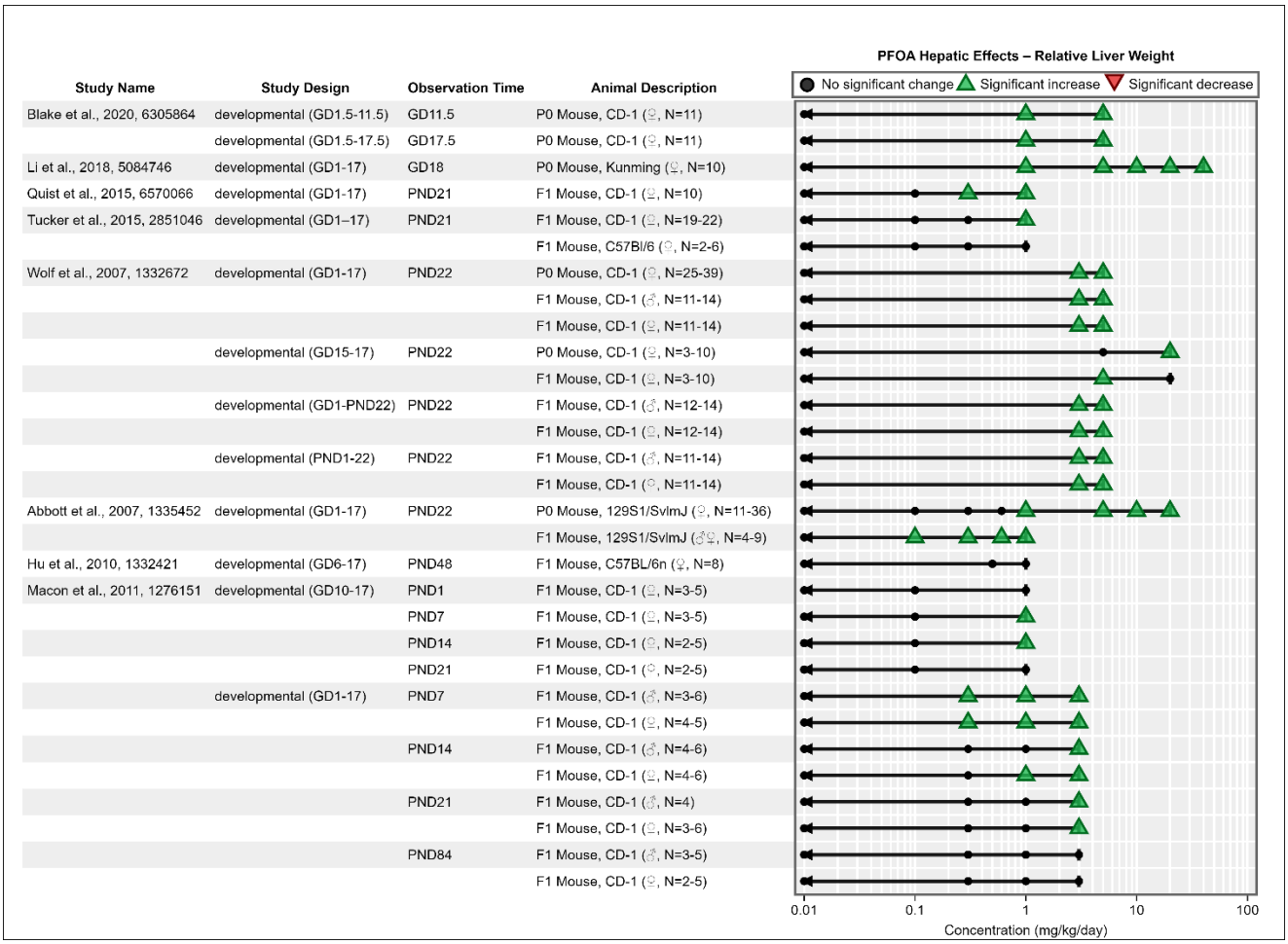


Figure 3-13. Relative Liver Weight in Rodents Following Exposure to PFOA (logarithmic scale)

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

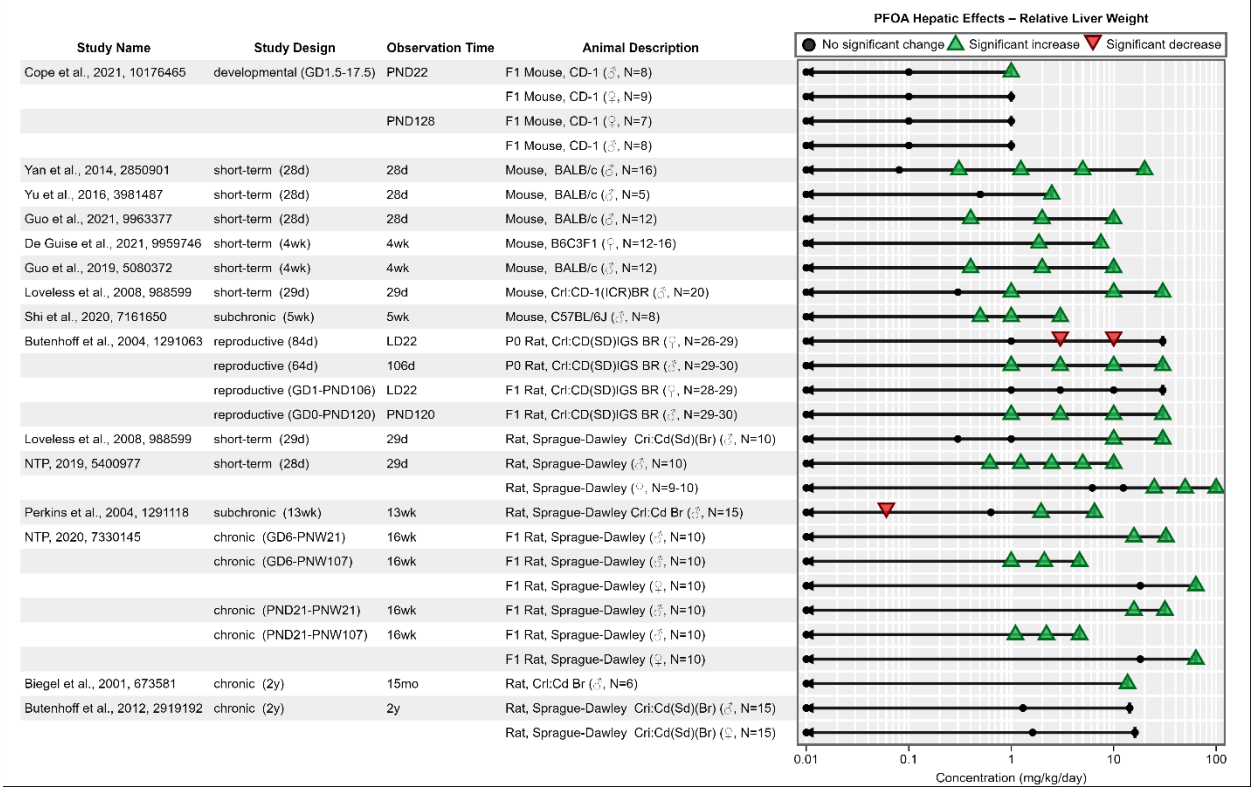


Figure 3-14. Relative Liver Weight in Rodents Following Exposure to PFOA (Continued, logarithmic scale)

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

3.4.1.2.2 Clinical Chemistry Measures

Albumin, a blood protein that plays a major role in PFOA toxicokinetics (Section 3.3), is synthesized by the liver. Increases in serum albumin were reported in several short-term and chronic studies in male rodents, with increases observed at doses as low as 0.4 and 1.3 mg/kg/day in mice and rats, respectively (NTP, 2020; Guo et al., 2019; Yan et al., 2014; Butenhoff et al., 2012). Females appeared to be less sensitive, with increased albumin at doses ≥25 mg/kg/day in rats after short-term or chronic exposures and no significant differences or inconsistent decreases in pregnant mice after gestational exposures (Blake et al., 2020; NTP, 2020, 2019; Butenhoff et al., 2012; Yahia et al., 2010). The albumin/globulin ratio was significantly increased in both adult males and females after PFOA exposure for 28 days or 16 weeks (NTP, 2020; Guo et al., 2019; NTP, 2019).

Similar to albumin, inconsistent results were observed for total protein, with statistically significant decreases observed in some studies in male rats (NTP, 2020, 2019) and pregnant female mice in one study (Blake et al., 2020), and increases or no significant changes observed in several other studies in adult male rats or mice (Guo et al., 2019; Butenhoff et al., 2012) and in female rats (NTP, 2020, 2019; Butenhoff et al., 2012).

Increases in enzymes including ALT, ALP, and AST following PFOA exposures were observed across multiple species, sexes, and exposure paradigms (Figure 3-15 (male mice), Figure 3-16 (male rats), Figure 3-17 (female rodents)). These enzymes are often useful indicators of hepatic enzyme induction, hepatocellular damage, or hepatobiliary damage as increased serum levels are thought to be due to hepatocyte damage resulting in release into the blood (U.S. EPA, 2002a). Alterations in serum enzymes are generally considered to reach biological significance and indicate potential adversity at levels ≥twofold compared with controls (i.e., ≥100% change relative to controls) (Hall et al., 2012; U.S. EPA, 2002a).

In adult male mice dosed with PFOA for 4–5 weeks, statistically significant increases in ALT and/or AST were observed at PFOA exposure levels ranging from 2 to 21.6 mg/kg/day (Crebelli et al., 2019; Guo et al., 2019; Yan et al., 2014; Minata et al., 2010). Increases in ALT were ≥100% above control values at doses as low as 1.25 mg/kg/day (Yan et al., 2014). Biologically significant increases in AST were only observed in two of these studies at doses ≥20 mg/kg/day (Yan et al., 2014; Minata et al., 2010). In the only short-term study examining ALP in male mice, ALP was significantly increased at concentrations of 5 and 20 mg/kg/day after 28-day exposure (Yan et al., 2014); serum ALP levels were ≥100% change at doses of 1.25 mg/kg/day and higher.

In male CD-1 mice gestationally exposed to 0.1 and 1 mg/kg/day from GD 1.5 to GD 17.5 and then fed either a high- or low-fat diet starting on PND 22, no significant changes were observed in ALT, AST, or ALP on PND 128 (Cope et al., 2021).

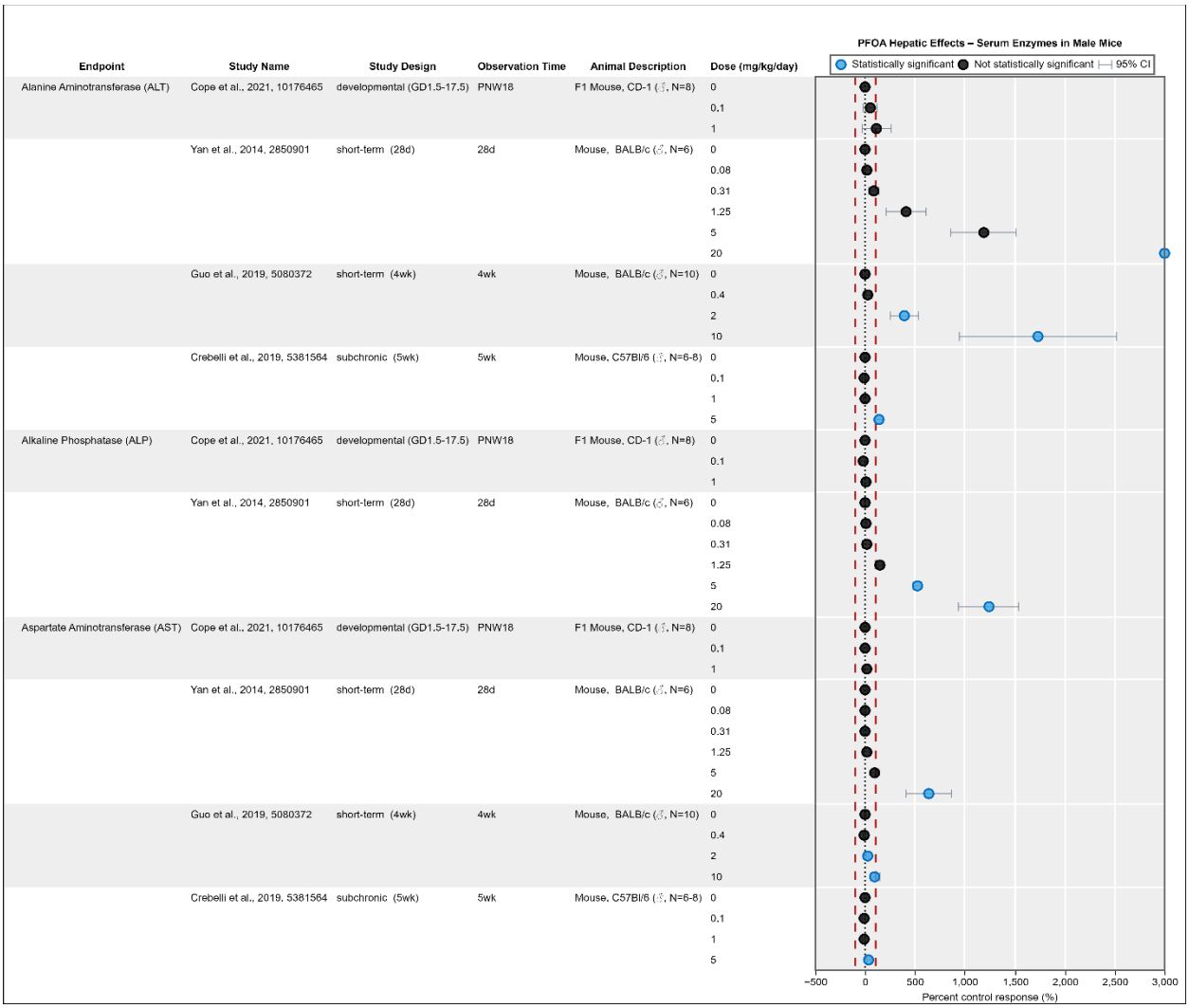


Figure 3-15. Percent Change in Serum Enzyme Levels Relative to Controls in Male Mice Following Exposure to PFOA[a,b]

Interactive figure and additional study details available on HAWC here and here.
ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; d = day; wk = week; CI = confidence interval.
[a] The red dashed lines indicate a 100% increase or 100% decrease from the control response.
[b] Results for Yan et al. (2014) are presented for six doses (0, 0.08, 0.31, 1.25, 5, and 20 mg/kg/day), and a statistically significant response of 7,000% occurred at the highest dose for the ALT endpoint. The axis has been truncated at 3,000% to allow results at lower doses for other studies and endpoints to be legible.

NTP (2020, 2019) reported significantly increased ALT and ALP at all doses tested in the 28-day and 16-week exposures of male Sprague-Dawley rats to PFOA (dose range of 0.625–32.1 mg/kg/day). However, increases in ALT did not exceed 100% change in either study. Similarly, increases in ALP did not exceed 100% change in the 28-day gavage study (NTP, 2019) and only exceeded 100% change with doses ≥15.6 mg/kg/day at the 16-week interim time point of the chronic dietary study (NTP, 2020). In another chronic dietary study, Butenhoff et al. (2012) generally observed increased ALT and ALP in male Sprague-Dawley rats dosed with 1.3 and 14.2 mg/kg/day PFOA at time points ranging from 3 months to 2 years of administration.

Increases in ALT were above or approximately 100% change in both dose groups at 6, 12, and 18 months of exposure. ALP levels were elevated at all time points with 14.2 mg/kg/day PFOA but were only above 100% change at the 18-month time point. AST was also less sensitive than ALT or ALP in male rats. NTP (2019) observed statistically significant but not biologically significant increases in AST at doses of 2.5 mg/kg/day and higher (up to 10 mg/kg/day) after 4 weeks. Butenhoff et al. (2012) did not observe biologically significant increases in AST at any time of assessment during the 2-year feeding study.

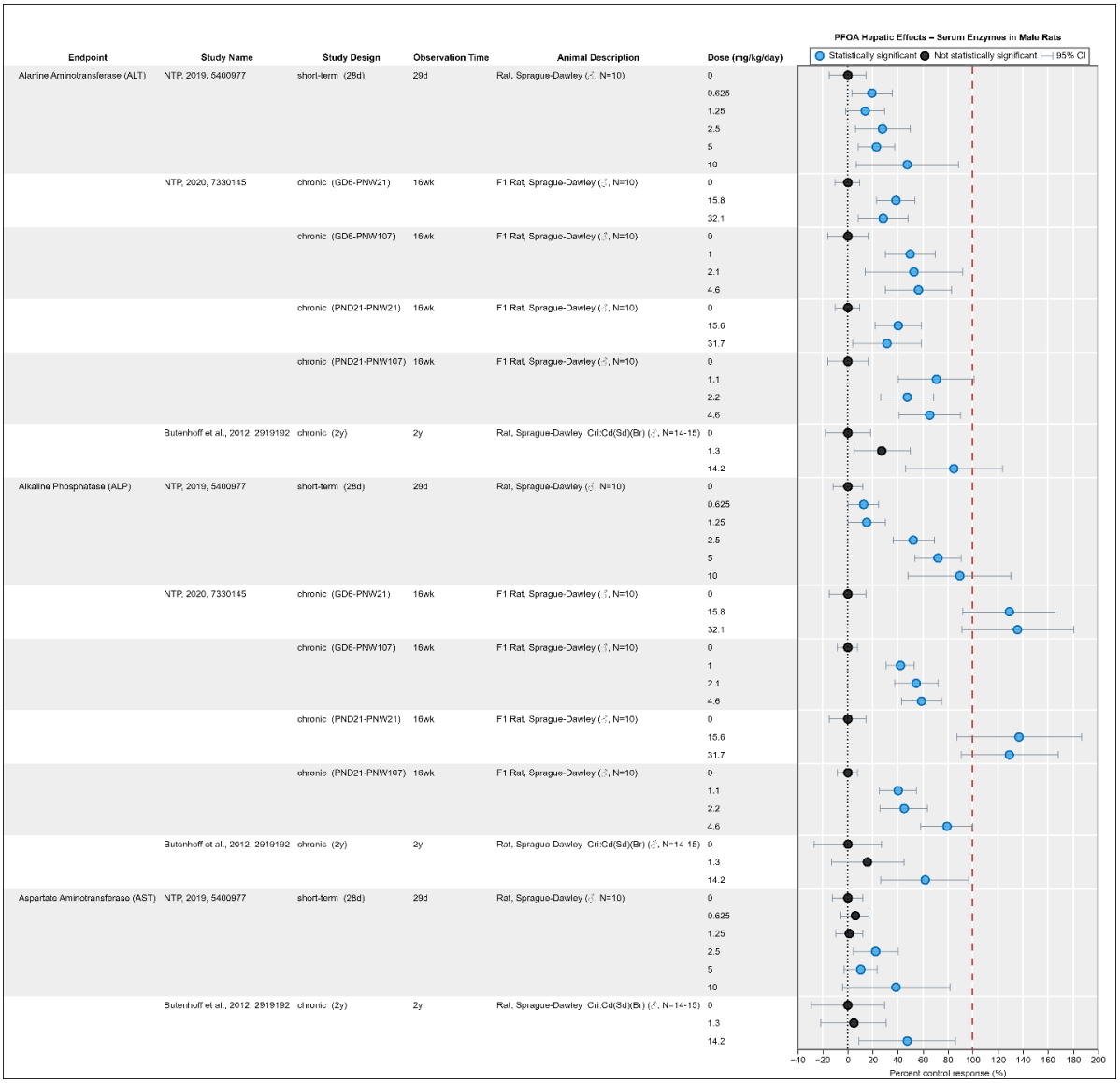


Figure 3-16. Percent Change in Serum Enzyme Levels Relative to Controls in Male Rats Following Exposure to PFOA[a]

Interactive figure and additional study details available on HAWC here and here.
ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GD = gestation day; PND = postnatal day; PNW = postnatal week; F_1 = first generation; d = day; wk = week; CI = confidence interval.
[a] The red dashed line indicates a 100% increase from the control response.

In addition to the findings in rodents, no consistent responses of serum enzymes were observed in the one available study in male cynomolgus monkeys dosed with PFOA for 26 weeks (Butenhoff et al., 2002).

The only available studies measuring ALT, AST, or ALP in female mice were after gestational PFOA exposures. Blake et al. (2020) reported no statistically significant effects on ALT or ALP levels in CD-1 dams after gestational PFOA exposure, and significantly increased AST (113% increase over control) only after exposure to the high dose of 5 mg/kg/day from GD 1.5 to GD 17.5. In contrast, Yahia et al. (2010) reported biologically significant increases in ALT and AST in dams after gestational exposure to 5 or 10 mg/kg/day PFOA (150% and 372% increase from control ALT levels, respectively; 312% and 813% increase from control AST levels, respectively). Biologically significant increases in ALT, ALP, and AST were only observed at the highest dose of 10 mg/kg/day. In a study in which female CD-1 mice were gestationally exposed to 0.1 or 1 mg/kg/day from GD 1.5 to GD 17.5 and then given a low-fat diet starting on PND 22, no significant changes were observed in ALT, AST, or ALP on PND 128 (Cope et al., 2021). However, in the group of females exposed to 1 mg/kg/day and then given a high-fat diet, statistically significant increases were observed in ALT (130% control), AST (23% control), and ALP (43% control).

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

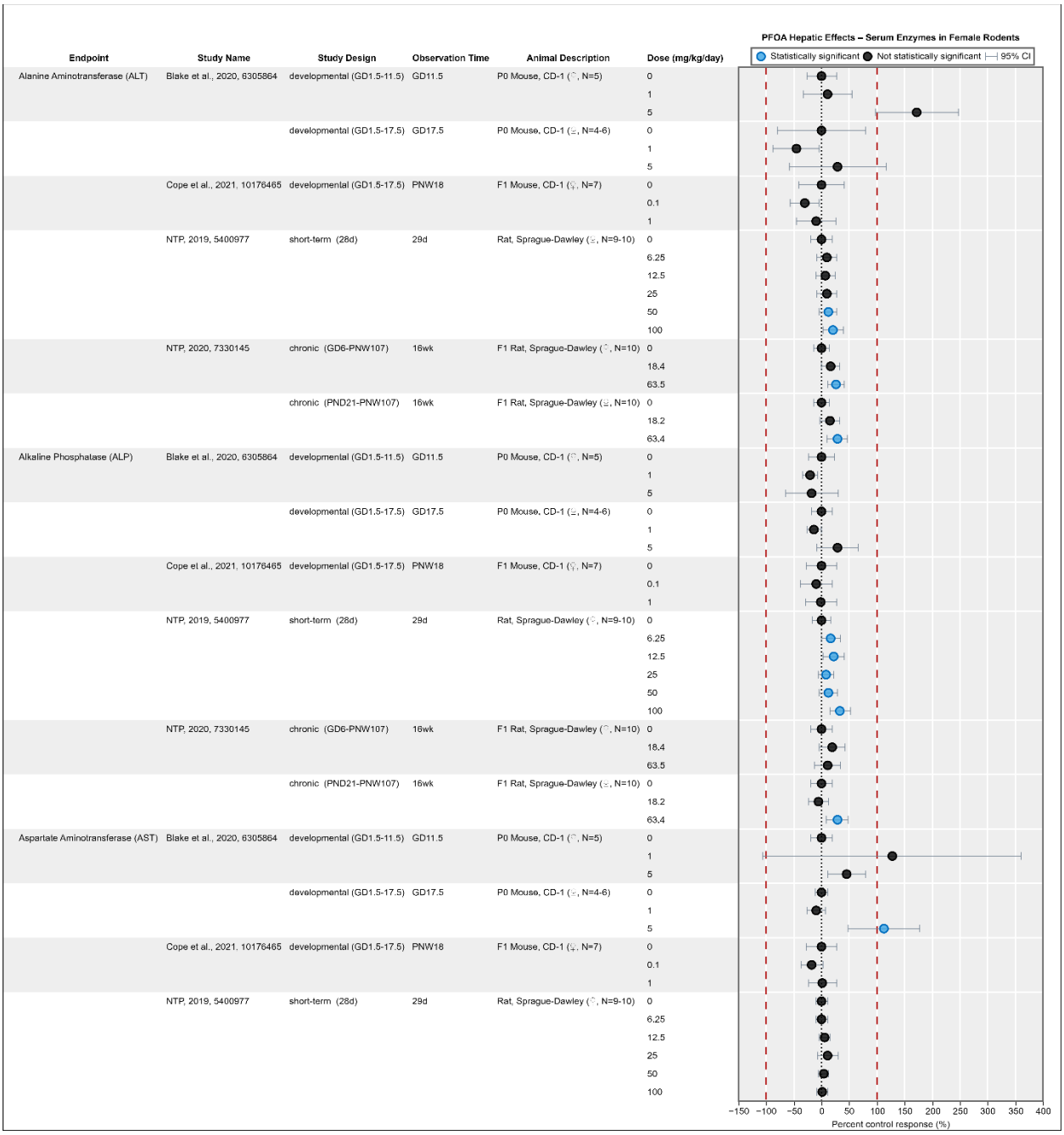


Figure 3-17. Percent Change in Enzyme Levels Relative to Controls in Female Rodents Following Exposure to PFOA[a]

Interactive figure and additional study details available on HAWC here and here.
ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; GD = gestation day; PND = postnatal day; PNW = postnatal week; P_0 = parental generation; F_1 = first generation; d = day; wk = week; CI = confidence interval.
[a] The red dashed lines indicate a 100% increase or 100% decrease from the control response.

3.4.1.2.3 Histopathology

The available animal toxicology literature provides evidence of alterations in liver histopathology were observed after PFOA exposure. Increased cell proliferation/division, bile duct hyperplasia, and hepatocellular hypertrophy were common responses across multiple studies. Loveless et al. (2008) reported increased incidence and severity of hepatocellular hypertrophy with increasing doses of PFOA (0.3–30 mg/kg/day) in male CD-1 mice dosed for 29 days (incidences of 0/19, 20/20, 20/20, 20/20, and 19/19 (all severity grades combined) in the 0, 0.3, 1, 10, and 30 mg/kg/day groups, respectively). Several other 28-day studies in adult male mice provided qualitative descriptions and images as evidence of increased hypertrophy, though results were not quantitatively reported (Guo et al., 2019; Li et al., 2017b; Yan et al., 2017; Minata et al., 2010).

Doses as low as 0.3 mg/kg/day PFOA resulted in increased incidence and severity of hypertrophy in male rats dosed for 28 or 29 days (NTP, 2019; Loveless et al., 2008; Perkins et al., 2004); female rats dosed for 28 days showed slight increases at 50 mg/kg/day (20%) and a 100% hypertrophy incidence rate at 100 mg/kg/day compared with 0% incidence at all lower doses (6.25, 12.5, or 25 mg/kg/day) and in controls (n = 10) (NTP, 2019). Butenhoff et al. (2012) reported significant increases in the incidence of hypertrophy in male and female adult Sprague-Dawley rats administered PFOA for 1 or 2 years at the highest dose tested for each sex (14.2 and 16.1 mg/kg/day for males and females, respectively). NTP (2020) also reported increased incidence of hepatocellular hypertrophy in male and female adult rats dosed with PFOA for 16 or 107 weeks (see study design details in Section 3.4.4.2.1.2). At the 16-week interim necropsy, males had significantly increased incidences of hypertrophy at all doses tested (1–32.1 mg/kg/day); significantly increased incidences of hypertrophy were only observed in females at the highest doses tested (63.4/63.5 mg/kg/day) at 16 weeks. At 107-weeks, significantly increased incidences of hypertrophy were observed in males and females at doses ≥1.1 mg/kg/day and ≥18.2 mg/kg/day, respectively.

In a developmental toxicity study, Blake et al. (2020) observed 100% incidence of hepatocellular hypertrophy with decreased glycogen and intensely eosinophilic granular cytoplasm at both the GD 11.5 and GD 17.5 time points with doses of 1 and 5 mg/kg/day compared with 0% incidence in controls (all n = 5–6); however, control CD-1 mouse dams at the GD 17.5 time point also exhibited what the authors characterized as hepatocellular hypertrophy consistent with pregnancy at that stage of gestation. Quist et al. (2015) similarly reported increased severity of hepatocellular hypertrophy with increasing PFOA doses (0.01–1 mg/kg/day) in PND 91 female CD-1 mouse offspring exposed from GD 1 to GD 17. In a standard 2-generation reproductive toxicity study, significant increases in the incidence of diffuse hepatocellular hypertrophy were reported for male F_1 Sprague-Dawley rat offspring at doses of 3 mg/kg/day and higher (Butenhoff et al., 2004a).

In addition to hepatocellular hypertrophy, significantly increased incidences of mitotic figures and bile duct hyperplasia were observed in adult male CD-1 mice exposed to 10 or 30 mg/kg/day PFOA for 29 days (Loveless et al., 2008). NTP (2020) reported significantly increased incidences of mitoses and bile duct hyperplasia in female Sprague-Dawley rats dosed with 63.5 mg/kg/day PFOA for 2 years, but not in males. In contrast, Filgo et al. (2015) reported the incidence and severity of bile duct hyperplasia in two strains of 18-month-old wild-type female mice exposed to PFOA during gestation and found no alterations in CD-1 mice and a significant

decrease in the severity of bile duct hyperplasia in 129/Sv mice. However, increased mitoses were observed (data not provided) in ICR mouse dams exposed to 1–10 mg/kg/day PFOA during gestation (Yahia et al., 2010).

Several studies reported cytoplasmic alterations including cytoplasmic vacuolization resulting from PFOA exposures. Male mice dosed with PFOA for 28 days were reported to have increased vacuolation at doses between 5.4–21.6 mg/kg/day (incidence data not provided) and significantly decreased numbers of nuclei per unit area with 28-day exposures to ≥0.4 mg/kg/day (Guo et al., 2019; Minata et al., 2010). Male rats were particularly susceptible to cytoplasmic alterations; NTP (2020, 2019) reported incidences of 90%–100% in animals receiving doses ≥1 mg/kg/day for 4 or 16 weeks compared with 0% incidences in controls (all n = 10). In the 2-year study, males receiving ≥2.1 mg/kg/day showed a 58% or greater incidence rate compared with 0% incidence rates in controls (all n = 50) (NTP, 2020).

Female rats receiving doses ≥25 mg/kg/day for 4, 16, or 107 weeks had 98%–100% incidence rates of cytoplasmic alterations compared with 0% incidence rates in controls (NTP, 2020, 2019). In CD-1 mouse dams, 100% incidence rates of cytoplasmic vacuolization were observed only at the highest dose of 5 mg/kg/day but at both gestational time points (GD 11.5 and GD 17.5) compared with 0% incidence rates in controls (n = 5–6) (Blake et al., 2020). In this study, the vacuoles frequently contained remnant membrane material as myelin figures.

Cell and tissue death[8] and degeneration was the final category of hepatic histological effects observed across multiple studies, species, and sexes (Table 3-3). Incidence rates of individual cell necrosis in male CD-1 mice dosed with PFOA for 29 days were above 50% at doses ≥1 mg/kg/day (Loveless et al., 2008). There was similarly a significantly increased percentage of necrotic liver cells, analyzed by flow cytometry, in male C57BL/6 mice administered 5 mg/kg/day PFOA in drinking water for 5 weeks (Crebelli et al., 2019). Significantly increased incidences of single-cell death were observed in male Sprague-Dawley rats after 16 weeks of exposure to doses as low as 1 mg/kg/day but were not increased in females at this time point (NTP, 2020). Incidence rates of single-cell death in male and female rats after 2-year exposures as reported in NTP (2020) are provided in Table 3-3 (see further study design details in Section 3.4.4.2.1.2). Apoptosis and single-cell necrosis were also observed in livers of pregnant CD-1 mice after gestational exposures of 1 and 5 mg/kg/day, with increasing length of exposure resulting in increased incidence rates (Blake et al., 2020). In male and female CD-1 mice gestationally exposed to 0.1 and 1 mg/kg/day from GD 1.5 to GD 17.5 and then given a low-fat diet on PND 22, incidences of single-cell necrosis were higher in the exposed groups but not significantly increased at PNW 18 (Table 3-3) (Cope et al., 2021). However, in females exposed to 1 mg/kg/day and then to a high-fat diet, incidences of single-cell necrosis were significantly increased at PNW 18.

In male CD-1 mice exposed to PFOA for 29 days, the incidence of hepatic focal necrosis increased with increasing PFOA doses between 1–30 mg/kg/day (Loveless et al., 2008). In the same study, increased incidences of necrosis were reported in male Sprague-Dawley rats only with the highest dose tested (30 mg/kg/day) (Loveless et al., 2008). Inconsistent incidences of

[8] In this document, EPA used the cell death nomenclature as reported in the individual studies to describe the observed effects. Cell "necrosis" is a type of cell death, the term for which is generally used when a specific method to distinguish necrotic cells from other dying cells (e.g., apoptotic cells) has been employed (Elmore et al., 2016). EPA did not evaluate the methods of individual studies to ensure that the nomenclature used by the authors accurately reflected the type of cell death reported.

hepatic necrosis were observed in male and female Sprague-Dawley rats administered PFOA in feed for 16 weeks, though there were increases reported after 2 years (NTP, 2020). Table 3-3 depicts the 2-year data for males and females. In a separate 2-year study, there were no significant differences in the incidences of hepatic necrosis in male or female Sprague-Dawley rats (Butenhoff et al., 2012). Blake et al. (2020) did not observe consistent increases in the incidence of focal necrosis in mouse CD-1 dams dosed with PFOA during gestation. However, Butenhoff et al. (2004a) reported significant increases in focal and multifocal necrosis in F_1 generation male Sprague-Dawley rats in a 2-generation reproductive toxicity study (data not provided).

Table 3-3. Associations Between PFOA Exposure and Cell Death or Necrosis in Rodents

Reference	Study Design	Endpoint Name	Incidence
		Males	
NTP (2019)	28-d Sprague-Dawley rat oral gavage dosing; 0, 0.625, 1.25, 2.5, 5, 10 mg/kg/d	Focal Hepatocellular Necrosis	0/10, 0/10, 0/10, 0/10, 1/10, 0/10
Loveless (2008)	29-d Crl:CD(SD)IGS BR rat oral gavage dosing; 0, 0.3, 1, 10, 30 mg/kg/d	Focal Necrosis	0/10, 0/10, 0/10, 1/10, 4/10
	29-d Crl:CD-1(ICR)BR mouse oral gavage dosing; 0, 0.3, 1, 10, 30 mg/kg/d	Individual Cell Necrosis	0/19, 0/20, 11/20, 20/20, 19/19
	29-d Crl:CD-1(ICR)BR mouse oral gavage dosing; 0, 0.3, 1, 10, 30 mg/kg/d	Focal Necrosis	0/19, 1/20, 3/20, 4/20, 7/19
Perkins (2004)[a]	4-wk Crl:CD®BR rat feeding study; 0, 0.06, 0.64, 1.94, 6.5 mg/kg/d	Coagulative Necrosis	0/15, 0/15, 0/15, 1/15, 2/14
	7-wk Crl:CD®BR rat feeding study; 0, 0.06, 0.64, 1.94, 6.5 mg/kg/d	Coagulative Necrosis	0/15, 0/15, 0/15, 0/15, 1/15
	13-wk Crl:CD®BR rat feeding study; 0, 0.06, 0.64, 1.94, 6.5 mg/kg/d	Coagulative Necrosis	0/15, 1/15, 0/15, 1/15, 0/15
Butenhoff (2012)	2-yr Crl:COBS® CD(SD)BR rat feeding study; 0, 1.3, 14.2 mg/kg/d	Focal Hepatocellular Necrosis	3/50, 5/50, 5/50
Cope (2021)[b]	Gestational CD-1 mouse gavage dosing from GD 1.5 to GD 17.5 (offspring); 0, 0.1, 1 mg/kg/d	Hepatocyte Single-Cell Necrosis	2/8, 5/9, 6/9
NTP (2020)	16-wk Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/150, 0/300, 150/150, and 300/300 ppm	Hepatocellular Single-Cell Death	0/10, 10/10, 10/10, 9/10, 10/10
		Necrosis	0/10, 6/10, 2/10, 2/10, 4/10
	16-wk Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/20, 0/40,	Hepatocellular Single-Cell Death	0/10, 7/10, 9/10, 10/10, 0/10, 5/10, 8/10, 10/10
		Necrosis	1/10, 1/10, 6/10, 4/10, 0/10, 2/10, 3/10, 1/10

Reference	Study Design	Endpoint Name	Incidence
	0/80, 300/0, 300/20, 300/40, 300/80 ppm		
	2-yr Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/20, 0/40, 0/80, 300/0, 300/20, 300/40, 300/80 ppm	Hepatocellular Single-Cell Death	1/50, 1/50, 11/50, 24/50, 1/50, 3/50, 5/50, 29/50
		Necrosis	2/50, 17/50, 23/50, 20/50, 1/50, 11/50, 14/50, 21/50
		Females	
NTP (2019)[c]	28-d Hsd:Sprague-Dawley SD rat oral gavage dosing; 0, 6.25, 12.5, 25, 50, 100 mg/kg/d	Focal Hepatocellular Necrosis	0/10, 0/10, 0/10, 0/10, 0/10, 0/10
Butenhoff (2012)	2-yr Crl:COBS® CD(SD)BR rat feeding study; 0, 1.6, 16.1 mg/kg/d	Focal Hepatocellular Necrosis	5/50, 6/50, 2/50
Blake (2020)	Gestational CD-1 mouse gavage dosing from GD 1.5 to GD 11.5 (dams); 0, 1, 5 mg/kg/d	Focal Necrosis	1/5, 0/5, 2/5
		Cell Death (including apoptosis and single-cell necrosis of individual hepatocytes)	0/5, 1/5, 3/5
	Gestational CD-1 mouse gavage dosing from GD 1.5 to GD 17.5 (dams); 0, 1, 5 mg/kg/d	Focal Necrosis	0/5, 0/5, 0/6
		Cell Death (including apoptosis and single-cell necrosis of individual hepatocytes)	0/5, 5/5, 6/6
Cope (2021)[b]	Gestational CD-1 mouse gavage dosing from GD 1.5 to GD 17.5 (offspring); 0, 0.1, 1 mg/kg/d	Hepatocyte Single-Cell Necrosis	1/9, 3/9, 4/10
NTP (2020)	16-wk Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/300, 0/1,000, 150/300, and 300/1,000 ppm	Hepatocellular Single-Cell Death	0/10, 0/10, 1/10, 0/10, 0/10
		Necrosis	0/10, 0/10, 2/10, 0/10, 0/10
	2-yr Hsd:Sprague-Dawley SD rat feeding study, with and without perinatal exposure; 0/0, 0/300, 0/1,000, 150/300, and 300/1,000 ppm	Hepatocellular Single-Cell Death	0/50, 4/50, 29/50, 5/50, 32/50
		Necrosis	0/50, 1/50, 8/50, 4/50, 5/50

Notes: GD = gestation day.
[a] Incidence data as reported by Perkins et al. (2004) were split into severity categories within the original study. For the purposes of this table, all non-grade 0 severities were considered an incidence (results for severity grades 1–3 were combined).
[b] Data are summarized for low-fat diet only from Cope et al. (2021).
[c] Incidence data not explicitly reported by NTP (2019).

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

in the incidence of cystic degeneration in the liver of male rats administered 4.6 mg/kg/day PFOA for 107 weeks.

3.4.1.2.4 Additional Hepatic Endpoints

A suite of other liver effects was observed but were either not included as endpoints of interest across multiple studies or had inconsistent results between studies, sexes, and/or species. These included serum measures of gamma-glutamyl transpeptidase (only measured in one short-term study of male BALB/C mice that showed increases at 2 and 10 mg/kg/day exposures) (Guo et al., 2021a), bile acids (study results generally showed no response or increases at high doses) (Guo et al., 2021a; Blake et al., 2020; NTP, 2020, 2019; Yan et al., 2014; Butenhoff et al., 2002), bilirubin (study results showed no change or minimal increases at high doses) (Guo et al., 2021b; NTP, 2019; Butenhoff et al., 2012; Yahia et al., 2010; Butenhoff et al., 2002), and histopathological findings such as hepatic inflammation (study results showed increased incidence/severity, decreased incidence, or no response) (NTP, 2020; Filgo et al., 2015; Quist et al., 2015), increased incidence of cellular infiltration (Cope et al., 2021; Butenhoff et al., 2012), and increased incidence of hepatocytomegaly (Zhang et al., 2020b). NTP (2020) also reported a variety of other histopathological outcomes including eosinophilic or mixed-cell foci (significant increases in male Sprague-Dawley rats) and pigmentation (significant increases in males and females). Butenhoff et al. (2004a) similarly reported increased discoloration of the liver in male F_1 Sprague-Dawley rats analyzed during a standard 2-generation reproductive toxicity study.

3.4.1.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse hepatic outcomes is discussed in Sections 3.2.1, 3.2.2, 3.2.3, 3.2.7, 3.2.8, 3.2.9, 3.3.2, 3.3.3, 3.3.4, 3.4.1, 3.4.2, 3.4.3, 3.4.4, and 4.2 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 81 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA 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 3-18.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling				
Atherogenesis And Clot Formation	0	0	1	1
Big Data, Non-Targeted Analysis	9	0	11	19
Cell Growth, Differentiation, Proliferation, Or Viability	17	1	36	50
Cell Signaling Or Signal Transduction	14	1	17	30
Extracellular Matrix Or Molecules	1	0	1	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	21	0	19	37
Hormone Function	6	1	1	8
Inflammation And Immune Response	5	1	3	9
Oxidative Stress	8	0	14	21
Renal Dysfunction				
Xenobiotic Metabolism	8	1	12	20
Other	0	0	3	3
Not Applicable/Not Specified/Review Article				
Grand Total	42	2	47	83

Figure 3-18. Summary of Mechanistic Studies of PFOA 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 PFOA to mediate hepatotoxicity via nuclear receptor activation has been investigated for several receptor-signaling pathways, including that of the peroxisome proliferator-activated receptors (PPARα, PPARδ, PPARγ), the pregnane X receptor (PXR), and the constitutive androstane receptor (CAR). PPARα is a major target for PFOA. A primary mechanism of hepatic injury associated with PFOA-mediated activation of PPARα relates to impacts on hepatic lipid metabolism caused by altered expression of genes and proteins within the PPARα signaling pathway (Li et al., 2019b; Pouwer et al., 2019; Wen et al., 2019c; Das et al., 2017; Hui et al., 2017; Rebholz et al., 2016; U.S. EPA, 2016c; van Esterik et al., 2015; Yan et al., 2015a; Yang et al., 2014; Wang et al., 2013). Activation of PPARα has been cited as a mechanism of action for PFAS, including PFOA (U.S. EPA, 2016c), because of the association between hepatic lesions and/or 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 PFOA. Additionally, many of the genes activated by PFOA are regulated by transcription factors other than PPARα, including CAR, PPARγ, PXR, Erα, and HNF4α (U.S. EPA, 2016c). PPARs, CAR, and PXR are nuclear receptors that can form heterodimers with one another to induce transcription of linked genes. Other factors impacting nuclear receptor activation in hepatocytes include dose and duration of PFOA exposure and the genetic background, diet, and sex of exposed animals. Sex-specific hepatic effects varied by strain, and long-term PFOA oral exposure in mice with pre-existing steatosis had protective effects against hepatic injury (Li et al., 2019c; NTP, 2019; Li et al., 2017b). Thus, the underlying mechanism(s) of PFOA-induced hepatotoxicity may involve multiple nuclear receptors. 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 a summary of studies that have examined PPARs, CAR, PXR, Erα, and HNF4α activation as potential mechanisms underlying the health effects induced by PFOA.

3.4.1.3.1.2 PPARα Receptor Binding and Activation

Receptor binding and activation assays have been performed to examine the association between activation of PPARs, CAR, and/or PXR, and PFOA-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, 2016c).

Several studies used luciferase reporter assays to examine the activation of PPARα by PFOA in vitro using human and animal cell lines transfected with mouse and human PPARα (Behr et al., 2020b; Rosenmai et al., 2018; Wolf et al., 2014; Buhrke et al., 2013). In African green monkey kidney COS-1 cells transfected with mouse PPARα, PFOA was the most potent activator of PPARα among the 5 PFAS tested, with PPARα activation observed at less than 1 μM after a 24 h exposure (Wolf et al., 2014). A study in human HEK293T cells found that human PPARα was activated at a concentration of 50 μM PFOA after a 24 h exposure (Behr et al., 2020b). Whether PFOA activates other nuclear receptors is less clear from studies conducted in HEK293 cells and may be cell type- and dose-dependent. PFOA had no activity in HEK293 cells transfected with constructs encoding other nuclear receptors, including PPARδ, CAR, PXR, the farnesoid X receptor (FXR), the liver X receptor α (LXRα), the retinoid X receptor α (RXRα) and retinoic acid receptor α (RARα), at concentrations up to 100 μM for 24 hours (Behr et al., 2020b). In a second study using a human PPARα construct in HEK293 cells, PFOA induced PPARα activation at concentrations of 25 μM and higher, whereas PFOA concentrations of at least 100 μM were necessary to activate PPARγ and PPARδ (Buhrke et al., 2013). Results from the single study conducted in a human hepatic cell line (HepG2) were consistent with results in other cell lines (Rosenmai et al., 2018). Of the 14 PFAS substances tested, PFOA was the most potent PPARα activator, showing significant elevation of luciferase activity after a 24-hour exposure to 30 and 100 μM PFOA. While luciferase levels were elevated at 10 μM of PFOA, the increase did not reach significance. These in vitro studies support PPARα activation by PFOA.

Another study measured the expression of hepatic carboxylesterases (*Ces*) that function in the metabolism of drugs, chemical toxicants, and endogenous lipids (Wen et al., 2019c). PFOA upregulated expression of the PPARα target gene, Cyp4a14, in the livers of male C57BL/6 NCrl mice after exposure to 3 mg/kg/day by gavage for 7 days. PFOA exposure also led to alterations to the expression of Ces genes: *Ces1d*, *1e*, *1f*, *1g*, *2c*, and *2e* mRNA levels were increased

between 1.5- and 2.5-fold, while *Ces1c* and *2b* transcripts were decreased. In a second study within Wen et al. (2019c), *Ces* genes were measured in the livers of C57BL/6NTac mice and PPARα-null mice also exposed to 3 mg/kg/day PFOA by gavage for 7 days. *Ces1e* and *1f* mRNA and protein levels were PPARα dependent, whereas *Ces1c, 1d, 1g, 2a, 2b*, and *2e* mRNA and CES2 protein levels were induced by PFOA in PPARα-null mice, implicating a CAR-mediated pathway for differential expression of these genes.

The mechanism by which PFOA activates PPARα is likely dependent on interactions with liver fatty acid binding protein (L-FABP). L-FABP facilitates the nucleo-cytoplasmic shuttling of activator ligands, such as fatty acids, for nuclear receptors, including PPAR activators, PXRs, and LXRs. PFOA is structurally similar to fatty acids, and both exhibit a strong binding affinity with L-FABP (Section 3.3.1.2). Thus, L-FABP is responsible for delivering PFOA to the nuclei of hepatic cells for access to nuclear receptors. Sheng et al. (2018) used circular dichroism (CD) spectroscopy, fluorescence displacement assays, and molecular docking approaches to evaluate the binding mode and capacity of PFOA as well as PFOS and PFAS replacement chemicals to purified human L-FABP (hL-FABP). The purified recombinant hL-FABP was calculated to consist of 15.7% α-helix and 54.4% β-sheet. In the presence of PFOA, α-helix content of the protein increased slightly, whereas the β-sheet content decreased. The dissociation constant (Kd) of PFOA to hL-FABP was 8.03 ± 2.10 μM, which was higher than PFOS and lower than some (but not all) replacement PFAS substances. By molecular docking, PFOA bonded with hL-FABP in a "head-out" mode, such that the carboxyl head of PFOA will interacted with R122 amino acid residue through hydrogen bonding and N111 amino acids residue through hydrophobic interactions. Introduction of oxygen molecules into the backbone could flip the binding prediction to a "head-in" mode characterized by interactions with amino acid residue N61. By comparing PFOA to PFOS and replacement PFAS chemicals, the authors demonstrated that these three parameters correlated both with cytotoxicity in human liver HL-7702 cells and binding affinity for hL-FABP. Notably, expression of select PPARα-regulated genes showed no significant change across the chemicals tested, with one exception, the Cd36 gene. Expression of other genes, including cell cycle genes, did correlate with these binding parameters. These findings suggest that binding of PFAS to hL-FABP can mediate toxicity in a manner that is not exclusively dependent on PPARα-mediated changes in gene expression in liver cells, but possibly through effects on other FABP-related events such as binding to the CD36 protein or effects on cell proliferation.

3.4.1.3.1.3 Receptor Binding and Activation of Other Nuclear Receptors

PFOA can activate PPARα in the liver of rodents and humans. However, the extent by which activation of PPARα mediates hepatoxicity may be species-specific, and activation of other receptors may also contribute to toxicity (U.S. EPA, 2016c). Indeed, studies in mice and rats indicate that PFOA may activate PPARα, CAR, and PXR in the liver (Li et al., 2019c; NTP, 2019; Wen et al., 2019c; Rose et al., 2016).

Several studies observed perturbations in lipid transport, fatty acid metabolism, triglyceride synthesis, and cholesterol synthesis in PFOA-exposed mice (Li et al., 2019b; Das et al., 2017; Rosen et al., 2017). A few of these studies, Das et al. (2017), Rosen et al. (2008b), and Rosen et al. (2017), investigated the effects of PFOA on lipid metabolism and homeostasis in the absence of PPARα by using knockout mouse models. After exposure to 10 mg/kg/day PFOA 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 wild-type (WT) mice. Increased expression of genes regulating fatty acid and triglyceride synthesis and transport into hepatocytes was attenuated but not entirely abolished in PFOA-exposed PPARα null mice compared with WT mice. Gene expression changes in PPARα null mice implicate a role for PPARβ/δ and/or PPARγ in the absence of PPARα (Rosen et al., 2008b). Mechanistically, these changes correlated with the development of steatosis in PFOA-exposed WT mice consistent with increased triglyceride accumulation. In contrast, elevated triglyceride levels and steatosis develop in PPARα null mice even in the absence of PFOA exposure. The authors propose that PFOA exposure alters lipid metabolism to favor biosynthesis and accumulation over β-oxidation, leading to hepatic steatosis. PFOA increased the expression of genes related to fatty acid β-oxidation, lipid catabolism, lipid synthesis, and lipid transport in both strains; however, gene induction was lower in PPARα null mice (Rosen et al., 2017; Rosen et al., 2008b). In fact, the authors suggest that the transcriptome of the mice resembled that of mice treated with PPARγ agonists, thus indicating a role for other PPAR isoforms in the dysregulation of lipid synthesis (Rosen et al., 2017). Furthermore, Rosen and colleagues (Rosen et al., 2017) demonstrated that PFOA significantly downregulated the Signal Transducer and Activator of Transcription 5B gene (STAT5B), a transcription factor and member of the STAT family, in a PPARα-dependent manner. STAT5B has been demonstrated in regulation of sexually dimorphic gene expression in the liver between males and females, raising the possibility that that PFOA exposure may promote feminization of the liver in male mice (Rosen et al., 2017; Oshida et al., 2016).

Increasing evidence links CAR activation as a mechanism of PFOA-induced liver toxicity (Li et al., 2019c; NTP, 2019; Wen et al., 2019c). The use of genetically modified mice and gene expression analyses has demonstrated that PFOA exposure activates both PPARα and CAR receptors (Li et al., 2019c; NTP, 2019; Wen et al., 2019c; Abe et al., 2017; Rosen et al., 2017; Oshida et al., 2015a; Oshida et al., 2015b).

Five recent studies also examined PFOA activation of CAR-specific genes (NTP, 2019; Wen et al., 2019c; Abe et al., 2017; Rosen et al., 2017; Rose et al., 2016). Additionally, one study used both a cell-based reporter assay and in silico approaches to examine PFOA activation of PXR (Zhang et al., 2020d), and one study examined other PFOA effects on other nuclear receptors in vitro (Buhrke et al., 2015). In support of PFOA as a CAR receptor activator, PFOA induced expression of the CAR target genes CYP2B6 in a human hepatocyte cell line in vitro (HepaRG), and *Cyp2b10* in wild-type mice but not CAR-null mice in vivo (Abe et al., 2017). Evidence of CAR-specific gene expression was also noted in male and female rats administered PFOA. Exposed animals exhibited significant increases in expression of PPARα-stimulated genes (*Acox1*, *Cyp4a1*) and CAR-specific genes (*Cyp2b1, Cyp2b2*) in livers compared with controls, suggesting increases in PPARα and CAR activity (NTP, 2019). Males were exposed to a range of doses between 0 and 10 mg/kg/day and females to between 0 and 100 mg/kg/day PFOA for 28 days. Gene expression in liver tissue was analyzed using qRT-PCR. Female rats displayed the greatest fold increase for the CAR-related genes *Cyp2b1* whereas males exhibited the greatest fold increase for *Cyp4a1* and *Cyp2b1* compared with controls.

Rosen et al. (2008b) postulated that gene expression changes in the liver should overlap between PFOA and phenobarbital, a known CAR activator. To test this, differentially expressed genes in wild-type or CAR-null mice treated with PFOA by gavage (3 mg/kg/day) for 7 days were compared with differentially expressed genes in the livers of mice exposed to 100 mg/kg/day

phenobarbital for three days (Rosen et al., 2017). Similarity in differentially expressed genes between the two studies (i.e., overlap) was analyzed using a Running Fisher Test for pairwise comparisons. As expected, there was significant similarity between the lists of differentially expressed genes for PFOA and phenobarbital in WT mice, but not in CAR-null mice. In fact, close to 15% of genes differentially expressed upon PFOA exposure in liver were considered PPARα-independent. Two gene expression compendium studies further analyzed these data using gene expression biomarker signatures built using microarray profiles from livers of WT mice, CAR-null mice (Oshida et al., 2015a), and PPARα-null mice (Oshida et al., 2015b). These analyses found that both CAR and PPARα were activated by PFOA, 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 PFOA (Oshida et al., 2015a).

Activation of CAR may occur via direct activation or indirect activation. Indirect activation of CAR by phenobarbital involves blockade of the downstream phosphorylation pathway of EGFR protein phosphatase 2A (PP2A), which dephosphorylates CAR to enable nuclear translocation. Using a COS-1 fibroblast cell-based reporter gene assay that is capable of detecting CAR ligands but not indirect activators, Abe et al. (2017) observed that PFOA failed to activate reporter gene expression. In a second study using primary mouse hepatocytes, PFOA exposure led to CAR-mediated expression of *Cyp2b10* even in the presence of okadaic acid, a PP2A drug inhibitor. Together these findings suggest the mechanism of PFOA-mediated CAR activation indirect and distinct from that of phenobarbital. Moreover, an analysis of historical and new data of gene expression in PPARα- and CAR-null mice indicate the pathway of PFOA-mediated CAR activation is PPARα-independent (Rosen et al., 2017). Thus, the precise mechanism of CAR activation by PFOA remains to be determined.

Several studies evaluated PFOA activation of other nuclear receptors. Rosen et al. (Rosen et al., 2017) noted that PFOA activated PPARγ and ERα in trans-activation assays from the ToxCast screening program. Zhang et al. (Zhang et al., 2020d) used a cell-based reporter assay and an in silico approach to estimate PFOA-mediated activation of the PXR receptor. The PFOA log EC50 was 5.04 M in the luciferase-based PXR reporter assay, a higher concentration (i.e., less potent) than observed for PPARα. These authors also developed classical QSAR and 3D-QSAR models that predicted very similar values of log EC50 of 4.92 M and 4.94 M, respectively. Both models suggested that molecular structural factors including molecular polarizability, charge, and atomic mass are key parameters dictating hPXR agonistic activity of PFOA and other perfluoroalkyl chemicals.

In addition to the key role of PPARα and other nuclear receptors discussed above, other transcription factors and epigenetic mechanisms influence PFOA-mediated changes in lipid metabolism and storage. Beggs et al. (2016) observed a decrease in hepatocyte nuclear factor alpha (HNF4α) protein, a master regulator or hepatic differentiation, in the livers of ten-week-old CD-1 mice exposed to 3 mg/kg/day PFOA once daily by oral gavage for 7 days. HNF4α regulates liver development (hepatocyte quiescence and differentiation), transcriptional regulation of liver-specific genes, and regulation of lipid metabolism. In this study, PFOA exposure correlated with downregulation of HNF4α target genes involved in differentiation (*Cyp7a1*) and induced pro-mitogenic genes including CCND1. Other genes altered by PFOA exposure mapped to pathways involved in lipid metabolism, liver cholestasis, and hepatic

steatosis. PFOA also led to diminished accumulation of HNFα protein. This decrease in HNF4α was not accompanied by a change in expression of the gene, suggesting that the decrease in HNF4α occurs post-translationally. The decreased HNFα correlated with upregulation of genes that are negative targets of HNF4α. 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 PFOA and PFOS interaction with this protein via in silico docking models, which showed a displacement of fatty acids by PFOA/PFOS, possibly tagging HNF4α for degradation. The authors hypothesize that steatosis, hepatomegaly, and carcinoma in rodents may be a consequence of the loss of this protein and also presents a mechanism for PFOA-induced hepatic effects in humans.

In primary human hepatocytes exposed to 1, 25, or 100 μM PFOA for 24 hours, the number of differentially regulated genes was 43, 109, and 215, respectively, as measured using a human genome gene chip (Buhrke et al., 2015). Given known activators of the differentially expressed genes, the authors suggest that in addition to PPARα, PPARγ and HNF4α may contribute to changes in expression of genes involved in carnitine metabolism. PFOA-mediated induction of ERα signaling was also predicted based on pathway analysis.

3.4.1.3.1.4 Host Factors Impacting PPARα Signaling

The effects of PFOA on PPARα activation depend on diet and pre-existing conditions (Li et al., 2019c). Mice were subjected to control diet or high-fat diet (HFD) for 16 weeks to induce nonalcoholic fatty liver disease (NAFLD), after which they were exposed to vehicle or 1 mg/kg/day PFOA by oral gavage for 2, 8, or 16 weeks; control diet and HFD were continued throughout this exposure period. Preexisting NAFLD in mice fed a HFD enhanced the induction of PPARα activation by PFOA early in the exposure but reduced the severity of macrovesicular steatosis and sinusoidal fibrosis induced by a HFD, and reversed HFD-induced increase in body weight and serum alanine aminotransferase (ALT). The authors hypothesized that PFOA exposure in animals with a lipid burden in the liver leads to PFOA-mediated inhibition of fatty acid biosynthesis pathways by the metabolic end-product feedback effect. The authors also observed reduced Tgf-β gene expression in PFOA-treated HFD-fed mice compared with vehicle-treated HFD-fed mice, which could account for the diminished level of hepatic stellate cell activation and collagen production associated with fibrosis. Furthermore, the duration of PFOA exposure impacted gene expression and hepatic injury. For example, PFOA induced Srebf1 and Srebf2 genes in the fatty acid biosynthesis pathway following 2 weeks of treatment, but this effect was not seen following 8 or 16 weeks of PFOA treatment. Notably, this increase in Srebf1 expression following 2 weeks of PFOA exposure was only observed with the co-treatment of PFOA and HFD; the Srebf1 effect was not observed in the PFOA-treated mice fed the control diet.

PFOA-driven changes in PPARα-mediated gene expression may also be modified be age, strain, or species. Pregnant Kunming mice were exposed to PFOA at doses of 1, 2.5, 5 and 10 mg/kg/day from gestational days 1–17, and female offspring were analyzed on postnatal day 21 (Li et al., 2019b). Genes involved in fatty acid β-oxidation including acyl-CoA synthetase (Acsl1), carnitine palmitoyl transferase I, Palmitoyl-CoA oxidase (Acox1), acyl-CoA thioesterase 1 (Acot1), and carnitine palmitoyltransferase 1a (Cpt1a) were significantly downregulated at the two highest doses, as was the PPARα gene. In this strain of mouse,

perinatal PFOA disrupts the gene expression of enzymes involved in fatty acid oxidation induced by PPARα, possibly through an epigenetic mechanism. In contrast, several studies have shown PFOA to upregulate expression of PPAR signaling pathway genes, including Acox in rats and mice (Li et al., 2019c; NTP, 2019; Cavallini et al., 2017). One such study proposed that the PFOA-mediated gene expression changes are due to changes in the activity of histone acetyltransferase (HAT) and HDAC (histone deacetylase) (Li et al., 2019b). In female offspring of pregnant Kunming mice treated with PFOA by oral gavage at doses between 0 and 10 mg/kg/day on GD 1–17, the overall levels of histone H3 and H4 acetylation were decreased in a dose-dependent manner in liver tissues in the pups at post-natal day 21. Histone acetylase (HAT) activity was reduced in pups at all doses except for the highest dose (10 mg/kg/day), in which there was no significant difference in HAT activity compared with controls. HDAC activity was increased in all dose groups. The changes in HAT and HDAC activity did not follow a dose-responsive pattern. Notably, gene-specific alterations in histone acetylation activity were not measured; thus, follow-up studies are needed to clarify the relationship between the global histone modifications and the gene expression changes.

Additional support for species specificity derives from studies demonstrating that PFOA-mediated gene expression changes were distinctly different in primary human hepatocytes compared with primary mouse hepatocytes (Rosen et al., 2013). Custom Taqman PCR arrays were generated to include transcripts regulated by PPARα as well as transcripts regulated independently of this nuclear receptor. Mouse and human hepatocytes were exposed to PFOA at doses ranging from 0 to 100 and from 0 to 200 μM, respectively, or the PPARα activator Wy14,643. In mouse cells, many fewer genes were altered by PFOA treatment compared with whole livers from mice exposed in vivo. Also, genes typically regulated by PPARα agonists were not altered by PFOA in mouse cells, including Acox1, Me1, Acaa1a, Hmgcs1, and Slc27a1. The CAR target gene Cyp2b10 was also unchanged in cultured mouse hepatocytes. In contrast, a larger group of genes were differentially expressed in primary human hepatocytes, including PPARα-independent genes (CYP2B6, CYP3A4, and PPARγ). These findings underscore some of the difficulty in extrapolating in vitro results from rodents to humans after PFOA exposure and suggest PPARα may elicit species-specific changes in gene expression.

3.4.1.3.1.5 Conclusions

Although activation of PPARα is a widely cited mechanism of liver toxicity induced by PFAS exposure, PFOA has been shown to activate a number of other nuclear receptors, including PPARγ, CAR/PXR, Erα, and HNF4α. Many of these nuclear receptors, including CAR and PPARγ, are also known to play an important role in liver homeostasis and have been implicated in liver dysfunction, including steatosis (Armstrong and Guo, 2019). Therefore, there is accumulating evidence that PFOA exposure may lead to liver toxicity through the activation of multiple nuclear receptors in both rodents and humans. However, the contribution of gene expression changes induced and associated toxicity by these other receptors is not clear. Also, it is possible that other receptors may play compensatory roles in PPARα null mice. In addition, PFOA-mediated changes in hepatic gene expression and toxicity exhibit strain, sex, and species specificity. Thus, the interplay between nuclear receptor activation and host factors may dictate the nature and severity of liver toxicity in response to PFOA exposure.

3.4.1.3.2 Lipid Metabolism, Transport, and Storage

3.4.1.3.2.1 Introduction

The liver is the prime driver of lipid metabolism, transport, and storage within an organism. 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) 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 (Huang et al., 2011). De novo cholesterol synthesis is another vital function of the liver. 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 can contribute to the development of diseases such as nonalcoholic fatty liver disease, steatosis, hepatomegaly, and obesity.

PFOA accumulates in liver tissue, and as such, not only influences lipid levels but can also alter gene expression for a variety of pathways involved in biological processes (U.S. EPA, 2016c). 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 that may have variable effects on serum triglyceride levels depending on species, sex, and exposure conditions (Li et al., 2019b; Liang et al., 2019; Das et al., 2017; Rosen et al., 2017; Beggs et al., 2016; Rosen et al., 2013). 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). Various studies have also shown that PFOA alters expression of genes directly involved in cholesterol biosynthesis (Li et al., 2019b; Pouwer et al., 2019; Das et al., 2017; Rosen et al., 2017) and in β-oxidation of fatty acids (e.g., Acox1 and/or carnitine palmitoyltransferase 1A (Cpt1a)) (Lee et al., 2020; Schlezinger et al., 2020; Li et al., 2019b; NTP, 2019; Cavallini et al., 2017; Rosen et al., 2013). Genes involved in lipid metabolism and homeostasis can be altered through PPARα, PPARγ, CAR, and HNF4α induction pathways and are dose-, lifestage-, species-, and sometimes sex-dependent.

3.4.1.3.2.2 In Vivo Models

3.4.1.3.2.2.1 Rats

Two studies conducted in Sprague-Dawley rats reported marked effects on lipid metabolism, including sex-dependent effects, of PFOA on hepatic outcomes (NTP, 2019; Cavallini et al., 2017).

The study conducted by NTP in 2019 (NTP, 2019) used an oral dosing paradigm of 0, 0.625, 1.25, 2.5, 5, or 10 mg/kg (males) or 0, 6.25, 12.5, 25, 50, or 100 mg/kg/day (females) for 28 days. Males exhibited higher plasma levels of PFOA despite receiving a 10-fold lower dose across the dose groups.

Serum cholesterol levels were decreased in PFOA-exposed males and females, whereas serum triglyceride levels were decreased in males but increased in females. In liver, PPARα- and CAR-

induced genes including Acox1, Cyp4a1, Cyp2b1, and Cyp2b2 were upregulated in both males and females compared with controls. In females, the CAR-induced Cyp2b1 and Cyp2b2 exhibited a greater increase than that of Acox1 and Cyp4a1, whereas Cyp4a1 and Cyp2b1 exhibited the greatest fold increase in males. Acox1 was more strongly upregulated in males than females. This gene expression profile indicates a stronger PPARα signal in males relative to females, and stronger CAR activation signal in females. Bile acid concentrations were increased at the two highest dose groups (5 and 10 mg/kg/day) in males, but were not measured in females.

PFOA is known to activate PPAR receptors and proliferation of peroxisomes, and increase expression of acyl-CoA oxidase (ACOX) activity, the first enzyme in the fatty acid beta-oxidation pathway. In one study, a single dose of PFOA (150 mg/kg) in male Sprague-Dawley 2-month-old rats caused increased liver weight associated with an eightfold and a 15-fold increase in ACOX after 2 and 4 days, respectively (Cavallini et al., 2017). PFOA exposure was associated with generation of new, ACOX rich peroxisomes. Autophagy was induced in fasted rats by an injection of an antilipolytic agent (3,5-dimethyl pyrazole (DMP)). In PFOA-treated rats, DMP-induced autophagy delayed the decrease in ACOX activity relative to controls. The authors hypothesized that autophagy may preferentially target older peroxisomes for degradation. However, another possibility not considered by the authors is that PFOA could disrupt drug-induced autophagy, which may represent an interesting area for further research.

3.4.1.3.2.2.2 Mice

Several studies were conducted to investigate the effects of PFOA on lipid accumulation in hepatocytes by histopathological and metabolomic methods using mice of different genetic backgrounds and lifestages, and mice genetically modified to mimic human lipid metabolism (Pouwer et al., 2019; Hui et al., 2017; Rebholz et al., 2016; van Esterik et al., 2015; Wang et al., 2013). Other studies focused on the transcription and translation of genes involved in lipid metabolism and biliary pathways. The focus of these studies was to identify key genes, gene products, and transcriptional regulators affected by PFOA exposure and to examine how PFOA alters metabolism of lipids (Zhang et al., 2020c; Li et al., 2019b; Wu et al., 2018; Das et al., 2017; Rosen et al., 2017; Beggs et al., 2016; Song et al., 2016; Yu et al., 2016; Yan et al., 2015a).

3.4.1.3.2.2.2.1 Changes in Hepatic Lipid Homeostasis

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, PFOA increased hepatic lipid levels including triglycerides, total cholesterol, and LDL, which correlated with histopathological changes that are often consistent with steatosis.

In Das et al. (2017), WT male SV129 mice administered 10 mg/kg/day PFOA for 7 days had increased lipid accumulation in liver, as seen by Oil Red O staining, as well as increased liver triglyceride levels. These effects were mainly attributed to activation of PPARα, as they were attenuated in PFOA-exposed PPARα null mice (Section 3.4.1.2). In contrast, in male BALB/c mice administered 0.08, 0.31, 1.25, 5, or 20 mg/kg/day PFOA for 28 days, liver cholesterol was significantly decreased at 0.31 mg/kg/day and above, while triglycerides were significantly decreased at 0.08 and 20 mg/kg/day and significantly increased at 1.25 mg/kg/day (no changes were seen at other concentrations) (Yan et al., 2015a). An increase in the transcriptional activity of PPARα and sterol regulatory element-binding proteins (SREBPs) was also observed. The

authors hypothesize that altered lipid metabolism is induced by PPARα activation, with increased SREBP activity as a mediator in this pathway.

One study evaluated PFOA effects on storage in hepatic lipid droplets (LDs) in BALB/c mice (Wang et al., 2013). LDs are storage structures for neutral lipids that form in the endoplasmic reticulum and release into the cytoplasm. In addition to lipid storage, they influence lipid metabolism, signal transduction, intracellular lipid trafficking, and protein degradation. Four-week-old BALB/c mice fed either regular or HFD were dosed with 5, 10, or 20 mg/kg/day PFOA by gavage for 14 days. Cytoplasmic LDs were apparent in both regular- and HFD-fed mice, though more were observed in HFD-fed mice. However, in PFOA-exposed mice, LDs transferred from the cytoplasm to the nucleus, forming hepatocyte intranuclear inclusions in a dose-dependent manner. The authors suggest that this translocation of LDs to the nucleus is a critical factor in PFOA-mediated liver toxicity. As discussed below (Section 3.4.1.3.2.2.2.2), at least two genes involved in lipid droplet formation, PLIN2 and PLIN4, were increased in PFOA-exposed HepaRG cells in vitro, supporting a role for PFOA in altering lipid droplets in hepatocytes (Louisse et al., 2020).

A targeted metabolomics approach was used to directly identify alterations in 278 metabolites in livers of BALB/c mice exposed to either 0.5 or 2.5 mg/kg/day PFOA for 28 days by gavage (Yu et al., 2016). A total of 274 of these metabolites were identified in liver and were mapped to KEGG metabolic pathways including amino acid, lipid, carbohydrate, and energy metabolism. In liver, nine metabolites mapped to lipid metabolism as evidenced by alterations in the relative concentrations of acylcarnitines, sphingomyelins, phosphatidylcholines, and oxidized polyunsaturated fatty acids. Among the 18 liver metabolites that were significantly different between exposed and control mice were six acylcarnitines, one phosphatidylcholine, and two polyunsaturated fatty acids, which could serve as potential biomarkers of PFOA exposure. The altered lipid profiles are consistent with the finding that PFOA upregulates hepatic nuclear receptors and their target genes directly involved in lipid metabolism and the β-oxidation of fatty acids (Lee et al., 2020). The profile of both phosphatidylcholine and fatty acid metabolites indicated a PFOA-mediated shift to phosphatidylcholines with more carbons and more double bonds. Because a change to fatty acids with more carbon atoms and double bonds is due to biosynthesis reactions of saturated and unsaturated fatty acids, these findings suggest PFOA exposure may stimulate fatty acid biosynthesis, which may account for the altered profile of both phosphatidylcholines and fatty acids in liver. Thus, PFOA may regulate both catabolic and anabolic lipid metabolism in liver.

3.4.1.3.2.2.2.2 Gene Expression and Metabolite Accumulation Impacting Lipid Homeostasis

Several studies probed the genes and pathways by which PFOA alters hepatic lipid homeostasis. Hui et al. (Hui et al., 2017) demonstrated that the expression of genes and proteins associated with lipid storage in was altered in the liver of PFOA-exposed BALB/c mice. Male mice were exposed to 1 or 5 mg/kg/day for 7 days and the expression of lipid metabolism genes was analyzed. Triglyceride and free fatty acid contents in serum were reduced, while hepatic triglyceride levels were increased in the PFOA-exposed mice compared with controls. In liver, transcript levels of hepatic lipoprotein lipase (Lpl) and fatty acid translocase (Cd36) were elevated, while apolipoprotein-B100 (ApoB) expression was diminished. LPL and CD36 regulate lipid intake through lipid hydrolysis and transport of lipids from blood to liver, whereas APOB is required for lipid export from liver. Protein levels aligned with the changes in transcript

levels for these genes. The authors suggest that dysregulation of lipid metabolism and, specifically, fatty acid trafficking, leads to decreased body weights and lipid malnutrition and deposition of lipids in liver. These findings are consistent with observations in male Kunming mice exposed to 5 mg/kg/day PFOA for 21 days (Wu et al., 2018). In these mice, PFOA exposure led to reduced APOB and elevated CD36 protein levels as measured immunohistochemically and correlated to increased liver triglyceride levels. In addition to genes directly involved in regulating lipid metabolism and storage, Eldasher et al. (2013) demonstrated that Bcrp mRNA and protein are increased in the livers, but not the kidneys of male C57BL/6 mice exposed to 1 or 3 mg/kg/day PFOA by gavage for 7 days. BCRP is an ATP-binding cassette efflux transporter protein involved in active transport of various nutrients and drugs and implicated in transport of xenobiotics. In addition, BCRP can function sterol transport and its ATPase activity can be stimulated with cholesterol (Neumann et al., 2017). Further studies are needed to elucidate the role of BCRP or other transport proteins in PFOA-mediated disruption of lipid metabolism.

MicroRNAs (miRNAs or miRs) are also altered after exposure to PFOA in mice in a dose-dependent manner. In serum of male BALB/c mice, 24 and 73 circulating miRNAs were altered in mice exposed to 1.25 and 5 mg/kg/day PFOA, respectively, for 28 days (Yan et al., 2014). Changes in expression of six miRNAs (miR-28-5p, miR-32-5p, miR-34a-5p, miR-200c-3p, miR-122-5p, miR-192-5p) were confirmed in liver, including two (miR-122-5p and miR-192-5p) considered to be biomarkers for drug-induced liver injury. MiRNAs may play a specific role in regulating expression of genes involved in lipid metabolism and storage.

Cui et al. (2019) observed that PFOA exposure (5 mg/kg/day PFOA for 28 day) led to a significant increase of miR-34a, but not miR-34b or miR-34c, in the livers of male BALB/c mice, consistent with the findings of Yan et al. (Yan et al., 2014).

Liver toxicity was evaluated by Cui et al. (2019) by measuring liver weight, elevated liver enzymes, and hepatic cell swelling manifested in both WT mice and in miR-34a-null mice generated on a C57BL/6J background. RNA-Seq analysis of hepatic tissue showed that expression of lipid metabolism genes was significantly altered in both WT mice and in miR-34a-null mice after PFOA exposure; however, fewer genes were altered in livers of miR-34a-null mice. Metabolism genes dominated those changed by miR-34a, including Fabp3, Cyp7a1, and Apoa4. On the basis of the transcriptome analysis, the authors found that miR-34a mainly exerts a metabolic regulation role, rather than the pro-apoptosis and cell cycle arrest role reported previously in vitro.

In addition to perturbed expression of genes as a consequence of activating PPARα and other nuclear receptors, PFOA may directly target enzymes involved in fatty acid metabolism. Shao et al. (2018) postulated that based on the electrophilic properties of PFOA, it may preferentially bind to proteins harboring reactive cysteine residues. To test this hypothesis, proteomic and metabolomic approaches were applied. Two cysteine-targeting probes were used to enrich putative target proteins in mouse liver extracts in the absence or presence of PFOA, resulting in the identification of ACACA and ACACB as novel target proteins of PFOA. Parallel reaction monitoring (PRM)-based targeted proteomics combined with thermal shift assay-based chemical proteomics was used to verify ACACA and ACACB as PFOA binding targets. Next, the authors used a metabolomic approach to analyze liver extracts from female C57BL/6 mice four hours after IP injection with a very high dose (300 mg/kg) of PFOA to confirm abnormal fatty acid

metabolism, including significantly elevated levels of carnitine and acyl-carnitines. ACACA and ACACB are acetyl-CoA carboxylases that can regulate fatty acid biosynthesis. The authors suggest PFOA interactions with these carboxylases leads to a downregulation of malonyl-CoA, required for the rate-limiting step of fatty acid biosynthesis and an inhibitor of carnitine palmitoyl transferase 1 (Cpt1). Despite the correlation to altered fatty acid profiles, additional studies are required to confirm PFOA binding to these lipid enzyme targets and changes in hepatic fatty acid metabolism.

3.4.1.3.2.2.2.3 Host Factors Influencing Lipid Metabolism and Storage

Rebholz et al. (2016) underscored the relevance of genetic background, sex, and diet in PFOA-mediated alterations of hepatic gene expression and highlighted the role of genes involved in sterol metabolism and bile acid production Young, sexually immature male and female C57BL/6 and BALB/c mice were placed on diets to target a dose of approximately 0.56 mg/kg/day of PFOA and supplemented with 0.25% cholesterol and 32% fat. Hypercholesterolemia developed in male and female C57BL/6 mice exposed to PFOA. Hypercholesterolemia was also observed in male BALB/c mice but to a lesser degree than C57BL/6, and did not manifest in female BALB/c mice. The PFOA-induced hypercholesterolemia appeared to be the result of increased liver masses and altered expression of genes associated with hepatic sterol output, specifically bile acid production. These data support genetic background and dietary levels of fat and cholesterol as important variables influencing PFOA-mediated changes in cholesterol. However, an important caveat in this study is that female mice in the control groups for both strains had higher than expected blood PFOA levels.

PFOA-mediated changes in lipid levels may be programmed during early life exposure. C57BL/6JxFVB hybrid mice were exposed during gestation and lactation via maternal feed (van Esterik et al., 2015) to seven doses of PFOA targeting 0.003–3 mg/kg/day. The dose range was chosen to be at or below the NOAEL used for current toxicological assessment. Liver morphology and serum lipids were analyzed at in the pups at 26 weeks (males) and 28 weeks (females) of age. Histopathological changes, including microvesicular steatosis and nuclear dysmorphology, were more frequent in PFOA-exposed mice compared with controls, though the incidence did not reach statistical significance over the dose range. However, perinatal exposure induced a sex-dependent change in lipid levels. In females only, serum cholesterol and triglycerides showed a dose-dependent decrease with a maximum change of −20% for cholesterol and −27% for triglycerides (BMDLs of 0.402 and 0.0062 mg/kg/day, respectively). The authors suggest that perinatal exposure to PFOA in mice alters metabolic programming in adulthood. On the basis of the sexually dimorphic lipid levels, as well as on extrahepatic changes, females appear more sensitive to PFOA-mediated alterations in metabolic programming.

The potential developmental effects of PFOA in liver are also of interest considering recent findings that PFOA regulates expression of homeobox genes involved in both development and carcinogenesis (Zhang et al., 2020c). Adult male C57BL/6 mice, PPARα-null mice, or CAR-null mice were given a single IP administration of 41.4 mg/kg and livers were collected on Day 5. PFOA induced mRNA expression of Hoxa5, b7, c5, d10, Pdx1 and Zeb2 in wild-type mice in a manner dependent on PPARα and CAR. Whether exposure to PFOA alters homeobox genes during perinatal exposure, and the potential for homeobox proteins to alter PFOA susceptibility in different lifestages remains to be determined.

One difference between human and rodent lipid metabolism relates to transfer of cholesterol ester from HDL to the APOB-containing lipoproteins in exchange for triglycerides. Mice lack cholesteryl ester transfer protein (CETP) and rapidly clear APOB-containing lipoproteins. In contrast, a higher proportion of HDL relative to LDL is observed in humans and primates due to the function of CETP. APOE*3-Leiden.CETP transgenic mice, a strain that expresses human CETP, exhibit a more human-like lipoprotein metabolism with transfer of cholesterol ester from HDL to the APOB-containing lipoproteins in exchange for triglycerides resulting in delayed APOB clearance. Pouwer et al. (2019) utilized these transgenic mice to evaluate the effect of PFOA on plasma cholesterol and the mechanism for the hypolipidemic responses observed with PFOA exposures. APOE*3-Leiden.CETP mice were fed a Western-type diet (0.25% cholesterol (wt/wt), 1% corn oil (wt/wt), and 14% bovine fat (wt/wt)) with PFOA (0.01, 0.3, or 30 mg/kg/day) for 4–6 weeks. The doses were chosen to parallel environmental and occupational exposures in humans. PFOA exposure did not alter plasma lipids at lower doses, but did decrease plasma triglycerides, total cholesterol, and non-HDL levels, and increased HDL levels. Overall, these findings mirrored a clinical trial in humans demonstrating PFOA-induced decreases in cholesterol levels. This lipid profile could be attributed to decreased very low-density lipoprotein (VLDL) production and increased VLDL clearance by the liver through increased lipoprotein lipase activity. The concomitant increase in HDL was attributed to decreased CETP activity subsequent to PPARα activation and the downregulation of hepatic genes involved in lipid metabolism, including Apoa1, Scarb1, and Lipc (genes involved in HDL formation, HDL clearance, and HDL remodeling, respectively). On the basis of the lipid profiles, gene expression analysis, and pathway analysis, the authors propose a mechanistic model in which high PFOA exposure increases VLDL clearance by the liver through increased LPL-mediated lipolytic activity. These changes lead to lower VLDL serum levels consistent with reduced VLDL particle formation and secretion from the liver due to reduced ApoB transcript levels and de novo synthesis.

To further explore mechanistic differences in PFOA-induced changes in lipid metabolism between humans and mice, Schlezinger et al. (2020) investigated PFOA-mediated lipid dysregulation in mice expressing human PPARα (hPPARα) and compared results to PPARα-null mice. Male and female mice were fed an American style diet (51.8% carbohydrate, 33.5% fat, and 14.7% protein, based on an analysis of what 2-to-19-year-old children and adolescents eat using NHANES data1) and exposed to PFOA (8 μM) in drinking water for 6 weeks that led to serum PFOA levels of 48 μg/mL. Both hPPARα-null and PPARα-null mice developed hepatosteatosis after PFOA exposure. Changes in gene expression and increased serum cholesterol that was more pronounced in males than females correlated with changes in expression of genes that regulate cholesterol homeostasis. PFOA decreased expression of Hmgcr in a PPARα-dependent manner. Ldlr and Cyp7a1 were also decreased but in a PPARα-independent manner. Apob expression was not changed. While many of the target genes analyzed were similarly regulated in both sexes, some sex-specific changes were observed. PFOA induced PPARα target genes in livers of both sexes including Acox1 (involved in fatty acid β-oxidation), Adrp (involved in coating lipid droplets), and Mogat1 (involved in diacylglyerol biosynthesis). PPARγ target genes were also upregulated in both sexes and included Fabp4 and Cd36 that contribute to lipid storage and transport as was the CAR target gene Cyp2b10. PFOA exposure decreased expression of Cyp7a1 required for conversion of cholesterol to bile acids and efflux, but more so in females than in males.

Sex-specific changes in hepatic gene expression in response to PFOA exposure was also observed in zebrafish (Hagenaars et al., 2013). Adult zebrafish were exposed to 0.1, 0.5, or 1 mg/L PFOA for 28 days. Livers were harvested and subjected to transcriptomic analysis. Similar to observations in mice, expression of genes regulating fatty acid metabolism and cholesterol metabolism and transport were generally upregulated in males and suppressed in females. Thus, sex-specific effects of PFOA on fatty acid and cholesterol metabolism is observed across different vertebrate species, but also exhibits species specificity. For example, genes in the cytochrome P450 family involved in cholesterol metabolism and transport were suppressed in female zebrafish but upregulated in male zebrafish (Hagenaars et al., 2013). However, Cyp2b genes downstream of CAR (e.g., Cyp2b1 and Cyb2b10) were more strongly upregulated in females compared with males in both rats and mice (Schlezinger et al., 2020; NTP, 2019). Differences in expression of Cyp450 genes may in part relate to species-specific activity of nuclear receptors, and the fact that no CAR orthologues have been identified in zebrafish nor any other fish species (Schaaf, 2017).

3.4.1.3.2.2.3 In Vitro Studies

In vitro studies reported genetic profiles and pathway analyses in mouse and human hepatocytes to determine the effect of PFOA treatment on lipid homeostasis and bile synthesis. Six studies investigated the effect of PFOA on lipid homeostasis using primary hepatocytes and human cell lines 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 addition, two studies evaluated PFOA-mediated changes to lipids using metabolomic approaches.

Franco et al. (2020a) exposed HepaRG cells to PFOA and PFOS and evaluated metabolomics at a dose range of 100 pM to 1 μM. The highest PFOA exposure levels (10–100 μM) were associated with significant increases in total lipid concentrations, especially at the three highest concentrations tested (10, 100, and 1,000 nM). Interestingly, hepatocyte lipids were decreased in response to increasing PFOS exposure in this system. The affected classes of lipids also diverged, with PFOA associated with increased diglycerides, triglycerides, and phosphatidylcholines, whereas PFOS was associated with decreased diglycerides, ceramides, and lysophosphatidylcholines. Staining of neutral lipids was also prominent in PFOA-treated hepatocytes, suggesting an obesogenic role PFOA that may directly impact hepatic steatosis. The authors further hypothesized that the concentration-dependent decrease in lipid accumulation associated with PFOS may be related to differential ability of these compounds to interact with PPARs, including PPARγ.

Peng et al. (2013) evaluated disturbances of lipids in the human liver cell line L-02 using metabolomic and transcriptomic approaches. Specifically, PFOA exposure was associated with altered mitochondrial metabolism of carnitine to acylcarnitines. The effect was dose-dependent and correlated with altered expression levels of key genes involved in this pathway. Downstream of this pathway, cholesterol biosynthesis was upregulated as measured by both increased cholesterol content and elevated expression levels of key genes. The profile of PFOA-associated disturbance in lipid metabolism was consistent with initial changes in fatty acid catabolism in cytosol that altered mitochondrial carnitine metabolism, ultimately impacting cholesterol biosynthesis.

In contrast to the findings of Peng et al. (2013) in L-02 cells, Das et al. (2017) reported that PFOA did not inhibit palmitate-supported respiration (mitochondrial metabolism) in HepaRG cells. There was no effect on oxidation or translocation of palmitoylcarnitine, an ester involved the in metabolism of fatty acids, as part of the tricarboxylic acid (TCA) cycle in the mitochondrial fraction. This may indicate less of a perturbation to fatty acid metabolism in this cell line. This suggests that intermediary steps in fatty acid activation, transport, and/or oxidation are affected. The authors suggest that PFOA effects on mitochondrial synthesis of fatty acid and other lipids are secondary and possibly compensatory to any mitochondrial-induced toxicity, rather than as the result of activation of peroxisomes, which are mediated by PPARs.

Rosen et al. (2013) exposed mouse and human primary hepatocytes to 0–100 or 0–200 μM PFOA, respectively. Gene expression was evaluated using microarrays and qRT-PCR. For PFOA-exposed murine hepatocytes, a much smaller group of genes was found to be altered compared with the whole liver. These genes included those associated with β-oxidation and fatty acid synthesis such as Ehhadh and Fabp1, which are upregulated by PFOA. In contrast to the transcriptome of primary mouse hepatocytes, a large group of genes related to lipid metabolism was differentially expressed in primary human hepatocytes including perilipin 2 (PLIN2) and CYPTA1, which were upregulated at 100 μM PFOA. 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 AhR (Section 3.4.1.3.1). Beggs et al. (2016) exposed human primary hepatocytes to 0.01–10 μM PFOA for 48 or 96 hours to determine pathways affected by PFOA exposure. PFOA treatment altered 40 genes (20 upregulated and 20 downregulated). Upregulated genes were primarily associated with lipid metabolism, hepatic steatosis and cholestasis, and liver hyperplasia. Among the top 10 upregulated genes were PLIN2, CYP4A22, and apolipoprotein A4 (APOA4).

Differential regulation of lipid metabolism and storage genes was also observed in HepG2 cells exposed to PFOA (dose range of 20–200 μM) for 48 hours (Wen et al., 2020). Some specific metabolic pathway genes were not altered, including genes encoding the acyl-CoA dehydrogenase enzyme. FABP1, which encodes for a key protein responsible for fatty acid uptake, transport, and metabolism, exhibited decreased expression. Acyl-CoA oxidase 2 (ACOX2), which is involved in the peroxisome-mediated degradation of fatty acids, was also decreased. In contrast, a number of genes involved in fatty acid anabolism were upregulated. The authors linked PFOA-mediated gene expression changes to diminished global methylation, implicating epigenetic factors in PFOA-mediated changes in gene expression.

In human hepatic cell lines such as HepaRG, PFOA treatment led to downregulation of genes involved in cholesterol homeostasis. Louisse et al. (2020) noted a concentration-dependent increase in triglycerides, a decrease of cholesterol at a high dose, and a downregulation of cholesterogenic genes especially after 24 hours of exposure to the high dose of 200 μM PFOA in HepaRG cells. Cellular cholesterol biosynthesis genes are regulated by SREBPs, which were also downregulated with PFOA exposure. In contrast, PPARα-responsive genes were upregulated with PFOA exposure, particularly at higher doses. Behr et al. (2020a) also exposed HepaRG cells to 0–500 μM PFOA 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 (SLC51B and ABCG1), and genes involved in bile acid and bilirubin detoxification (CYP3A4, UGT1A1). The gene profiles after 48 hours of exposure were similar, except at the high dose, at which there was an attenuation of the response in cholesterol synthesis and transport. Cholesterol content was significantly higher in the supernatant at the highest dose of 500 μM but there was no significant difference after 48 hours between treated cells and controls, which aligns with the attenuation of gene expression changes. Both studies also observed a PFOA-associated decrease in CYP7A1, a key enzyme involved in the initial step of cholesterol catabolism and bile acid synthesis.

3.4.1.3.2.2.4 Conclusions

Despite some inconsistencies in the literature, an emerging picture of PFOA-related dyslipidemia is largely initiated by activation of nuclear receptors targeted by PFOA, primarily PPARα, PPARγ, and CAR. A primary consequence of this interaction is altered expression of genes regulating hepatic lipid homeostasis. Gene expression profiles of lipid metabolism genes were observed both in vivo and in vitro, and in a diverse set of study designs. While changes in gene expression were consistently observed, the magnitude of the changes varied according to dose, dose duration, and model system. PPARα appears to be the primary driver regulating gene expression. However, studies in PPARα-null mice and analysis of nuclear receptor-specific genes implicate PPARγ, CAR, and possibly PPARδ as important contributors to the changes in PFOA-mediated gene expression. It should be noted, however, that a thorough analysis of potential compensatory changes in gene knockout mice was not discussed in the literature reviewed here.

Two of the primary pathways targeted by PFOA-induced changes in gene expression include metabolism of fatty acids leading to triglyceride synthesis and metabolism of cholesterol and bile acids. In both mice and rats, gene expression changes generally correlated with increased triglyceride levels in liver, and decreased levels of circulating serum triglycerides. For cholesterol, in vitro studies were conflicting but suggest hepatic cholesterol content generally increases in PFOA-exposed animals. However, serum cholesterol levels were reduced in rats but were generally elevated in mice. Hepatic changes in lipid-regulating gene expression appear to influence circulating levels of lipids in serum in a manner that varies by sex, species, and lifestage. For example, adult male rats exhibited decreases in serum triglycerides, whereas adult female rats exhibited increases (NTP, 2019). However, in mice exposed perinatally and then examined in adulthood, females, but not males, exhibited decreased serum levels of triglycerides, a treatment effect that was not observed in males (van Esterik et al., 2015). Male Kunming mice also exhibited a dose-dependent decrease in serum triglycerides and an increase in liver triglycerides (Wu et al., 2018). For cholesterol, serum levels were decreased in PFOA-exposed male rats and increased in female rats (NTP, 2019). In contrast, young male and female C57BL/6 mice exhibited hypercholesterolemia after PFOA exposure, though this was less striking male among BALB/c mice and did not manifest in female BALB/c mice (Rebholz et al., 2016). Elevated serum cholesterol was also more pronounced in males than females in mice expressing human PPARα (Schlezinger et al., 2020).

Importantly, changes in gene expression and lipid content in liver ultimately manifest in altered hepatocyte morphology. Most strikingly and consistently, steatosis manifests in PFOA-exposed animals. Other pathogenetic changes associated with PFOA included hepatomegaly, cholestasis,

hyperplasia, and carcinoma. The finding of steatosis is interesting in light of observation that PFOA exposure downregulates expression of HNF4α in liver with concomitant changes in HNF4α target genes because HNF4α-deficient mice develop steatosis in the absence of exposure to toxicants.

While the precise events that lead to steatosis have yet to be elucidated, the current studies conducted in animals and in vitro studies supports the following key molecular and cellular events related to PFOA-mediated hepatoxicity specific to changes in lipid metabolism: (1) PFOA accumulation in liver activates nuclear receptors; (2) nuclear receptors, including PPARα, then alter expression of genes involved in lipid homeostasis and metabolism; (3) the products of the genes altered by activated nuclear receptors modify the lipid content of liver to favor triglyceride accumulation, and possibly also cholesterol accumulation; (4) altered lipid content in liver leads to accumulation of lipid droplets promoting development of steatosis and other changes leading to liver dysfunction; and (5) alterations in lipid metabolism leads to alterations in serum levels of triglycerides and cholesterol. An intriguing possibility that may be concurrent to these events is direct binding of PFOA to ACACA and ACACB enzymes in a manner that interferes with fatty acid biosynthesis. Although this series of events is plausible, significant gaps remain in understanding this process, including how these events interface with other cellular processes such as cell growth and survival, oxidative stress, and others in understanding the mechanisms of PFOA-mediated hepatoxicity.

There are challenges in the extrapolation of results from research related to PFOA-mediated changes to lipid metabolism in animals to humans. As presented in the 2016 PFOA HESD (U.S. EPA, 2016c), serum lipid levels were variably altered in humans exposed to PFOA in their environments. In occupationally exposed humans and humans exposed to high levels of PFOA, there was a general association with increased serum total cholesterol and LDL, but not HDL. At least one obstacle to extrapolating from rodent to humans is that the cholesteryl ester transfer protein encoded by the CETP gene in humans is absent in rodents. Mice lack CETP and rapidly clear apoB-containing lipoproteins. In contrast, a higher proportion of HDL relative to LDL is observed in humans and primates due to the function of CETP. New models designed to develop mice that are “humanized” for lipid metabolism, including APOE*3-Leiden.CETP (Pouwer et al., 2019), and mice expressing human nuclear receptors (Schlezinger et al., 2020), are likely to accelerate the extrapolation of mechanistic information from animals to humans.

3.4.1.3.3 Hormone Function and Response

While much of the literature relevant to hormone function and response is focused on reproductive or endocrine outcomes (see Appendix, (U.S. EPA, 2024a)), recent literature has also shown a relationship between hepatic hormonal effects and PFOA exposure. PFOA has been found to affect thyroid mechanisms in hepatic cells. Huang et al. (2013) studied the effect of 5, 10, 25, or 50 mg/L PFOA in a human nontumor hepatic cell line (L-02 cells) and found that PFOA exposure downregulated thyroid hormone binding protein precursor.

While there are a small number of studies regarding hormone function and response specifically within the liver, there is evidence that PFOA has the potential to perturb hormonal balance in hepatic cells, particularly regarding thyroid function. This could have implications for hormone function and responses in other organ systems and may also be important for MOA considerations for hepatotoxicity.

3.4.1.3.4 Xenobiotic Metabolism

Xenobiotic metabolism is the detoxification 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 PFOA is not metabolized in humans or other species. However, several studies have investigated how PFOA could alter xenobiotic metabolism in the liver by downregulating or upregulating the gene expression of enzymes and transporters.

Li et al. (2017a) summarized the literature on molecular mechanisms of PFOA-induced toxicity in animals and humans. The authors noted how Elcombe et al. (2007) and Guruge et al. (2006) reported PFOA activation of PXR/CAR and subsequent manipulation of the expression of genes responsible for xenobiotic metabolism (Li et al., 2017a). For instance, Cheng and Klaassen (Cheng and Klaassen, 2008b) concluded that PFOA induced the gene expression of CYP2B10 in mice.

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

3.4.1.3.4.1 In Vivo Models

Three studies investigated xenobiotic metabolism endpoints in in vivo models with two using mice (Li et al., 2019c; Wen et al., 2019c) and one using zebrafish (Jantzen et al., 2016b).

Li et al. (2019c) examined 5–6-week-old male C57BL/6 mice administered PFOA (1 mg/kg/day) via oral gavage for 2, 8, or 16 weeks. CYP2B and CYP3A activity were assessed via PROD and BQ assays as an indicator of CAR/PXR activity in the liver. As discussed in Section 3.4.1.3.1, the authors reported upregulation of Cyp2b and Cyp3a gene expression with downstream effects to CAR/PXR activation and xenobiotic metabolism. Similarly, Wen et al. (2019c) investigated CYP gene expression (including Cyp1a1, Cyp2b10, and Cyp3a11) with a focus on the activation of the nuclear receptor PPARα and downstream alteration of metabolism and excretion of xenobiotics. Adult, male wild-type C57BL/6NTac and PPARα-null mice were administered PFOA (3 mg/kg/day) for 7 days (Wen et al., 2019c). Expression of a targeted list of genes, including Cyp1a1, Cyp2b10, and Cyp3a11, was quantified by qRT-PCR. In PFOA-treated wild-type mice, gene expression of Cyp1a1 and Cyp3a11 were not significantly changed. Conversely, in PFOA-treated PPARα-null mice, gene expression of Cyp2b10 and Cyp3a11 were significantly altered compared with the wild-type mice (11-fold increase for Cyp2b10 and 1.7-fold increase for Cyp3a11). Authors noted the differences between wild-type and PPARα mice were consistent with a previous study (Corton et al., 2014).

One study examined the expression of four genes related to xenobiotic metabolism in zebrafish (Jantzen et al., 2016b). Zebrafish embryos (AB strain) were exposed to 2.0 µM PFOA dissolved in water from 3 to 120 hours post-fertilization (hpf) and evaluated 180 days post-fertilization (dpf) at adult lifestage for gene expression. Females and males both had significant reductions in slco1d1 expression; however, only males had significant reductions in slco2b1 expression (Jantzen et al., 2016b). Jantzen et al. (2016b) noted that in their previous study (Jantzen et al., 2016a), PFOA exposure from 5 to 14 dpf resulted in significantly increased slco2b1 expression. Given the fluctuation in gene expression from short-term to long-term, further studies with additional timepoints are needed to elucidate the effect of PFOA exposure on OATPs expression.

3.4.1.3.4.2 In Vitro Models

CYP2B6 is expressed in the liver and is predominately responsible for xenobiotic metabolism; similar to previous studies, Behr et al. (2020b) investigated activation of nuclear receptors by PFAS. Authors exposed HEK293T cells and HepG2 cells to varying concentrations of PFOA (0, 50, 100, or 250 μM) for 24 hours. As discussed further in Section 3.4.1.3.1, the authors reported the downstream effects of PFOA-mediated PPARα activation. At the highest concentration of 250 μM, Behr et al. (2020b) reported that PFOA significantly induced gene expression of CYP2B6 by 11.2-fold. CYP2B6 gene expression was assessed in an additional study that used primary human and mouse hepatocytes (Rosen et al., 2013). In primary human hepatocytes, PFOA concentrations ranged between 0 and 200 μM; in mouse hepatocytes, concentrations ranged between 0 and 100 μM. Results varied between human and mouse hepatocytes, with CYP2B6 upregulated in human hepatocytes but not in mouse hepatocytes. The authors noted that the differences between gene expression of the human and mouse hepatocytes were unclear; however, cell density, collection methods, and time in culture were possible factors.

Franco et al. (2020b) assessed the expression of genes encoding several phase I and II biotransformation enzymes following exposure to PFOA concentrations (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) for 24 or 48 hours. Gene expression of phase I enzymes (CYP1A2, CYP2C19, and CYP3A4) varied across concentrations and between the 24- and 48-hour exposures. For CYP1A2, after 24 hours, expression was significantly upregulated at concentrations $\geq 10^{-9}$ M; however, after 48 hours, expression was significantly downregulated at concentrations $\geq 10^{-8}$ M. CYP2C19 was downregulated across all concentrations after both 24- and 48-hour exposures; downregulation was significant for concentrations after both 24- and 48-hour exposures with the exception of 10^{-8} M after 24-hours. The authors concluded that PFOA exposure can significantly reduce expression of phase I biotransformation enzymes.

Evidence varied across studies for the effect of PFOA on the expression of CYP3A4, a phase I enzyme involved in bile acid metabolism and detoxification by hydroxylation and xenobiotic metabolism, 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; Rosen et al., 2013; Shan et al., 2013). Franco et al. (2020b) reported that after 24-hours, there were not significant changes in CYP3A4 expression. However, after 48 hours, there was a fivefold reduction in the expression. Conversely, Behr et al. (2020a) and Louisse et al. (2020) reported upregulation of CYP3A4 enzyme activity following 24- or 48-hour PFOA exposure in HepaRG cells; specifically, Behr et al. (2020a) reported significant upregulation at 50 and 100 μM after both 24- and 48-hour PFOA exposure.

Rosen et al. (2013) also reported upregulation of CYP3A4 expression following PFOA exposure (0–100 μM) in human hepatocytes; however, significant changes were not reported for mouse hepatocytes. Lastly, Shan et al. (2013) reported no significant changes in CYP3A4 enzyme activity following PFOA exposure (0, 100, 200, 300, or 400 μM) in HepG2 cells.

Franco et al. (2020b) also assessed gene expression of phase II enzymes, glutathione-s-transferase mu1 (GST-M1) and UDP glucuronosyltransferase-1A1 (UGT-1A1), which were not significantly affected by exposure to PFOA after 24 or 48 hours. The authors noted that it was unclear where and how PFOA alters gene expression of phase I enzymes and not phase II enzymes. Further research is needed to determine whether altered gene expression occurs by

interference with cytoplasm receptors, inhibition of nuclear translocation, and/or inhibition of the interaction of nuclear translocator complexes with DNA sequences (Franco et al., 2020b).

Orbach et al. (2018) focused on the gene expression of the CYP2E1 enzyme. PFOA was added to primary human hepatocytes and primary rat hepatocytes at either ½ LC50 or LC50 (500 μM for both humans and rats) for 24 hours. CYP2E1 enzymatic activity was estimated by the conversion of 7-methoxy-4-trifluoromethylcoumarin (MFC) to 7-hydroxytrifluoromethylcoumarin (HFC). However, in both human and rat hepatocytes, there were no significant changes in CYP2E1 activity.

Song et al. (2016) analyzed the expression of over 1,000 genes by expression microarray analysis following exposure of HepG2 cells with increasing concentrations (0–1,000 μM) of PFOA for 48 hours. As a result, 1,973 genes expressed ≥1.5-fold changes in the exposed groups compared with the control group, including 20 genes responsible for metabolism of xenobiotics by cytochrome P450.

3.4.1.3.4.3 Conclusions

Several studies are available that assessed xenobiotic metabolism endpoints as a response to PFOA exposure, including studies in mice (Li et al., 2019c; Wen et al., 2019c), zebrafish (Jantzen et al., 2016b), primary hepatocytes (Orbach et al., 2018; Rosen et al., 2013), or hepatic cell lines (Behr et al., 2020b; 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 altered gene expression of CYP enzymes, the direction and magnitude of change varied across doses and exposure durations. Jantzen et al. (2016b) and Franco et al. (2020b) both noted the need for further research to elucidate any potential relationships between PFOA exposure and xenobiotic metabolism.

3.4.1.3.5 Cell Viability, Growth and Fate

3.4.1.3.5.1 Cytotoxicity

Several in vitro studies have examined the cytotoxic effect of PFOA on cell viability assays in both primary hepatic cell cultures (Xu et al., 2019b; Beggs et al., 2016) and in hepatic cell lines (Behr et al., 2020a; Franco et al., 2020b; Franco et al., 2020a; Ojo et al., 2020; Wen et al., 2020; Zhang et al., 2020a; Lv et al., 2019; Rosenmai et al., 2018; Sheng et al., 2018; Song et al., 2016; Cui et al., 2015; Wielsøe et al., 2015; Yan et al., 2015a; Hu et al., 2014; Huang et al., 2014; Shan et al., 2013; Florentin et al., 2011), with varying results depending on the exposure concentration and duration, cell line, and culturing methods.

In mouse primary hepatocytes, cell viability as determined by cell counting Kit-8 (CCK-8) assay did not significantly change at concentrations of PFOA in the range of 10–500 μM; however, a 41% decrease in viability was observed after 24 hours of exposure to 1000 μM PFOA (Xu et al., 2019b). In primary rat hepatocytes exposed to PFOA for 24 hours showed no changes in cell viability at concentrations ≤25 μM, but cell viability was increased by approximately 16% in the 100 μM concentration (Liu et al., 2017a).

PFOA exposure duration and concentration affect cytotoxicity. In HepG2 cells, 100 μM PFOA did not affect cell viability after 1–3 hours of exposure (Shan et al., 2013; Florentin et al., 2011). However, after 72 hours, cell viability as determined by neutral red assay was reduced by nearly

80% in the same cell line (Buhrke et al., 2013), suggesting that PFOA cytotoxicity is increased with long-term exposure. Additionally, in human HEPG2 cells treated at different concentrations of PFOA for 24 hours, viability as determined by MTT assay did not change with 100 μM PFOA, but was significantly reduced by 14% at 200 μM, 22% at 400 μM, 47% at 600 μM, and 69% at 800 μM, suggesting a concentration-dependent reduction in cell viability (Florentin et al., 2011). In contrast, cell viability dropped below 80% in HepaRG cells exposed to 100 μM PFOA at 24 hours (Franco et al., 2020b). Another study in HepaRG cells (Louisse et al., 2020) showed no effect on cell viability up to concentrations of 400 μM for 24 hours. Although some results are conflicting, overall, these studies suggest that exposure duration and concentration, type of cell lines, species, and viability assessment methods are determinants of PFOA-induced cytotoxicity.

IC50 values in hepatic cell lines ranged from approximately 42 μM PFOA after 72 hours (Buhrke et al., 2013), 102–145 μM after 24 hours (Franco et al., 2020b; Ojo et al., 2020), to 305 μM after 48 hours of exposure in HepG2 cells (Song et al., 2016). In a fetal liver cell line (HL-7702), IC50 values were 647 μM after 24 hours exposure and 777 μM after 48 hours exposure (Sheng et al., 2018; Hu et al., 2014). One study in zebrafish liver cells reported IC50 values of 84.76 μg/mL after 48 hours exposure (Cui et al., 2015).

3.4.1.3.5.2 Apoptosis

To determine the mechanism underlying PFOA-induced cytotoxicity, several studies have interrogated the apoptosis pathway as a potential mechanism (Li et al., 2017b; Cui et al., 2015; Buhrke et al., 2013). Apoptosis is characterized by biochemical and morphological changes in cells. Flow cytometry has been used to quantify the percentage of apoptotic cells and their phase in cells exposed to PFOA. The percentage of apoptotic cells in the early and late phases of apoptosis nearly doubled in isolated C57BL/6J mice hepatocytes exposed to 500 μM and 1,000 μM PFOA for 24 hours (Xu et al., 2019b). In zebrafish liver cells exposed to the IC50 (84.76 μg/mL) and IC80 (150.97 μg/mL) for 48 hours, the percentage of dead cells in the late phase of apoptosis did not change in cells exposed to the IC50 compared with control, while a significant increase in the percentage of apoptotic cells in the late phase of apoptosis was observed in the cells exposed to the IC80 (Cui et al., 2015).

Activation of cysteine aspartic acid-specific protease (caspase) family is essential for initiation and execution of apoptosis. PFOA-induced apoptosis via caspase activities have been examined in primary mouse hepatocytes, mouse cell lines, and human cell lines after exposure to various PFOA concentrations (Xu et al., 2020b; Sun et al., 2019; Li et al., 2017b; Cui et al., 2015; Buhrke et al., 2013; Huang et al., 2013). In mouse hepatocytes, PFOA induced caspase activity in a dose-dependent manner (Li et al., 2017b). In male C57BL/6J mouse hepatocytes treated with PFOA for 24 hours, caspase 3 activity did not change at doses below 1,000 μM but increased by more than 1,000% at 1,000 μM (Xu et al., 2020b). In a spheroid model of mouse liver cells (AML12), increased activity of caspase 3/7 was detected from 14 to 28 days of ≥100 μM PFOA exposure (Sun et al., 2019). In contrast, 100 μM PFOA did not change caspase 3/7 activity in HepG2 cells exposed for 48 hours (Buhrke et al., 2013).

Another key feature of cells undergoing apoptosis is the release of lactate dehydrogenase (LDH). Many studies have reported intracellular release of LDH in hepatocytes treated with PFOA (Sun et al., 2019; Wielsøe et al., 2015; Yan et al., 2015b; Shan et al., 2013). In male C57BL/6J mouse

primary hepatocytes treated with PFOA for 24 hours, 35% increase in LDH was observed at the 10 mM dose compared with control. However, for all concentrations below 10 mM, the difference was not significant (Xu et al., 2020b).

Changes in mRNA and protein expression of apoptotic genes is a hallmark of apoptosis. Increased expression of p53, Bcl-2, Bcl-2 associated X-protein (Bax), caspase-3, nuclear factor kappa B (NF-κB) mRNA and protein was observed in zebrafish liver (Cui et al., 2015). In human hepatoma SMM-721 cells treated with 10 or 100 μg/mL PFOA for 3 hours, BAX mRNA was significantly increased while B cell lymphoma 2 (Bcl-2) decreased compared with control (Lv et al., 2019). Proteomic analysis of 28 proteins differentially expressed in PFOA-exposed human nontumor hepatic cells (L-02) led the authors to conclude that PFOA induces apoptosis by activating the p53 mitochondria pathway (Huang et al., 2013). This result is consistent with several studies showing that PFOA-induced liver apoptosis is in part mediated through p53 activation (Sun et al., 2019; Li et al., 2017b). In a third study that examined miRNA expression in the mouse liver, an increase in the expression of miR-34a-5p, which has been shown to be involved in p53-mediated apoptosis, was observed (Yan et al., 2014).

PFOA has been shown to induce apoptosis through morphological changes to the mitochondrial membrane (Xu et al., 2020b; Li et al., 2017b). One study in Balb/c male mice gavaged with PFOA (0.08–20 mg/kg/day) for 28 days suggested that hepatocyte apoptosis following exposure to PFOA may be caused by endoplasmic reticulum stress, mediated by the induction of ER stress markers including phosphorylated eukaryotic initiation factor 2α (p-elf2α), spliced X box-binding protein 1 (XBP1), and C/EBP homologous protein (CHOP) (Yan et al., 2015b).

An RNA-sequencing study in primary human hepatocytes found that PFOA 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 PFOA induced intracellular oxidative stress in Sprague-Dawley rats leading to apoptosis (Liu et al., 2017a). Other mechanisms underlying PFOA-induced apoptosis include DNA damage (Wielsøe et al., 2015), autophagosome accumulation (Yan et al., 2017; Yan et al., 2015b), induction of ER stress biomarkers and oxidative stress (Li et al., 2017b; Wielsøe et al., 2015; Huang et al., 2013; Panaretakis et al., 2001), and reduction of mitochondrial ATP (Sun et al., 2019; Mashayekhi et al., 2015). Although many studies have reported oxidative stress as a potential mechanism underlying PFOA-induced apoptosis, Florentin et al. (2011) did not observe an increase in DNA damage or ROS at doses that proved cytotoxic to HEPG2 cells, leading the authors to conclude that PFOA-induced apoptosis is not related to DNA damage nor oxidative stress.

PFOA-induced apoptosis has been shown to differ between males and females. In male and female Balb/c mice gavaged with PFOA at doses ranging from 0.01 to 2.5 mg/kg/day for 28 days, caspase-9 activity and dissipation of the mitochondrial membrane potential were higher in females than males. Specifically, mitochondrial membrane dissipation was 25% in males and 39% in females for mice in the 2.5 mg/kg/day groups. In the 0.05 mg/kg/day group, caspase-9 activity was elevated by 72% in females compared with 40% in males. The sexual dimorphic changes in caspase-9 and mitochondrial membrane dissipation were accompanied by morphological changes in the mitochondria characterized by increased mitochondrial vesicle formation and swelling in female than male hepatocytes, suggesting that female livers are more susceptible to PFOA-induced apoptosis than males (Li et al., 2017b).

3.4.1.3.5.3 Cell Cycle and Proliferation

Alterations in cell proliferation and cell cycle were also seen in many in vivo and in vitro studies (Wen et al., 2020; Zhang et al., 2020a; Lv et al., 2019; Beggs et al., 2016; Song et al., 2016; Zhang et al., 2016a; Buhrke et al., 2015; Buhrke et al., 2013). In mice exposed to 3 mg/kg/day PFOA for 7 days by oral gavage, proliferation in the liver, as seen through proliferation cell nuclear antigen (PCNA) staining, was increased relative to control (Beggs et al., 2016). HL-7702 cells were treated with PFOA at concentrations of 50–400 μM for 48 or 96 hours (Zhang et al., 2016a). All except the highest dose (400 μM) group showed an increase in cell proliferation compared with control at 48 hours. Other studies have reported a similar pattern for which proliferation is significantly increased at low doses and decreased at high doses of PFOA in human primary hepatocytes (Buhrke et al., 2015), HepG2 (Buhrke et al., 2013), and HepaRG cells (Behr et al., 2020a). Together these studies suggest that higher concentration of PFOA may interfere with cell cycle progression by reducing cell proliferation rather than severely inducing apoptosis.

In contrast, a study in primary hepatocytes of Sprague-Dawley rats found increased proliferation at the highest dose and no proliferative effect at low doses. Approximately 16% increase in proliferation was observed with PFOA exposures of 100 μM for 24 hours compared with controls (Liu et al., 2017a). However, no changes in cell number as measured by MTT assay was observed at the PFOA concentration range of 0.4–25 μM at the same duration, adding to the evidence that PFOA-induced proliferation is dose-dependent and may vary by cell type.

PFOA has also been shown to disrupt cell cycle progression. Using flow cytometry, Zhang et al. (2016a) found that in HL-7702 cells, the proportion of cells in the G0/G1 phase (nondividing) significantly decreased while cells in the S-phase increased after 48 hours of exposure to 50 and 100 μM PFOA. However, at the 200 μM and 400 μM exposure for 48 hours, percentage of cells in the G0/G1 phase increased while cells in the G2/M/S phase (interphase growth/mitosis) decreased significantly compared with control. Interestingly, the same trend was observed in cells incubated at the same dose for 96 hours (Zhang et al., 2016a). A second study in immortalized nontumor cells derived from human normal liver tissue (L-02 cells) also used flow cytometry to examine changes in the cell cycle after 72 hours at 25 and 50 mg/L and found that PFOA increased the percentage of cells in G2/M phases but decreased the number of cells in G0/G1 and S phases (Huang et al., 2013). Additionally, the percentage of cells in apoptotic sub-G1 (G1-) phase increased significantly from 19% to 33% compared with 10% of cells in the G1-phase in the control group, leading the authors to conclude that PFOA treatment disrupt cell cycle in L-02 cells by arresting cells in G2/M phase while inducing apoptosis. A third study in a zebrafish liver cell line also used flow cytometry to identify changes in the cell cycle after 85 and 151 μg/mL PFOA exposure for 48 hours. In corroboration with the study in L-02 cells, PFOA concentration of 151 μg/mL showed an increase in the percentage of cells in the G2/M/S stage and a decrease in the percentage of cells in the G1/G0 phase (Cui et al., 2015). Together, these studies suggest that PFOA interferes with the balance between apoptosis and proliferation by disrupting cell cycle progression.

PFOA-induced changes in cell proliferation and cell cycle progression are often accompanied with changes in mRNA and protein expression of genes implicated in cell cycle progression. Pathway analysis of protein expression in human HL-7702 normal liver cells exposed to 50 μM PFOA for 48 and 96 hours identified 68 differentially expressed proteins that are related to cell

proliferation and apoptosis (Zhang et al., 2016a). Western blot analysis from the same study showed differential protein expression of positive cell cycle-regulators, including cyclins and cyclin-dependent kinases (Cyclin/CDKs) that are known to control G1/G2/S/M cell cycle progression, as well as negative regulators (p53, p21, MYTI, and WEE1). Interestingly, expression of cell cycle regulations was dose-dependent. Significant induction of cyclin D1, CDK6, cyclin E2, cyclin A2, CDK2, p-CDK1, p53, p21, p-WEE1 and myelin transcription factor 1 (MYT1) was observed at low dose (50 or 100 μM). However, cyclin A2, cyclin B1 and p21 proteins were significantly inhibited at high dose (400 μM) at the same duration (48 hours) (Zhang et al., 2016a). In primary human hepatocytes treated with 10 μM PFOA, CCND1 and Aldo-keto reductase family 1 member B10 (AKR1B10) mRNA were significantly induced after 96 hours (Beggs et al., 2016). AKR1B10 is a promitogenic gene that has been associated with the progression of hepatocellular carcinoma (Matkowskyj et al., 2014). In addition, two microarray studies in hepatic cell lines found that PFOA exposures ranging from 100 to 305 μM for up to 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).

PFOA has been shown to decrease the expression of hepatocyte nuclear factor 4-alpha (HNF4α), a regulator of hepatic differentiation and quiescence, in multiple studies and is thought to mediate steatosis following PFOA exposure (Behr et al., 2020a; Beggs et al., 2016). One study suggested that PFOA-induced proliferation may be mediated by the degradation of HNF4α (Beggs et al., 2016). This study, using wild-type CD-1 and HNF4α knockout mice, reported that 11 out of 40 genes altered by PFOA exposure were regulated by HNF4α. PFOA exposure decreased the expression of HNF4α in both male mice and primary human hepatocytes and increased the expression of Nanog, a stem cell marker, suggesting that PFOA may be de-differentiating hepatocytes. Increased relative liver weight in PFOA-exposed mice was observed in this study and the authors concluded that hepatomegaly, along with other liver effects such as steatosis, may be mediated by PFOA-induced dysregulation of HNF4α.

3.4.1.3.5.4 Conclusions

Hepatotoxicity is widely cited as a type of toxicity induced by PFOA exposure. PFOA has been shown to trigger apoptosis at high doses and induce cell proliferation at low doses. PFOA-induced apoptosis is activated through a cascade of mechanisms including activation of caspase activity, intracellular release of LDH, induction of apoptotic genes, morphological changes to the mitochondria membrane, and activation of p53 mitochondria pathway. Additionally, PFOA induced hepatocyte proliferation both in vivo and in vitro by disrupting cell cycle progression leading to liver dysfunction, including steatosis and hepatomegaly. Therefore, PFOA exposure may lead to liver cytotoxicity through a myriad of intracellular events.

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, resulting 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 PFOA on the immune function of these cell types has not been thoroughly investigated.

3.4.1.3.6.1 In Vivo Studies

Investigations into the liver immune response have been conducted in a single human study in the C8 Health Project cohort (Bassler et al., 2019), and in several rodent studies (Li et al., 2019c; Wu et al., 2018; Hui et al., 2017; Liu et al., 2016; Yu et al., 2016; Botelho et al., 2015). 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 proinflammatory cytokines. PFOA levels were significantly correlated with decreases in serum levels of the proinflammatory cytokine tumor necrosis factor α (TNFα). In contrast, both interferon γ (IFNγ) and cleaved complement 3 (C3a) were positively associated with PFOA levels. 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).

A study in mice acutely exposed to PFOA also linked hepatic injury to activation of the complement system. In contrast to the human study (Bassler et al., 2019), a decrease in serum C3a was observed in mice (Botelho et al., 2015). C57BL/6 mice exposed to a 10-day dietary treatment with PFOA (0.002–0.02%, w/w) exhibited hepatomegaly, elevated serum triglycerides, elevated alanine aminotransferase (ALAT), hepatocyte hypertrophy, and hepatocellular necrosis at all doses. At the highest dose only, PFOA-induced hepatic injury coincided with deposition of the complement factor C3a fragment in the hepatic parenchyma. The findings support activation of the classical, but not alternative complement cascade in liver, and correlated with diminished C3 levels in serum. In serum, commercial hemolytic assays indicated attenuation of both the classical and alternative complement pathways. These authors proposed that that PFOA-mediated induction of hepatic parenchymal necrosis is the initiation event that leads to activation of the complement cascade and pro-inflammatory responses.

In another study in mice, the effects of PFOA exposure on inflammatory changes in liver varied depending on the presence of pre-existing NAFLD (Li et al., 2019c). Mice were subjected to control diet or HFD for 16 weeks to induce NAFLD, after which they were exposed to vehicle or 1 mg/kg/day PFOA by oral gavage for 2, 8, or 16 weeks; the control diet and HFD were continued throughout the exposure period until necropsy. In mice on the control diet, inflammatory changes were not observed in the first 8 weeks of PFOA treatment. However, after 16 weeks of PFOA treatment, mild hepatic lobular inflammation was observed in 3 of 5 animals, suggesting that chronic exposure to PFOA induces inflammatory changes in liver. In HFD-fed mice, focal inflammation was seen as early as 2 weeks after initiating PFOA treatment and inflammatory foci were observed in 2 of 5 mice after 16 weeks of PFOA exposure. Gene expression of Tnfα measured by qRT-PCR was elevated in the HFD group exposed to PFOA for

all three treatment durations (2, 8, or 16 weeks of PFOA). Similarly, Liu et al. (2016) observed an induction of TNFα in liver homogenates, measured by ELISA, in male Kunming mice fed a regular diet (Liu et al., 2016) and exposed to a higher dose of PFOA (10 mg/kg/day for 2 weeks). This study observed significantly elevated levels of both TNFα and IL-6 in liver homogenates.

Li et al. (2019c) also confirmed increased expression of inflammatory genes using an RNA-Seq transcriptomic approach. Compared to mice on the control diet, the HFD group exposed to PFOA resulted in 537 differentially expressed genes. The inflammatory response was among the top enriched Gene Ontology (GO) terms for the gene set specific to the PFOA-exposed HFD. Analysis using Ingenuity Pathway Analysis showed significant upregulation of chemokines and chemokine-related genes and toll-like receptor (TLR) related genes in the PFOA-exposed HFD group compared with mice fed the control diet. Taken together with the histopathological findings, these gene expression changes suggest that preexisting fatty liver may enhance PFOA-mediated inflammatory changes in liver.

Another potential nexus between changes in hepatic lipid metabolism and inflammation comes from a high-throughput metabolomics study in male BALB/c mice (Yu et al., 2016). After a 28-day exposure to 0, 2.5 or 5 mg/kg/day PFOA, livers were subjected to metabolomic analysis. Metabolite analysis indicated PFOA altered polyunsaturated fatty acid metabolism including the arachidonic acid pathway. Arachidonic acid is a precursor in production of inflammatory mediators including prostaglandins, thrombaxanes, and leukotrienes. Prostaglandins (PGD2, PGE2, and PGF2α) were slightly elevated but increases did not reach statistical significance. However, the ratio of the thromboxane A2 (TXBA2) metabolite thromboxane X2 (TXB2) to prostaglandin I2 (PGI2) was significantly decreased in PFOA-exposed mice. Given the prothrombotic role of TXBA2 and the vasodilatory role of PGI2, the authors suggest these changes are consistent with ischemic liver injury that is characterized by vasodilation of microvasculature, lessened adherent leukocytes, and improved flow velocity in liver. Two leukotrienes, LTD4 and LTB4 were significantly lower in the high dose group. Both leukotrienes can also regulate vascular permeability and the authors suggest these changes are consistent with PFOA-induced inflammation in liver. PFOA also upregulates CD36 gene expression in hepatocytes (Wu et al., 2018; Hui et al., 2017), which is a negative regulator of angiogenesis (Silverstein and Febbraio, 2009). Together with the PFOA-mediated changes in abundance of prostaglandins and thrombaxanes, these findings raise the possibility that PFOA-mediated alterations of the hepatic microvasculature are key events in the development or persistence of liver inflammation.

3.4.1.3.6.2 In Vitro Studies

In a study investigating the hepatic effects of PFOA in vitro, Song et al. (2016) evaluated gene expression changes in human liver hepatocellular carcinoma HepG2 cells using a whole genome expression microarray. After exposing these cells to 306 μM PFOA (the IC20 dose for cell viability inhibition) for 48 hours, gene expression changes were evaluated. PFOA exposure led to differential regulation of 1,973 genes. Through KEGG pathway analyses, the authors reported that genes related to immune response were among the most differentially expressed biological process out of the 189 processes with altered genetic profiles. The authors identified 17 immune-associated genes that were differentially expressed. These genes mapped to the TNF signaling pathway, nucleotide-binding and oligomerization domain (NOD)-like receptor signaling,

cytokine-cytokine receptor interactions, and the complement and coagulation cascade system. These findings support a role for PFOA in dysregulating innate immune mechanisms.

Alterations in cytokines associated with regulation of adaptive immunity were also observed using multicellular hepatic organotypic culture models composed of primary human or rat cells (Orbach et al., 2018). This system involved seeding primary liver sinusoidal epithelial cells and Kupffer cells encapsulated in extracellular matrix proteins above the hepatocytes. This culture system forms a stratified three-dimensional (3D) structure designed to more accurately mimic liver tissue. Organotypic cultures were exposed to 500 μM PFOA for 24 hours (the LC50 in human cultures). PFOA exposure led to a 62% decrease in IL-10 levels. In addition to being a key cytokine in development of T helper lymphocytes, IL-10 has anti-inflammatory properties. Thus, the decrease in IL-10 observed in organotypic culture is consistent with the proinflammatory changes in liver associated with PFOA exposure. Using a proteomic approach, another cytokine, IL-22, has also been shown to be downregulated in PFOA-exposed human hepatic L-02 cells (Huang et al., 2013). IL-22, a member of the IL-10 cytokine family, exerts protective effects in liver during acute inflammation and alcoholic liver injury (Ki et al., 2010; Zenewicz et al., 2007). T helper (Th22) cells are a T cell subset responsive to IL-22. Th22 cells function in maintaining the integrity of the epithelial barriers (Hossein-Khannazer et al., 2021). As such, diminished levels of IL-22 in the liver suggest that PFOA could interfere with the protective effects of IL-22 and Th22 cells.

3.4.1.3.6.3 Conclusions

The limited number of studies reviewed support a role PFOA in inducing hepatic inflammation through dysregulation of innate immune responses. This includes elevated levels of TNFα as well as changes in prostaglandin and thromboxane levels. Gene expression studies also suggest a role for chemokines in elaborating inflammation in liver. Expression of genes coding for products involved in innate immune defense systems were altered, including TLRs, molecules involved in NOD signaling, and C3a, a key indicator of complement cascade activation. Far less is known regarding PFOA effects on adaptive immunity in liver. PFOA exposure caused a reduction in IL-10 levels in organotypic culture of liver. IL-10 has anti-inflammatory properties in addition to promoting differentiation of Th2 CD4+ T cells. Intriguingly, IL-22 levels were diminished in PFOA-exposed hepatic cells. This cytokine may impact the function of Th22 T lymphocytes and impact the epithelial barriers in liver. Moreover, IL-22 reduction may reduce the protective effects of this cytokine during inflammation. Altogether, induction of inflammation appears to be an important mechanism that impacts liver pathogenesis in response to PFOA exposure, though the contribution of specific populations of resident or infiltrating liver immune cells and the series of events that produce inflammation have yet to be elucidated. Adaptive immune responses are disrupted in PFOA-exposed animals (Section 3.4.2.2). However, whether alterations in adaptive immunity impact pathogenetic mechanisms in liver remain unknown.

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). PFOA has been implicated as a chemical that can induce these indicators of oxidative stress, inflammation, and cell damage.

3.4.1.3.7.2 In Vivo Models

3.4.1.3.7.2.1 Mouse

Yan et al. (2015b) examined livers from male Balb/c mouse following PFOA exposure of 0.08, 0.31, 1.25, 5, or 20 mg/kg/day for evidence of oxidative stress, including changes in expression of oxidative stress-related genes. While no change was observed in Cat expression levels, increases in Sesn1, Sod1, and Sod2 were observed in livers from mice exposed to 1.25, 5, and 20 mg/kg/day PFOA, respectively. PFOA exposure led to increased CAT activity and decreased SOD activity in mouse livers. MDA contents were decreased at all dose levels, and levels of the antioxidant GSH increased at 5 and 20 mg/kg/day PFOA. Authors concluded that the changes in SOD, CAT, GSH, and MDA reflect PFOA-induced disruptions to the antioxidant defense system in the livers of exposed mice. However, no significant oxidative damage was observed.

Li et al. (2017b) explored the role of ROS accumulation in apoptosis in male and female Balb/c mice dosed with 0.05, 0.5, or 2.5 mg/kg/day PFOA for 28 days. The authors explored how activation of PPARα and suppression of the electron transport chain (ETC) sub-unit Complex I influenced ROS generation. Excluding the lowest male dose group, PFOA exposure significantly increased 8-OHdG levels in the liver, a key indicator of oxidative DNA damage. 8-OHdG levels were higher among dosed females compared with males, which authors suggest signals stronger genotoxicity in females. Authors explored the connection between the oxidative stress and apoptosis through the p53 signal pathway. Increases in p53 levels occurred in the same dose groups with elevated 8-OHdG, which authors suggest indirectly links oxidative stress to apoptosis. Authors posited that ROS hypergeneration led to increased 8-OHdG levels, and DNA damage then leads to increases in programmed cell death protein 5 (PDCD5), which activates p53 to induce apoptosis. At 0.5 and 2.5 mg/kg/day, PFOA exposure decreased expression of electron transport chain (ETC) proteins, which corresponds to an increase in ROS generation and accumulation. For two ETC subunits, ACP and NDUV2, expression was increased, which also indicates an accumulation of ROS and an increase in antioxidant activity to counter ROS generation. At 0.05 mg/kg/day, female mice showed more oxidative stress than males. In these females, Complex I suppression drove ultimate apoptosis, while PPARα activation drove apoptosis among males.

Two studies examined changes in oxidative stress endpoints in male Kunming mice exposed to PFOA (Liu et al., 2016; Yang et al., 2014), and an additional two studies evaluated oxidative stress endpoints in pregnant female Kunming mice and their pups (Li et al., 2019a; Song et al.,

2019). In the livers of male Kunming mice exposed to 2.5, 5, or 10 mg/kg/day PFOA for 14 days, MDA at all doses and H_2O_2 at 5 and 10 mg/kg/day levels were significantly increased compared with controls (Yang et al., 2014). Liu et al. (2016) explored grape seed proanthocyanidn extract (GSPE) as a protective agent against PFOA damage in the liver. The authors reported significantly increased MDA and H_2O_2, significantly decreased Nrf2 protein levels, and significantly decreased SOD and CAT activity in the liver following PFOA exposure. Additionally, expression of SOD and CAT, measured via qRT-PCR, were significantly decreased in the livers of exposed mice. Li et al. (2019b) found that serum levels of SOD and 8-OHdG were significantly increased in pups of females dosed at 2.5, 5, and 10 mg/kg/day PFOA. Serum levels of CAT were increased at 5 and 10 mg/kg/day PFOA. PFOA-induced changes in SOD, CAT, and 8-OHdG reflect increased antioxidant activity in response to increased oxidative stress and increased DNA damage. In their study examining the protective effects of lycopene against PFOA-induced damage, Song et al. (2019) exposed pregnant mice to 20 mg/kg/day PFOA via oral gavage from gestational days (GD) 1–7. After sacrifice on GD 9, levels of MDA were significantly increased in livers of pregnant mice treated with 20 mg/kg/day PFOA, while SOD and GSH-Ps levels were significantly decreased compared with controls, providing evidence of oxidative damage in the liver following PFOA exposure.

Three studies dosed C57Bl/6 mice with PFOA to study impacts on oxidative stress endpoints (Crebelli et al., 2019; Wen et al., 2019c; Kamendulis et al., 2014). In male C57Bl/6 mice dosed with 28 mg/L PFOA, Crebelli et al. (2019) found slightly decreased T-AOC, but the results were not statistically significant. MDA levels were below detection limits in all collected samples. Additionally, there was no statistically significant change in the levels of liver TBARS that would indicate lipid peroxidation. Kamendulis et al. (2014) exposed male C57Bl/6 mice to 5 mg/kg/day and found that PFOA exposure led to a 1.5-fold increase in 8-iso-PGF2α levels, a measure of lipid peroxidation that indicates oxidative damage. Additionally, PFOA led to a nearly twofold increase in mRNA levels of Sod1 in liver cells extracted from mice dosed at 2.5 and 5 mg/kg/day PFOA. mRNA levels of Sod2 and Cat were increased threefold and 1.3-fold, respectively. The same doses of PFOA also led to a nearly twofold increase in Nqo1 mRNA levels. The induction of genes for detoxifying enzymes following PFOA exposure suggests PFOA causes increased oxidative stress activity. In a different study (Wen et al., 2019c), 1 and 3 mg/kg/day PFOA exposure in wild-type C57BL/6 NCrl male mice increased gene expression of Nrf2 and Nqo1, measured via qRT-PCR assays, by 50%–300%.

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 PFOA exposure was obtained from a previously published paper by Rosen et al. (2008b). In WT (129S1/SvlmJ) and Pparα-null male mice, Nrf2 activation was observed (as seen by increases in gene expression biomarkers) after a 7-day exposure to 3 mg/kg/day PFOA via gavage. Similar to Nrf2, CAR was also activated in both mouse strains after PFOA exposure. The authors proposed that CAR activation by chemical exposure (PFOA or otherwise) leads to Nrf2 activation, and that oxidative stress may be a mediator.

3.4.1.3.7.3 In Vitro Models

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 levels of PFOA ranging from 0 to 200 μM. PFOA exposure led to a decrease in the expression of the heme oxygenase 1 (Hmox1) gene in human primary hepatocytes. There were no changes observed in the nitric oxide synthase 2 (Nos2) gene nor in either gene in primary mouse hepatocytes.

Orbach et al. (2018) examined the impacts of 500 μM PFOA exposure in multicellular organotypic culture models (OCM) of primary human and rat hepatocytes and in collagen sandwich (CS) models via high-throughput screening. In exposed rat and human cells, PFOA decreased GSH levels by <10%. The authors suggest that PFOA did not bind to or oxidize GSH. In human OCMs, mitochondrial integrity decreased 37% following PFOA exposure. In human CS models, the decrease was 39%. In rat OCMs, exposure decreased mitochondrial integrity by 47%, and by 45% in rat CS models.

In primary rat hepatocytes incubated with 100 μM PFOA for 24-hours, Liu et al. (2017a) found that intracellular oxidant intensity increased to more than 120% of control levels as measured by mean fluorescence intensity of 2′,7′-dichlorofluorescein (DCF). In addition, cells incubated with 6.25, 25, or 100 μM PFOA displayed significantly increased levels of mitochondrial superoxide, measured by MitoSOX fluorescence. In cells exposed to 100 μM PFOA, mitochondrial superoxide levels were elevated to 130% of those of controls. Authors suggest that these results indicate that mitochondrial superoxide is a more sensitive marker of oxidative stress than intracellular ROS levels.

Two studies examined oxidative stress endpoints following PFOA exposure in mitochondria isolated from Sprague-Dawley rats (Das et al., 2017; Mashayekhi et al., 2015). Mashayekhi et al. (2015) examined oxidative damage in the mitochondria, an important organelle in the oxidative stress pathway, associated with PFOA exposure. In mitochondria isolated from the livers of male Sprague-Dawley rats, significant increases in the percent ROS formation were observed following exposure to 0.75, 1, or 1.5 mM PFOA for up to 20 minutes. At 30 minutes and longer, significant increases were observed at the two highest concentrations only. Mashayekhi et al. (2015) also observed significantly increased levels of ROS formation in complexes I and III of the mitochondrial respiratory chain, key sources of ROS production. Disruption to the chain can lead to accumulation of ROS and, ultimately, oxidative stress. In complex II, activity levels were significantly decreased at 0.75 and 1.5 mM PFOA exposure. There was no significant difference in MDA of GSH content in liver mitochondria following PFOA exposure. PFOA exposure from 0.5–1.5 mM significantly decreased mitochondrial membrane potential and ATP levels and significantly increased mitochondrial swelling, suggesting a decrease in mitochondrial function following exposure to PFOA.

Xu et al. (2019b) exposed mouse hepatic primary cells from C57Bl/6J male mice to 0.01, 0.1, 0.5, or 1 mM PFOA for 24 hours. ROS levels, measured by a CM-H2DCFA fluorescent probe, were significantly increased in cells exposed to 0.5 and 1 mM PFOA. Interestingly, SOD activity was significantly increased in cells exposed to 0.5 and 1 mM PFOA, up to 123% with 1 mM, while CAT activity was reduced to 7.7% in cells at the highest concentration. Increasing PFOA exposure also led to alterations in the structure of SOD, resulting in a significantly decreased

percentage of α-helix structures (20%) and an increased percentage of β-sheet structures (29%), providing evidence of polypeptide chain unfolding and decreased helical stability. These structural changes suggest that PFOA interacts directly with SOD, resulting in polypeptide chain extension and, ultimately, diminished antioxidant capacity. Additionally, GSH content was increased by 177% and 405% in cells exposed to 0.5 mM and 1 mM PFOA, respectively. The authors suggest that increases in GSH may reflect cellular adaptations to oxidative stress and can lead to detoxification of oxidized GSSG to GSH.

Xu et al. (2020b) exposed cultured primary mouse hepatocytes to 0.01, 0.1, 0.5, or 1 mM of PFOA for 24 hours to examine oxidative stress-related apoptosis. The authors examined the impact of PFOA exposure on endogenous levels of lysozyme (LYZ), an enzyme that inhibits oxidative stress-induced damage, and demonstrated that PFOA 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 PFOA, which inhibited enzyme activity. At concentrations of 100 μM and above, LYZ enzyme activity decreased to 91% of control levels. Such an impact on LYZ activity was deemed to be related to the high affinity of PFOA for key central binding sites on the LYZ molecule.

In human HL-7702 liver cells, 24 hours of PFOA exposure at 1, 2.5, or 7.5 μg/mL led to a dose-dependent increase in 8-OHdG levels in cells exposed to the two highest concentrations (Li et al., 2017b). The authors noted that DNA damage, which frequently accompanies increases in 8-OHdG, was observed in their in vivo models following PFOA exposure, suggesting increased oxidative stress following exposure. In human non-tumor hepatic cells (L-02) exposed to 25 or 50 mg/L PFOA for 72 hours, Huang et al. (2013) observed concentration-dependent increases in ROS levels measured via DCFH-DA fluorescent probe, evidence of the role of PFOA in inducing oxidative stress.

Six additional studies examined oxidative stress endpoints following PFOA exposure in HepG2 cell lines (Wan et al., 2016; Wielsøe et al., 2015; Yan et al., 2015b; Shan et al., 2013; Florentin et al., 2011; Panaretakis et al., 2001). Four studies reported increases in ROS levels following PFOA exposure (Wan et al., 2016; Wielsøe et al., 2015; Yan et al., 2015b; Panaretakis et al., 2001), while two studies did not observe statistical differences in ROS levels following 1- or 24-hour PFOA exposures up to 400 μM (Florentin et al., 2011) or following 3-hour PFOA exposures up to 400 μM (Shan et al., 2013).

Wielsøe et al. (2015) incubated HepG2 cells with up to 2×10^{-4} M PFOA to detect changes in ROS, T-AOC, and DNA damage. PFOA exposure significantly increased ROS production, as measured with the carboxy-H2DCFDA, and significantly decreased T-AOC at all concentrations by 0.70–0.82-fold compared with controls. Additionally, PFOA induced DNA damage, specifically, increased mean percent tail intensity, an indicator of strand breaks, measured via comet assay. In cells exposed up to 400 μM PFOA for up to 24 hours, Panaretakis et al. (2001) observed increased ROS levels, measured via DCFH-DA and dihydroethidium fluorescent probes, following 3 hours PFOA exposure. H_2O_2 levels were detectable in 91% and 98% of the cell population at 200 and 400 μM PFOA, respectively. Additionally, superoxide anion levels were detectable in 43% and 71% of cells exposed to 200 and 400 μM PFOA, respectively. Authors reported evidence of depolarized mitochondrial membranes in cells exposed up to 24 hours. Yan et al. (2015b) observed significantly increased ROS levels in cells incubated with

100 and 200 μM PFOA for 24 hours, but no changes were observed in superoxide anion levels. After 72 hours of exposure, however, ROS levels decreased at those concentrations, with statistically significant results observed at 200 μM PFOA. Activity levels of SOD and CAT were not altered in exposed cells compared with controls, nor were MDA or GSH contents. Similarly, in HepG2 cells treated with PFOA for 24 hours, Yan et al. (2015b) found ROS levels significantly increased, but no significant changes were observed in SOD and CAT activity or MDA and GSH levels. Yarahalli Jayaram et al. (2018) examined the impacts of PFOA exposure on oxidative stress endpoints and small ubiquitin-like modifiers (SUMO), which play a key role in posttranslational protein modifications. SUMOylation of a protein has been identified as a key part of the oxidative stress pathway. In cells incubated with 250 μM PFOA, ROS levels were significantly increased. Cells incubated with PFOA also showed increased levels of nitric oxide (NO). Additionally, expression levels of genes related to SUMOylation were measured. PFOA treatment significantly increased levels of SUMO2 in HepG2 cells, but did not impact SUMO1, SUMO3, or UBC9 mRNA levels.

In cells exposed to 10 and 200 μM PFOA for 24 hours, Florentin et al. (2011) observed significant increases in the percentage of DNA tails, an indicator of DNA damage measured via comet assay. However, no such changes were observed at the 1-hour time point or at other concentrations (5, 50, 100, or 400 μM) after 24 hours. Additionally, no significant changes in ROS generation were observed. Shan et al. (2013) exposed HepG2 cells to 100 μM PFOA for 3 hours and found an increase in ROS generation, though the effect was not statistically significant. Additionally, no changes were observed in the GSH/GSSG ratio.

In two cell lines derived from Hepa1c-1c7 mouse cells, CR17 and HepaV cells, Melnikov et al. (2018) found that Hmox1 gene expression was significantly decreased in cells exposed to PFOA for 24 hours compared with controls. Additionally, exposed HepaV cells showed significantly decreased expression of Gclc and Gclm. There were no significant changes in GSH levels after exposure to 100 μM PFOA for 24 hours. CR17 cells have increased glutamate-cysteine ligase (GCL) activity, leading to increased GSH content. Authors anticipated that the elevated GSH levels in the CR17 cell line would better resist PFOA toxicity. They concluded that the observed changes in gene expression in PFOA-exposed HepaV cell lines, but not in CR17 cell lines, supported this hypothesis.

Sun et al. (2019) examined the impacts of PFOA exposure on both a monolayer and a scaffold-free three-dimensional spheroid model of mouse liver cells (AML12). Monolayer cells were exposed to 6.25–2,000 μM PFOA for 24 and 72 hours. The spheroid cell model was exposed to 50, 100, and 200 μM PFOA for up to 28 days. In monolayer cells exposed to 200 μM PFOA for 72 hours, ROS levels, measured via an ROS-Glo assay kit, increased 1.6-fold compared with controls. In the spheroid cell models, however, ROS levels decreased in cells exposed to 100 and 200 μM PFOA for 24 and 72 hours, which authors report suggests that monolayer cells demonstrate higher PFOA toxicity due to the absence of an endogenous extracellular matrix with the potential to inhibit PFOA diffusion. After 14 days of exposure, ROS levels in spheroid cells significantly increased at all concentrations. Gene expression of glutathione S-transferases alpha 2 (Gsta2), Nqo1, and Ho-1 increased with increasing PFOA concentration and duration of exposure, which provides additional evidence of PFOA's effect on oxidative stress.

3.4.1.3.7.4 Conclusions

Results from new studies published since the 2016 PFOA HESD (U.S. EPA, 2016c) further support the 2016 conclusions that PFOA can cause oxidative stress and related cellular damage. Evidence of increased oxidative stress in the liver, including increased ROS levels, changes in GSH and GSSG levels, and decreases in T-AOC, was observed following both in vivo and in vitro exposures to PFOA. PFOA 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. There was also evidence that PFOA 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 understand the underlying mechanisms of PFOA-induced oxidative stress responses, it is clear that PFOA induces oxidative stress in hepatic tissues.

3.4.1.4 Evidence Integration

There is *moderate* evidence for an association between PFOA exposure and hepatic effects in humans based on associations with liver biomarkers, especially ALT, in several *medium* confidence studies. Across the studies in the 2016 PFOA HESD (U.S. EPA, 2016c) and this updated systematic review, there is consistent evidence of a positive association between exposure to PFOA and ALT in adults (Jain, 2019; Jain and Ducatman, 2019c; Nian et al., 2019; Salihovic et al., 2018; Darrow et al., 2016; Gleason et al., 2015; Yamaguchi et al., 2013; Gallo et al., 2012; Lin et al., 2010). An exposure-response gradient observed in one *medium* quality study that examined categorical exposure in adults (Darrow et al., 2016) increases certainty in the association. These associations were observed in studies of the general population, in communities with high exposure from water due to contamination events, and in occupational studies. Consistency in the direction of association across these different population sources increases certainty in the results and reduces the likelihood that they can be explained by confounding across PFAS. For example, studies in communities with high exposure from water and occupational participants are less susceptible to potential confounding from other PFAS due to PFOA exposure predominating over other PFAS. In addition, the single general population that performed multipollutant modeling (Lin et al., 2010) found no attenuation of the association, further increasing confidence in the association between PFOA exposure and increased 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). Associations for other hepatic outcomes were less consistent, including for functional outcomes such as liver disease. This may be due to a relative lack of *high* confidence studies of these outcomes.

The animal evidence for an association between PFOA exposure and hepatic toxicity is *robust* based on 27 *high* or *medium* confidence animal toxicological studies. 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). Many of the studies discussed in this section reported dose-dependent increases in liver weight and hepatocellular hypertrophy in rodents of both sexes. However, a limited number of these studies additionally examined functional or histopathological hepatic impairment to

provide evidence that the enlargement of hepatic tissue was an adverse, and not adaptive, response (Blake et al., 2020; NTP, 2020; Crebelli et al., 2019; Guo et al., 2019; Yan et al., 2014; Minata et al., 2010; Loveless et al., 2008).

EPA identified the following studies as providing the most comprehensive evidence of dose-dependent hepatoxicity resulting from oral PFOA exposure: a chronic dietary study in male and female Sprague-Dawley rats (NTP, 2020) (see study design details in Section 3.4.4.2.1.2); a developmental study in male and female CD-1 mice (Cope et al., 2021); and a 29-day oral gavage study in male rats and mice (Loveless et al., 2008). NTP (2020) conducted histopathological examinations of liver tissue in male and female rats and reported dose-dependent increases in the incidence of hepatocellular hypertrophy and hepatocellular cytoplasmic vacuolation, as well as increases in the incidence of hepatocellular single-cell death and hepatocellular necrosis at the same dose levels. Cope et al. (2021) also provides evidence of hepatic lesions in adult male and female CD-1 mice offspring exposed gestationally from GD 1.5 to GD 17.5. When the offspring were weaned, they were placed on a low- or high-fat diet. At 18 weeks there were increases in the incidence and severity of hepatocellular single-cell death in females on either the low- or high-fat diets and males on the low-fat diet. Loveless et al. (2008) similarly provides concurrent evidence of liver enlargement and hepatic lesions in male mice gavaged with PFOA for 29 days. Increases in the incidence and severity of hepatocellular hypertrophy and individual cell or focal cell necrosis were dose-dependent. Similar to the NTP (2020) study, Loveless et al. (2008) provides a comprehensive report of hepatotoxicity, with a low-dose range resulting in dose-dependent increases in histopathological outcomes indicating adversity.

An important element of understanding the underlying mechanism(s) of toxicity is species specificity and relevance of data collected from laboratory models in relation to observed human effects as well as in consideration of human hazard. There are several studies that have proposed potential underlying mechanisms of the hepatotoxicity observed in rodents exposed to PFOA, such as induction of hepatocytic proliferation leading to hypertrophy or nuclear receptor activation leading to lipid droplet accumulation and steatosis. Generally, mechanistic evidence supports the ability of PFOA to induce hepatotoxicity which may explain elevated serum ALT levels in humans (and animals). However, mechanistic studies did not specifically relate (or, "anchor") mechanistic data with serum ALT levels in animals, and challenges exist in the extrapolation of evidence for PFOA-mediated changes in rodents to humans. For example, there is substantial evidence that PFOA-induced liver toxicity, specifically alterations to lipid metabolism and accumulation, occurs via the activation of multiple nuclear receptors, including PPARα. Activation of PPARα by PFOA has been demonstrated in multiple studies across various model systems, both in vivo and in vitro. Several studies examined the activation of PPARα in vitro in both human and animal cell lines transfected with mouse and human PPARα using luciferase reporter assays, the results of which demonstrate that PFOA can activate human PPARα in vitro. In addition to PPARα, evidence also exists indicating that PFOA can activate CAR, PXR, PPARγ, ERα, and HNFα, as evidenced by receptor activation assays as well as changes in target genes of these receptors. PFOA showed the highest potency for PPARα in comparison to PPARγ and PPARδ, although PFOA did activate these receptors at concentrations of 100 μM (compared with 25 μM for PPARα). Like PPARα, PPARγ and CAR are known to play important roles in liver homeostasis, and dysregulation of these nuclear receptors can lead to steatosis and liver dysfunction, potentially presenting an important mechanism for the liver

effects observed in rodent studies. Beyond receptor activation assays, individual target genes that represent reliable markers of CAR and PPARα activation (e.g., *Cyp2b1* and *Cyp4a1*, respectively) have been clearly demonstrated to be altered by PFOA, and changes to these nuclear receptors have important implications regarding hepatotoxicity, specifically steatosis. PPARα has a vastly different expression in rodents compared with humans, and this species difference is known to play a major role in differences in liver effects between the two species. PPARα is the most demonstrated nuclear receptor to be activated by PFOA, and it should be noted that using PPARα-null mice to study PPARα-independent effects of PFOA may lead to compensatory mechanisms involving other nuclear receptors.

Another example of species specificity for an effect of PFOA is the presence or absence of a transfer protein that is important in cholesterol accumulation, CETP, which is expressed in humans but not in rodents. Transgenic mice that express human CETP exhibit a more human-like lipoprotein metabolism. Laboratory models that are designed to better predict human-relevant mechanisms, such as mice expressing human CETP or PPARα, will continue to aid in accuracy of the extrapolation of mechanistic findings in rodents to humans. Despite these challenges, the evidence that PFOA leads to hepatotoxicity via activation of hepatic nuclear receptors and dysregulation of lipid metabolism and accumulation is clear.

When considering the evidence from both in vivo and in vitro studies, PFOA-mediated hepatoxicity specific to changes in lipid metabolism leading to steatosis, the most commonly reported hepatocytic morphological alteration in PFOA-exposed animals, likely occurs through the following molecular and cellular events: (1) PFOA accumulation in liver activates nuclear receptors, including PPARα; (2) expression of genes involved in lipid homeostasis and metabolism is altered by nuclear receptor activation; (3) gene products (translated proteins) modify the lipid content of liver to favor triglyceride accumulation and potentially cholesterol accumulation; (4) altered lipid content in the liver leads to accumulation of lipid droplets, which can lead to the development of steatosis and liver dysfunction; and (5) alterations in lipid metabolism lead to alterations in serum levels of triglycerides and cholesterol. Although individual studies have not demonstrated every step of this proposed process, each event has been demonstrated for PFOA, including steatosis in PFOA-exposed animals. It has also been suggested that PFOA could interfere with fatty acid biosynthesis by binding to the Acetyl-CoA carboxylase 1 and Acetyl-CoA carboxylase 2 enzymes; however, only a single study has demonstrated such a binding event and further research is needed to understand the plausibility of this binding occurring across species and exposure scenarios.

In addition (and potentially related) to the abundance of evidence related to hepatic nuclear receptors, PFOA also alters apoptosis and cell proliferation in the liver. Specifically, PFOA exposure at high doses causes apoptosis through a cascade of mechanisms including activation of caspase activity, intracellular release of LDH, induction of apoptotic genes, morphological changes to the mitochondria membrane, autophagy, and activation of the p53 mitochondria pathway. PFOA has been shown to induce hepatocytic proliferation at low doses by disrupting cell cycle progression, leading to steatosis, hepatomegaly, and liver dysfunction in general.

There are other mechanisms that may be involved in PFOA-induced hepatotoxicity, but the evidence for such is limited and the relevance to liver outcomes is less clear. These include hormone perturbation, inflammatory response, and oxidative stress. There are very limited data demonstrating the potential of PFOA to perturb hormone balance, particularly related to thyroid

function. There are also a limited number of studies that reported inflammation in the liver, including changes in cytokine levels and the expression of genes involved in innate immunity. PFOA can cause oxidative stress in the liver, as demonstrated by standard indicators of oxidative stress including increased ROS levels, changes in GSH and GSSG levels, and decreased total antioxidant capacity in both in vivo and in vitro exposures to PFOA. The direct relevance of oxidative stress to liver pathology induced by PFOA requires further study, but it is clear that PFOA can cause oxidative stress. These other mechanisms that have a limited evidence base may also occur in relation to the more well-characterized mechanisms of PFOA-induced hepatotoxicity. For example, while the role of alterations in adaptive immunity in PFOA-induced liver pathology is not clear, it is plausible that the inflammatory response is related to fatty liver and associated liver dysfunction, such as the liver outcomes observed in humans and rodents, which can occur via nuclear receptor-mediated pathways.

3.4.1.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the *evidence indicates* that PFOA exposure is likely to cause hepatotoxicity in humans under relevant exposure circumstances (Table 3-4). This conclusion is based primarily on coherent liver effects in animal models following exposure to doses as low as 0.3 mg/kg/day PFOA. In human studies, there is consistent evidence of a positive association with ALT in adults, at median PFOA levels as low as 1.3 ng/mL. The available mechanistic information provides 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.

Table 3-4. Evidence Profile Table for PFOA 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 17 *Medium* confidence studies 5 *Low* confidence studies	Studies in adults consistently reported significant increases in ALT (9/11). Findings in adults were generally positive for AST (5/7) and GGT (7/10). Some studies reported conflicting or nonsignificant associations, however, these were mostly of *low* confidence. Occupational studies generally reported significant increases in ALT (4/7), but there were some nonsignificant associations based on type of analysis, location, or years analyzed. In occupational studies, findings for liver enzymes other than ALT were mixed, varying at times by time, location, or sex. Findings in studies of children were limited, but one study observed	• *Medium* confidence studies that reported an effect • *Consistent direction* of effect for ALT • *Coherence* of findings across biomarkers	• *Low* confidence studies	⊕⊕⊙ *Moderate* Evidence for hepatic effects is based on increases in ALT in adults, including increases in ALT in occupational populations. Supporting evidence includes increases in other liver enzymes such as AST and GGT, and increased incidence of liver disease mortality in occupational settings. Minor uncertainties remain regarding mixed liver enzyme findings in children and coherence across biomarkers and limited availability of	*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 PFOA 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

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**	
				high-quality studies on liver disease.	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.
	significant positive associations for ALT, AST, and GGT in girls (1/3).				
Liver disease or injury 4 *Medium* confidence studies 3 *Low* confidence studies 1 *Mixed* confidence study	A limited number of studies examined liver disease or injury in general population adults and occupational populations. One occupational study reported significantly higher mortality from cirrhosis of the liver compared with a group of similar, non-exposed workers (1/1). Two occupational and one general population study reported no significant	• No factors noted	• Association only observed in *Low* confidence studies • *Lack of coherence* of across measures of liver inflammation		

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**	
	association with any form of liver disease (0/3). Other measures of inflammation in the liver were mixed and lacked coherence.				
Serum protein 4 *Medium* confidence studies 2 *Low* confidence studies	Significant increases in albumin were consistently observed in adults (4/5), while findings from the single occupational study were nonsignificant. Findings for total serum protein and fibrinogen were mixed or imprecise.	• *Medium* confidence studies • *Consistent direction of effect* for albumin	• *Low* confidence studies • *Imprecision* of findings for fibrinogen and other serum proteins		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
Serum 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 outcome		
Evidence from In Vivo Animal Toxicological Studies (Section 3.4.1.2)					
Histopathology 3 *High* confidence studies 11 *Medium* confidence studies	Histopathological alterations in liver were observed in male and female rodents exposed to PFOA for various durations (14/14). Increased hepatocellular hypertrophy (10/14) and necrosis (5/12) were the most common lesions. Other lesions included inflammation or cellular infiltration (5/14), cytoplasmic alteration or vacuolation (3/12), mitosis or mitotic figures (3/12), bile duct hyperplasia (2/13), cystic/cystoid degeneration	• *High* and *medium* confidence studies *Consistent direction of effects* across study design, sex, and species • *Dose-dependent response* • *Coherence* of findings across other endpoints indicating liver damage (i.e., increased serum biomarkers and liver weight) • *Large magnitude of effect,* with some responses reaching 100%	• No factors noted	⊕⊕⊕ *Robust* Evidence is based on 26 *high* or *medium* confidence animal toxicological studies indicating increased incidence of hepatic nonneoplastic lesions, increased liver weight, and elevated serum biomarkers of hepatic injury. However, it is important to distinguish between alterations that may be non-adverse (e.g., hepatocellular hypertrophy alone) and those	

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**	
	(2/12), fatty change (2/13), and/or pigment (1/12).	incidence in some dose groups (i.e., hypertrophy, vacuolation, single-cell death) or are considered severe		that indicate functional impairment or lesions. EPA considers responses such as increased	
		(i.e., cell or tissue death/necrosis and cystoid degeneration)		relative liver weight and hepatocellular hypertrophy adverse when accompanied by hepatotoxic effects such as necrosis, inflammation, or biologically significant (i.e., greater than 100% change) increases in enzymes indicative of hepatobiliary damage. Many of the studies discussed in this section reported dose-dependent increases in liver weight and	

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**	
			hepatocellular hypertrophy in rodents of both sexes. Although a limited number of these studies additionally examined functional or histopathological hepatic impairment, several provide evidence of adverse hepatic responses.		
Liver weight 5 *High* confidence studies 21 *Medium* confidence studies	Absolute (17/21) and relative (18/22) liver weights were increased in male and female rodents exposed to PFOA for various durations. Several studies that included both males and females suggested that males may be more sensitive than females (4/7).	• *High* and *medium* confidence studies • *Consistent direction* of effects across study design, sex, and species • *Dose-dependent response* • *Coherence* of effects with other responses indicating	• *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**	
		increased liver size (e.g., hepatocellular hypertrophy)			
Serum biomarkers of hepatic injury 3 *High* confidence studies 7 *Medium* confidence studies	Increases were observed in ALT (6/9), AST (6/7), ALP in (4/6), and GGT (1/1). Biologically significant changes (≥100%) in an enzyme level were observed in 6/9 studies. Albumin (5/6) and albumin/globulin ratio (3/3) were increased. Bile acids were increased in males (4/4) and	• *High* and *medium* confidence studies • *Consistent direction of effects* across study design, sex, and species • *Dose-dependent response* • *Coherence* of findings with other responses indicating	• *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation						Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**		**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	unchanged in females (3/3). Inconsistent changes in	hepatobiliary damage (i.e., histopatho logical lesions)				
	bilirubin were observed with direct bilirubin increased in males (2/2) or females (0/1), increased indirect bilirubin in males (1/1), and mixed effects on total bilirubin in males (2) and transient effects in females (1). Total protein was decreased in males (3/5) and females (1/4).	• *Large magnitude of effect*, with evidence of biologically significant increases (i.e., ≥100% control responses) in serum liver enzymes indicating adversity				

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**	
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 human PPARα in vitro. • Increased expression of PPARα-target genes in vitro in rat and human hepatocytes, and cells transfected with rat or human PPARα. • Altered expression of genes involved in lipid metabolism and lipid homeostasis. **Limitations:** • Increased hepatic lipid content has also been reported for PFOA in the absence of a strong PPARα response.		Overall, studies in rodent and human in vitro and in vivo models suggest that PFOA 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 PFOA.		
Molecular or cellular initiating events – other pathways	**Key findings and interpretation:** • Increased apoptosis is a high dose effect demonstrated in vivo, as well as in vitro, occurring through a cascade of mechanisms: ○ activation of caspase activity, intracellular release of LDH, induction of apoptotic genes, morphological changes to the mitochondria membrane, autophagy, and activation of p53 mitochondria pathway. • Inflammation of the liver (e.g., changes in cytokine levels and the expression of genes involved in innate				

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**	
	immunity) has been reported in a limited number of studies. • Induction of oxidative stress in vivo and in vitro, including increased ROS levels, changes in GSH and GSSG levels, and decreased total antioxidant capacity. • Indirect evidence of activation of alternative pathways, including activation of other nuclear receptors, primarily CAR and PPARγ, following observations in knockout or humanized PPARα mice. **Limitations:** • The direct relevance of oxidative stress to liver pathology induced by PFOA requires further study. • Very limited database for other pathways, with the exception of apoptosis and cell cycle changes.				

Notes: ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; CAR = constitutive androstane receptor; EPA = Environmental Protection Agency; GGT = gamma-glutamyl transferase; GSH = glutathione; GSSG = glutathione disulfide; LDH = lactate dehydrogenase; MOA = mode of action; PPARγ = peroxisome proliferator-activated receptor gamma; PPARα = peroxisome proliferator-activated receptor alpha; ROS = reactive oxygen species.

3.4.2 Immune

EPA identified 50 epidemiological and 13 animal toxicological studies that investigated the association between PFOA and immune effects. Of the epidemiological studies, 1 was classified as *high* confidence, 29 as *medium* confidence, 12 as *low* confidence, 6 as *mixed* (6 *medium/low*) confidence, and 2 were considered *uninformative* (Section 3.4.2.1). Of the animal toxicological studies, 3 were classified as *high* confidence, 9 as *medium* confidence, and 1 was considered *mixed* (*medium*/*low)* confidence (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 13 epidemiological studies (14 publications[9]) from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and immunosuppresive effects. Study quality evaluations for these 14 studies are shown in Figure 3-19. Results from studies summarized in the 2016 PFOA HESD are described in Table 3-5 and below.

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

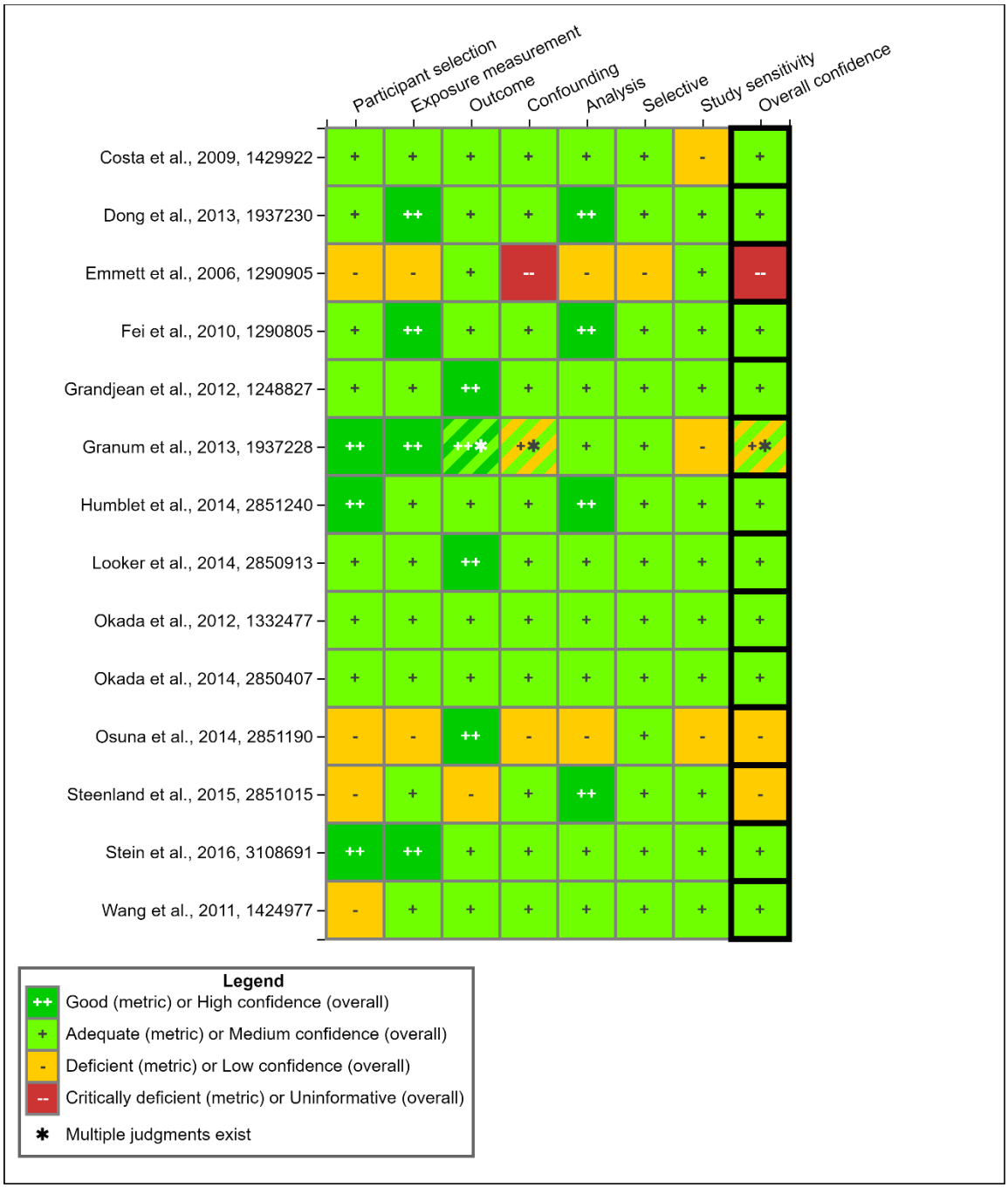


Figure 3-19. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Immune Effects Published Before 2016 (References in 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Three studies reported decreases in response to one or more vaccines in relation to higher PFOA exposure in children (Granum et al., 2013; Grandjean et al., 2012) and adults (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 PFOA concentrations were inversely associated with childhood anti-diphtheria antibody response at all measured timepoints, and the association was significant for anti-diphtheria antibody response at age seven in separate models for prenatal and age five serum PFOA concentrations. The association was less pronounced when examining anti-tetanus antibody responses in relation to prenatal PFOA measurements, but the anti-tetanus antibody response (age seven) was significantly decreased in relation to PFOA measured in child serum at five years of age. Another study on Faroese children conducted a pilot investigation on the association between elevated PFOA exposure and autoantibodies to antigens indicating tissue damage, but the results were unclear (Osuna et al., 2014). Prenatal PFOA 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 PFOA concentrations among three-year-old children. Stein et al. (2016b) reported significant inverse associations between PFOA exposure and mumps and rubella antibody concentrations in adolescents (12–19 years old) from multiple NHANES cycles (1999–2000, 2003–2004), but no association was observed for measles. A C8 Health Project study examining influenza vaccine responses in highly exposed adults (Looker et al., 2014) observed that pre-vaccination PFOA concentrations were inversely associated with GM A/H3N2 antibody titer rise, but no association was found with antibody titers for A/H1N1 and influenza type B. In the studies of children, there was concern that the associations were also seen with other correlated PFAS, but this was not considered a limitation in the study in adults, which was conducted in a population with known high PFOA exposure (the C8 Health Project study).

Associations between prenatal PFOA exposure and risk of infectious diseases (as a marker of immune suppression) were not observed in one study, although there was some indication of effect modification by gender (i.e., associations seen in females but not in males). Fei et al. (2010b) examined hospitalizations for infectious diseases in early childhood in a Danish birth cohort with mean maternal PFOA concentration of 0.0056 μg/mL. A slightly higher risk for hospitalizations was observed in females whose mothers had higher PFOA concentrations (incidence rate ratio [IRR] = −1.20, 1.63, 1.74 for quartile 2 [Q2], quartile 3 [Q3], and quartile 4 [Q4], respectively compared with quartile 1 [Q1]; see Appendix D, (U.S. EPA, 2024a)), and the risk for males was below 1.0 for each quartile. Overall, there was no association between hospitalizations due to infectious diseases and maternal PFOA exposure.

Overall, the 2016 PFOA HESD (U.S. EPA, 2016c) found consistent evidence of an association between PFOA exposure and immunosuppression.

Table 3-5. Associations Between Elevated Exposure to PFOA and Immune Outcomes from Studies Identified in the 2016 PFOA 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]	White Blood Cell Count[a]
Costa 2009, 1429922 *Medium*	Cohort	Occupational	NA	NA	NA	NA	NA	NA	NA	NA	↑
Dong, 2013, 1937230 *Medium*	Case-control	Children	NA	NA	NA	NA	NA	↑↑	NA	NA	NA
Fei, 2010, 1290805 *Medium*	Cohort	Children	NA	NA	NA	NA	–	NA	NA	NA	NA
Grandjean, 2012, 1248827 *Medium*	Cohort	Children	↓↓	↓↓	NA	NA	NA	NA	NA	NA	NA
Granum, 2013, 1937228 *Mixed*[c]	Cohort	Children	–	NA	↓↓	NA	↑↑	–	–	NA	NA
Humblet, 2014, 2851240 *Medium*	Cross-sectional	Adolescents	NA	NA	NA	NA	NA	↑↑	NA	NA	NA
Looker, 2014, 2850913 *Medium*	Cohort	Adults	NA	NA	NA	↓↓	–	NA	NA	NA	NA
Okada, 2014, 2850407 *Medium*	Cohort	Children	NA	NA	NA	NA	↑	↑	–	NA	NA
Steenland, 2015, 2851015 *Low*	Cohort	Adults	NA	NA	NA	NA	NA	NA	NA	↑↑	NA
Stein, 2016, 3108691 *Medium*	Cross-sectional	Adolescents	NA	NA	↓↓	NA	NA	↑	NA	NA	NA
Wang, 2011, 1424977 *Medium*	Cohort	Children	NA	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.

Emmett et al., 2006, 1290905 was not included in the table due to their *uninformative* overall study confidence ratings.

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).

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

3.4.2.1.2 Immunosuppression Study Quality Evaluation and Synthesis from the Updated Literature Review

There are 27 epidemiological studies identified from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated associations between prenatal, childhood, or adult PFOA exposure and immunosuppression since publication of the 2016 PFOA HESD. Study quality evaluations for these 27 studies are shown in Figure 3-20 and Figure 3-21.

One study from the 2016 assessment (Grandjean et al., 2012) was updated during this period, and the update was included in the systematic review (Grandjean et al., 2017a).

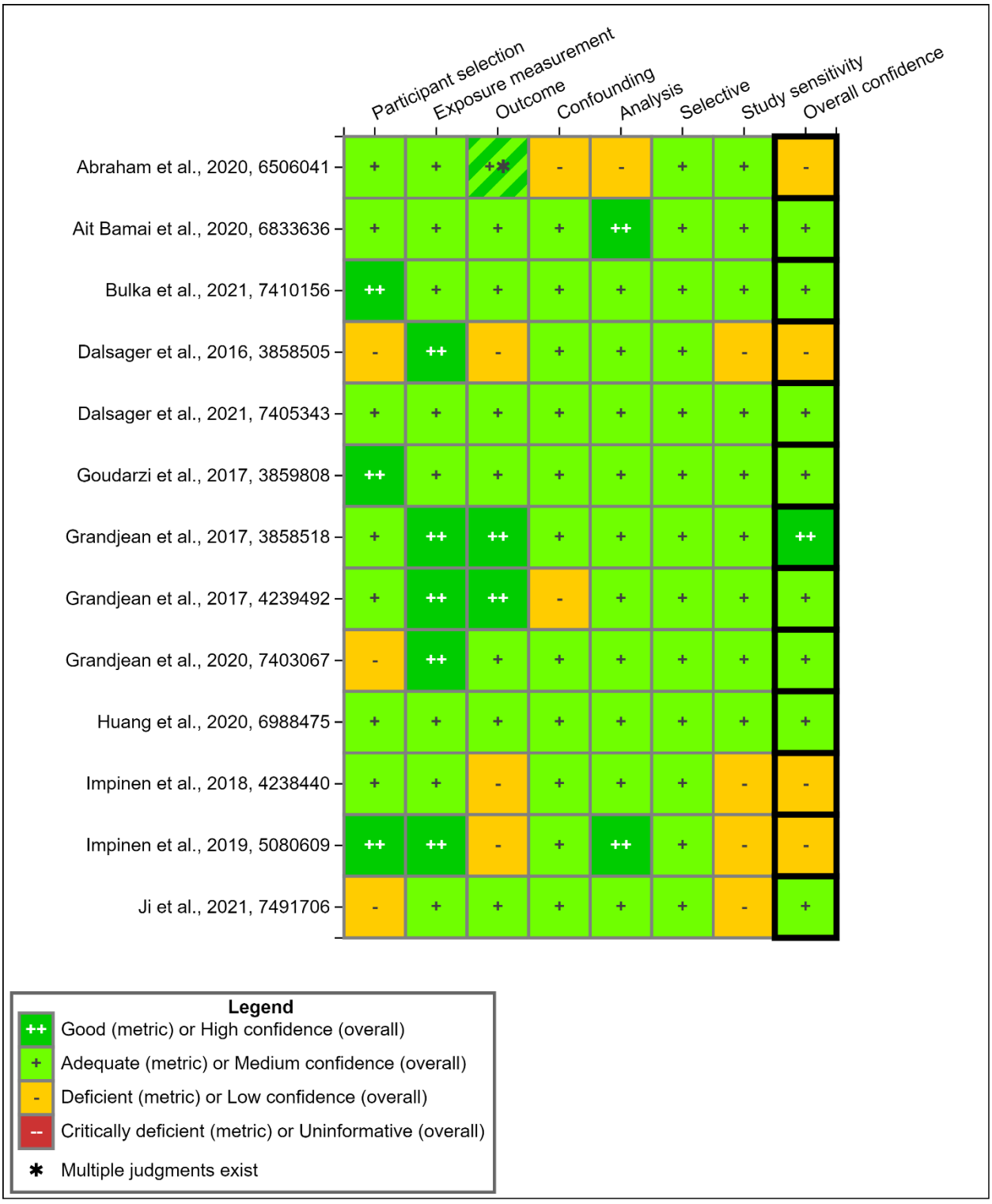


Figure 3-20. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Immunosuppression Effects

Interactive figure and additional study details available on HAWC.

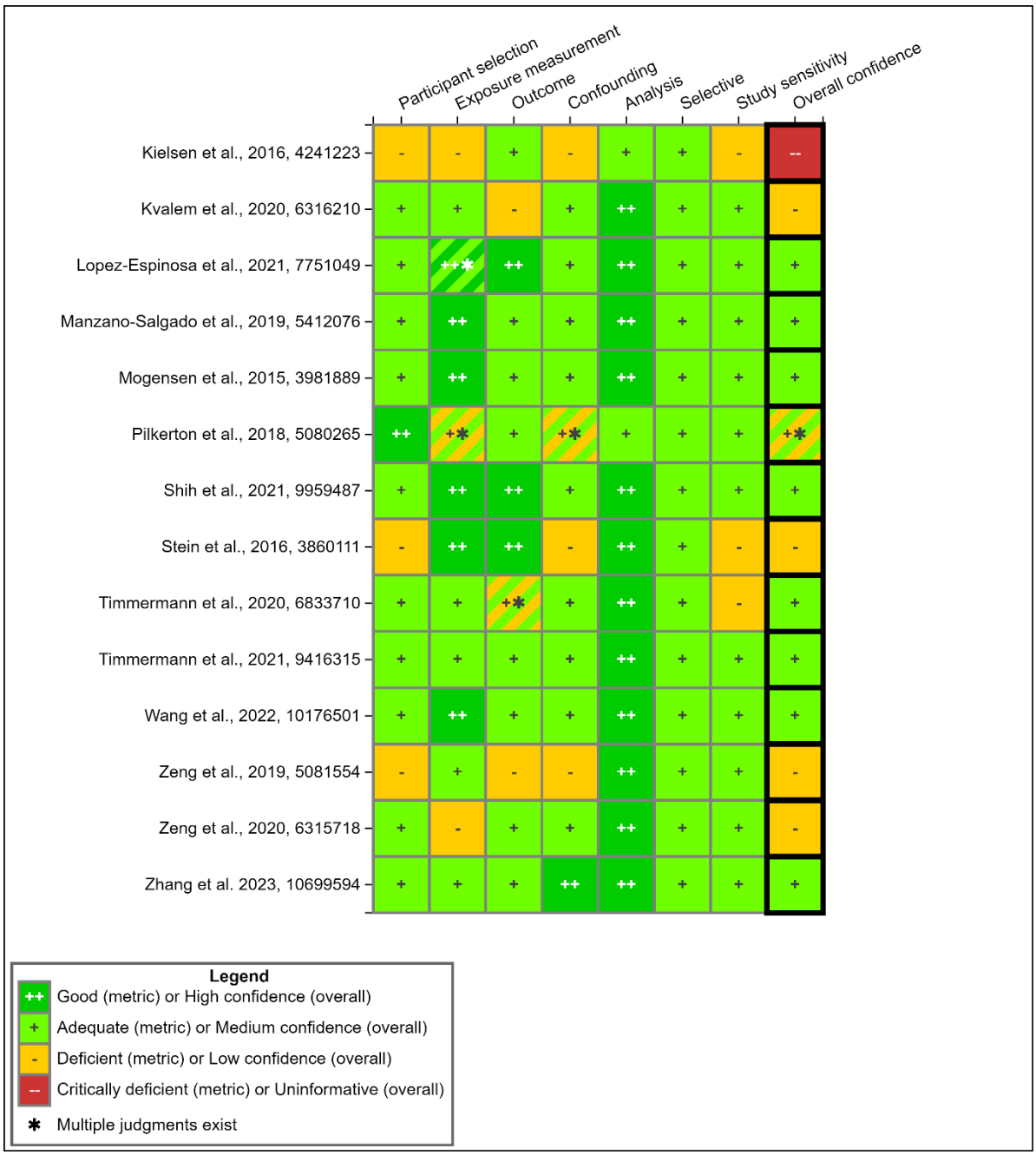


Figure 3-21. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Immunosuppression Effects (Continued)

Interactive figure and additional study details available on HAWC.

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix D, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

3.4.2.1.2.1 Vaccine Response

Ten studies (11 publications[10][11]) studied the relationship between antibody response to vaccination and PFOA exposure. Five of these studies (six publications) investigated antibody response to vaccination in children (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 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 to these studies on vaccine response, one study (Zeng et al., 2019b) measured natural antibody response to hand, foot, and mouth disease (HFMD), and one study (Zeng et al., 2020) measured antibody response to hepatitis B infection in adults. Overall, eight studies were *medium* confidence (Zhang et al., 2023; Shih et al., 2021; Timmermann et al., 2021; Timmermann et al., 2020; Pilkerton et al., 2018; Grandjean et al., 2017b; Grandjean et al., 2017a; Mogensen et al., 2015a), four were *low* confidence (Abraham et al., 2020; Zeng et al., 2020; Stein et al., 2016a Zeng, 2019, 5081554), and one study (Kielsen et al., 2016) was *uninformative*.

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 also 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 five (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; 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 influenza* type b (Hib) antibodies (Abraham et al., 2020).

The results for this set of studies in children are shown in Table 3-6 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 PFOA and lower antibody levels against tetanus and diphtheria in children at birth, 18 months, age 5 years (pre-and post-booster), and at age 7 years, with some being statistically significant. These studies measured PFOA exposure levels in maternal blood during the perinatal period and at later time periods from children (at ages 5, 7, and 13 years). There are a few results in the opposite direction for sub-

[10] Multiple publications of the same study: the study populations are the same in Grandjean et al. (2017a) and Mogensen et al. (2015a).
[11] 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.

analyses of the Faroe Island cohorts (Grandjean et al., 2017b; Grandjean et al., 2017a), such as maternal PFOA exposure and anti-tetanus antibodies at 7 years (Table 3-6). No biological rationale has been identified as to whether one particular time period or duration of exposure or outcome measurement is more sensitive to an overall immune response to PFOA exposure. Changes in tetanus and diphtheria antibody concentrations in children from all *high* and *medium* confidence studies are provided in Figure 3-22 and Figure 3-23.

It is plausible that the observed associations between decreased antibody concentration and PFOA exposure observed in the Faroe Islands cohort could be partially explained by confounding across the PFAS (e.g., exposure levels to PFOS were higher than PFOA (PFOS 17 ng/mL, PFOA 4 ng/mL); there was a moderately high correlation between PFOA and PFOS, PFHxS, and PFNA (0.50, 0.53, 0.54, respectively) (Grandjean et al., 2017b; Grandjean et al., 2017a). To investigate this, the authors assessed the possibility of confounding in a follow-up paper (Budtz-Jørgensen and Grandjean, 2018). In these analyses, estimates were adjusted for PFOS and there was no notable attenuation of the observed effects. The other available studies did not perform multipollutant modeling, so it is difficult to determine the potential for highly correlated PFAS to confound the effect estimates. However, as described above, one study (Looker et al., 2014) observed an association with PFOA in a population where PFOA exposure predominated (the C8 Health Project population), and this is not likely to be confounded by other PFAS. Overall, the available evidence suggests that confounding is unlikely to explain the observed effects.

Reference, Confidence Rating	Exposure Levels	Comparison	Sub-population	Exposure Age	Outcome Age	EE
Grandjean et al. (2017a, 3858518), High	PFOA at 7 years median (25th-75th percentile)= 4.4 ng/mL (3.5-5.7 ng/mL)	percent change (per doubling of PFOA)	--	Age 7	Age 13	9.4
	PFOA at 13 years median (25th-75th percentile)=2.0 ng/mL (1.6-2.5 ng/mL)	percent change (per doubling of PFOA)	--	Age 13	Age 13	3.3
Grandjean et al. (2012, 1248827), Medium	Age 5 PFOA: Geometric mean=4.06 ng/mL (25th-75th percentile=3.33-4.96 ng/mL)	Percent difference (per doubling in age 5 PFOA)	--	Age 5	Age 7	-35.8
			Adj for Age 5 Ab	Age 5	Age 7	-28.2
			Pre-booster	Age 5	Age 5	-13.3
			Post-booster	Age 5	Age 5	-9.7
	Maternal PFOA: Geometric mean=3.20 ng/mL (25th-75th percentile=2.56-4.01 ng/mL)	Percent difference (per doubling in maternal PFOA)	--	Prenatal	Age 7	7.4
			Adj for Age 5 Ab	Prenatal	Age 7	12.3
			Pre-booster	Prenatal	Age 5	-10.5
			Post-booster	Prenatal	Age 5	14.5
Grandjean et al. (2017b, 4239492), Medium	--	Percent change (per doubling of PFOA)	Cohort 3	Age 1.5	Age 5	-19.24
				Age 5	Age 5	-13.28
				Cord blood	Age 5	-10.46
			Cohort 3 and 5	Age 1.5	Age 5	-16.47
				Age 5	Age 5	-18.75
				Cord blood	Age 5	-17.59
			Cohort 5	Cord blood	Age 5	-22.25
	Median = 2.2 ng/mL (25th - 75th percentile: 1.8 - 2.8 ng/mL)	Percent change (per doubling of PFOA)	Cohort 5	Age 5	Age 5	-25.26
	Median = 2.8 ng/mL (25th - 75th percentile: 2.0 - 4.5 ng/mL)	Percent change (per doubling of PFOA)	Cohort 5	Age 1.5	Age 5	-16.31
Mogensen et al. (2015, 3981889), Medium	median=4.4 ng/ml (25th-75th percentile=3.5-5.7 ng/ml)	Percent change per doubling of PFOA	Age 7	Age 7	Age 7	-20.5
Timmermann et al. (2022, 9416315), Medium	median=2.13 ng/mL (25th - 75th percentiles: 1.68 - 2.54 ng/mL)	percent difference (per unit increase in maternal PFOA concentration)	Ages 7-12	Prenatal	Age 7-12	-7
	median=2.28 ng/mL (25th - 75th percentiles: 1.89 - 2.88 ng/mL)	percent difference (per unit increase in child PFOA concentration)	Ages 7-12	Age 7-12	Age 7-12	-8

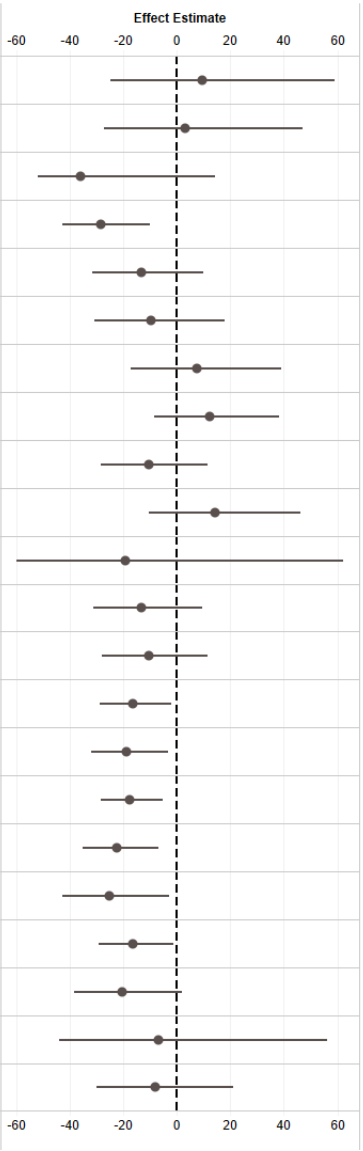


Figure 3-22. Overall Tetanus Antibody Levels in Children from Epidemiology Studies Following Exposure to PFOA

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

Reference, Confidence Rating	Exposure Levels	Comparison	Sub-population	Exposure Age	Outcome Age	EE
Grandjean et al. (2017a, 3858518), High	PFOA at 7 years median (25th-75th percentile)= 4.4 ng/mL (3.5-5.7 ng/mL)	percent change (per doubling of PFOA)	--	Age 7	Age 13	-4.1
	PFOA at 13 years median (25th-75th percentile)=2.0 ng/mL (1.6-2.5 ng/mL)	percent change (per doubling of PFOA)	--	Age 13	Age 13	-17.5
Grandjean et al. (2012, 1248827), Medium	Age 5 PFOA: Geometric mean=4.06 ng/mL (25th-75th percentile=3.33-4.96 ng/mL)	Percent difference (per doubling in age 5 PFOA)	--	Age 5	Age 7	-25.2
			Adj for Age 5 Ab	Age 5	Age 7	-23.4
			Pre-booster	Age 5	Age 5	-6.8
			Post-booster	Parental	Age 5	-6.1
	Maternal PFOA: Geometric mean=3.20 ng/mL (25th-75th percentile=2.56-4.01 ng/mL)	Percent difference (per doubling in maternal PFOA)	--	Prenatal	Age 7	-22.8
			Adj for Age 5 Ab	Prenatal	Age 7	-16.8
			Pre-booster	Prenatal	Age 5	-16.2
			Post-booster	Prenatal	Age 5	-6.2
Grandjean et al. (2017b, 4239492), Medium	--	Percent change (per doubling of PFOA)	Cohort 3	Age 1.5	Age 5	30.49
				Age 5	Age 5	-6.84
				Cord blood	Age 5	-35.17
			Cohort 3 and 5	Age 1.5	Age 5	5.44
				Age 5	Age 5	3.38
				Cord blood	Age 5	-17.82
	Median = 2.2 ng/mL (25th - 75th percentile: 1.8 - 2.8 ng/mL)	Percent change (per doubling of PFOS)	Cohort 5	Age 5	Age 5	18.31
	Median = 2.8 ng/mL (25th - 75th percentile: 2.0 - 4.5 ng/mL)	Percent change (per doubling of PFOA)	Cohort 5	Age 1.5	Age 5	4.19
Mogensen et al. (2015, 3981889), Medium	median=4.4 ng/ml (25th-75th percentile=3.5-5.7 ng/ml)	Percent change per doubling of PFOA	Age 7	Age 7	Age 7	-25.4
Timmermann et al. (2022, 9416315), Medium	median=2.13 ng/mL (25th - 75th percentiles: 1.68 - 2.54 ng/mL)	percent difference (per unit increase in maternal PFOA concentration)	Ages 7-12	Prenatal	Age 7-12	44
	median=2.28 ng/mL (25th - 75th percentiles: 1.89 - 2.88 ng/mL)	percent difference (per unit increase in child PFOA concentration)	Ages 7-12	Age 7-12	Age 7-12	-22

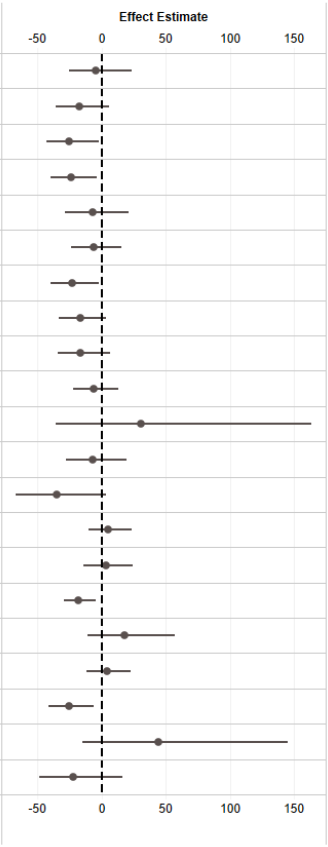


Figure 3-23. Overall Diphtheria Antibody Levels in Children from Epidemiology Studies Following Exposure to PFOA

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

Table 3-6. Associations between PFOA Exposure and Vaccine Response in Faroe Islands Studies

Exposure measurement timing, PFOA levels (ng/mL)[a]	Diphtheria Antibody Associations with PFOA by Age at Assessment			Tetanus Antibody Associations with PFOA 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: 3.20 (2.56–4.01)	↓ (C3; age, sex)[b] BMD/BMDL (C3 and 5; sex, birth cohort, log-PFOA)[c]	↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 yr)[b]	–	↓ (C3; age, sex)[b] BMD/BMDL (C3 and 5; sex, birth cohort, log-PFOA)[c]	↑ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 yr)[b]	–
Birth (modeled)	↓ (C3; age, sex)[d] ↓↓ (C3 and 5; age, sex)[d] ↓↓ (C5; age, sex)[d]	–	–	↓ (C3; age, sex)[d] ↓↓ (C3 and 5; age, sex)[d] ↓↓ (C5; age, sex)[d]	–	–
18 mo C3: NR C5: 2.8 (2.0–4.5)	↑ (C3; age, sex)[d] ↑ (C3 and 5; age, sex)[d] ↑ (C5; age, sex)[d]		–	↓ (C3; age, sex)[d] ↓↓ (C3 and 5; age, sex)[d] ↓↓ (C5; age, sex)[d]		–
5 yr C3: GM: 4.06 (3.33–4.96) C5: 2.2 (1.8–2.8)	↓ (C3; age, sex)[b] ↓ (C3; age, sex)[d] ↑ (C3 and 5; age, sex)[d] ↑ (C5; age, sex)[d]	↓↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 yr)[b] BMD/BMDL (C3; sex, age, and booster type at age 5 yr)[e] BMD/BMDL (C3; sex, booster type at age 5 yr, log-PFOA)[c]	–	↓ (C3; age, sex)[b] ↓ (C3; age, sex)[d] ↓ (C3 and 5; age, sex)[d] ↓↓ (C5; age, sex)[d]	↓↓ (C3; age, sex, booster type, and the child's specific antibody concentration at age 5 yr)[b] BMD/BMDL (C3; sex, age, and booster type at age 5 yr)[e] BMD/BMDL (C3; sex, booster type at age 5 yr, log-PFOA)[c]	–

Exposure measurement timing, PFOA levels (ng/mL)[a]	Diphtheria Antibody Associations with PFOA by Age at Assessment			Tetanus Antibody Associations with PFOA 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 yr C3: 4.4 (3.5–5.7)	–	↓↓ (C3; age, sex, booster type)[f] ↓ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]	↓ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]	–	↓ (C3; age, sex, booster type)[f] ↑ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]	↑ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]
13 yr C3: 2.0 (1.6–2.5)	–	–	↓ (C3; sex, age at antibody assessment, booster type at age 5 yr)[g]	–	–	↑ (C3; sex, age at antibody assessment, booster type at age 5 yr)[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 PFOA 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); *high* confidence

A cross-sectional study of these antibody levels 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. A subset of the study population did have maternal samples available, and those results were also inconsistent by vaccine. However, this study was the only one to examine the OR 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: 1.41 (0.91, 2.19)).

In children from Guinea-Bissau, West Africa, Timmermann et al. (2020) observed nonsignificant associations between elevated levels of PFOA and decreased adjusted anti-measles antibody levels across time in the group with no measles vaccination at age 9 months. This association was not seen in the group with one measles vaccination. The same pattern was observed at the 2-year follow-up.

Two *medium* cross-sectional studies of adolescents examined associations between elevated levels of PFOA 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 mumps per 2.7-fold increase in serum (Figure 3-24). 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) reporting on adolescents from the same NHANES cycles (i.e., 1999–2000 and 2003–2004) observed a significant inverse association in adolescents seropositive for rubella.

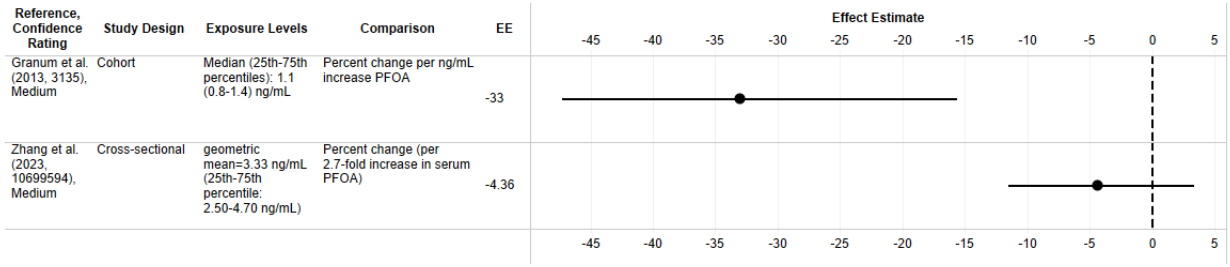


Figure 3-24. Overall Rubella Antibody Levels in Children and Adolescents from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
Adolescent regression coefficients from Pilkerton, 2018, 5080265 were not reported quantitatively.
Regression coefficients from Granum, 2013 were re-expressed as percent change.

Lastly, the *low* confidence cross-sectional study of one-year-old children in Germany, Abraham et al. (2020), reported statistically significant correlations between PFOA concentrations and adjusted levels of antibodies against tetanus, Hib, and diphtheria.

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). The

medium confidence study by Shih et al. (2021) measured PFOA in cord blood and at multiple points through childhood to early adulthood in people in the Faroe Islands, with outcome measurement at age 28 years. The study by Stein et al. (2016a) was rated *low* confidence because it utilized convenience sampling to recruit participants, had low seroconversion rates, and was at high risk of residual confounding. 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.

In adults and adolescents, results were less consistent than in children. Shih et al. (2021) reported inverse associations for all exposure windows in the total cohort (not statistically significant) for hepatitis B antibodies but for other vaccines (diphtheria, tetanus, and hepatitis A), the direction of association was inconsistent across exposure windows. Results also differed by sex for all vaccines, but without a consistent direction (i.e., stronger associations were sometimes observed in women and sometimes in men). Similar to the results in 13-year-old children in the other Faroe Islands cohorts, this may indicate that by age 28, the effect of developmental exposure is less relevant. Pilkerton et al. (2018) observed statistically significant associations between high-quartile PFOA levels and decreased rubella IgA levels compared with low-quartile PFOA levels in adult men. Stein et al. (2016a) reported no immunosuppression based on seroconversion following FluMist vaccination.

Despite the imprecision (i.e., wide CIs) of some of the exposure-outcome analysis pairs, the findings are generally consistent with respect to an association between PFOA exposure and immunosuppression in children. Changes in antibody levels of 10%–20% per doubling of exposure were observed in the Faroe Islands cohorts (Grandjean et al., 2017b; Grandjean et al., 2017a). The variability in some of the results could be related to differences in etiological relevance of exposure measurement timing, vaccine type, and timing of the boosters, as well as differences in timing of antibody measurements in relation to the last booster. However, these factors cannot be explored further with currently available evidence. Overall, the evidence indicates an association between increased serum PFOA levels and decreased antibody production following routine vaccinations, particularly in children.

In addition to these studies of antibody response to vaccination, there are two studies that examined antibody response to HFMD (Zeng et al., 2019b) and hepatitis B infection (Zeng et al., 2020). This birth cohort study 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 PFOA 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 ($p > 0.05$) between serum PFOA concentration and hepatitis B surface antibody; however, there are study limitations due to concurrent measurement of exposure and outcome and potential for reverse causality, and this study was rated *low* confidence.

In a C8 Health Project study, Lopez-Espinoza et al. (2021) measured serum PFAS and white blood cell types in 42,782 adults in 2005–2006 and 526 adults in 2010 from an area with PFOA drinking water contamination in the Mid-Ohio Valley (USA). Generally positive monotonic associations between total lymphocytes and PFOA were found in both surveys (difference range: 1.12%–5.50% for count and 0.36–1.24 for percentage, per PFOA IQR increment). Findings were inconsistent for lymphocyte subtypes. However, the magnitude of the differences was small.

3.4.2.1.2.2 Infectious Disease

Overall, 10 studies (11 publications)[12] measured associations between PFOA exposure and infectious diseases (or disease symptoms) in children with follow-up ranging between 1 and 16 years. Infectious diseases measured included common cold, respiratory tract infections, respiratory syncytial virus, otitis media, pneumonia, chickenpox (varicella), bronchitis, bronchiolitis, ear infections, gastric flu, urinary tract infections, and streptococcus. Of the studies measuring associations between infectious disease and PFOA exposure, 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). Six studies measured PFOA concentrations from mothers during pregnancy (Wang et al., 2022; Ait Bamai et al., 2020; Impinen et al., 2019; Manzano-Salgado et al., 2019; Goudarzi et al., 2017; Dalsager et al., 2016). Two studies (Huang et al., 2020; Impinen et al., 2018) measured PFOA concentrations from cord blood at delivery. Two studies measured PFOA concentrations in children's serum at age one year (Abraham et al., 2020) 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, six studies were *medium* confidence (Wang et al., 2022; Dalsager et al., 2021; Ait Bamai et al., 2020; Huang et al., 2020; Manzano-Salgado et al., 2019; Goudarzi et al., 2017) and five were *low* confidence (Abraham et al., 2020; 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 PFOA exposure was observed, although results were not consistent across studies (see Appendix D, (U.S. EPA, 2024a)). The most commonly examined types of infections were respiratory, including pneumonia/bronchitis, upper and lower respiratory tract, throat infections, and common colds. Dalsager et al. (2021) reported higher rates of hospitalization for upper and lower respiratory tract infections with higher PFOA exposure (statistically significant only 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 PFOA concentrations and increased risk of developing pneumonia in 0- to 3-year-old children (Impinen

[12] 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.

et al., 2019) and 7-year-old children (Ait Bamai et al., 2020); one other *low* and one other *medium* confidence study did not report an increase in infections (Wang et al., 2022; Abraham et al., 2020). Huang et al. (2020), a *medium* confidence study, examined recurrent respiratory infections and found no association. Two *low* confidence studies and one *medium* confidence study found positive associations with lower respiratory tract 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). In addition, non-statistically significant positive associations were reported with upper respiratory tract infection (Dalsager et al., 2021) and throat infection (Impinen et al., 2019). There were also statistically significant associations seen for PFOA in relation to respiratory syncytial virus, rhinitis, throat infection, and pseudocroup (Ait Bamai et al., 2020; Kvalem et al., 2020; Impinen et al., 2019), but findings were inconsistent across studies. No positive associations were reported with common cold (Kvalem et al., 2020; Impinen et al., 2019). Outside of respiratory tract infections, two *medium* confidence studies examined total infectious diseases. Dalsager et al. (2021) reported higher rates of hospitalization for any infections with higher PFOA exposure (not statistically significant), while Goudarzi et al. (2017) reported higher odds of total infectious disease incidence in girls ($p > 0.05$) but not boys. Results for other infection types, including gastrointestinal, generally did not indicate a positive association. Lastly, one study (Dalsager et al., 2016) measured common infectious disease symptoms in children aged 1-to-4 years and found a positive association with fever and nasal discharge, but not cough, diarrhea, or vomiting. Overall, the observed associations provide some coherence with the associations observed with vaccine response, but inconsistency across studies reduces confidence in the evidence.

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) (see 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 PFOA exposure (OR (95% CI) per log-2 SD increase in PFOA: 2.73 (1.71, 4.55)). 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 PFOA exposure and increased COVID-19 severity. Bulka et al. (2021) used NHANES data from 1999–2016 in adolescents and adults and examined immunoglobulin G (IgG) antibody levels to several persistent infections, including cytomegalovirus, Epstein Barr virus, hepatitis C and E, herpes simplex 1 and 2, 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 and total pathogen burden with higher PFOA exposure in adults but no association with other individual pathogens.

3.4.2.1.3 Immune Hypersensitivity Study Quality Evaluation and Synthesis from the Updated Literature Review

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

In the 2016 PFOA HESD, two *medium* confidence epidemiological studies reported higher odds of asthma with higher PFOA exposure in children (Humblet et al., 2014; Dong et al., 2013). A case-control study (Dong et al., 2013) of children in Taiwan reported increased odds of asthma with increasing childhood PFOA 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 for IgE in serum, absolute eosinophil counts, eosinophilic cationic protein, and asthma severity score. A study on NHANES (1999–2000, 2003–2008) adolescents also reported significantly increased odds of 'ever asthma' per doubling of concurrent PFOA measurements, where 'ever asthma' was defined as ever having received an asthma diagnosis from a healthcare professional (Humblet et al., 2014). Results were less consistent for measures of hypersensitivity (e.g., food allergy, eczema); however, among female infants, decreased cord blood IgE (Okada et al., 2012) was significantly associated with prenatal PFOA exposure.

There are 23 epidemiological studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and hypersensitivity (i.e., asthma, allergy, and eczema) effects. Study quality evaluations for these 23 studies are shown in Figure 3-25. *High* and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix D, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

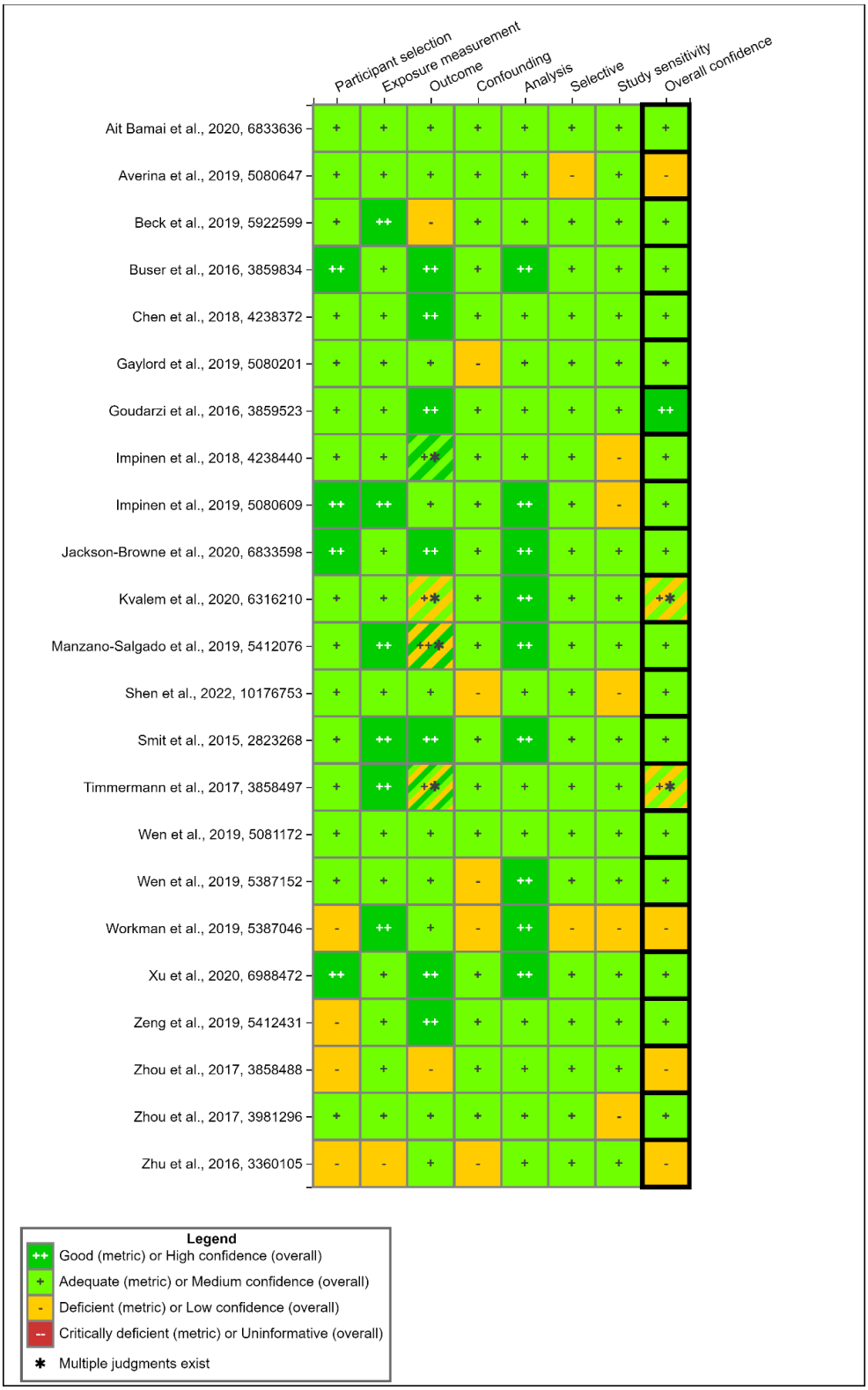


Figure 3-25. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Immune Hypersensitivity Effects

Interactive figure and additional study details available on HAWC.

Thirteen studies (15 publications)[13] examined asthma (or asthma symptoms) and PFOA exposure. Nine of these studies were cohorts (Kvalem et al., 2020; Averina et al., 2019; Beck et al., 2019; Impinen et al., 2019; Manzano-Salgado et al., 2019; Workman et al., 2019; Zeng et al., 2019a; Timmermann et al., 2017a; 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, (Gaylord et al., 2019; 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). Nine studies measured 'ever asthma' for at least one time point (Jackson-Browne et al., 2020; Kvalem 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., 2017a; Smit et al., 2015). Incident or recurrent wheeze was examined in one study (Workman et al., 2019). For asthma, 10 publications were rated *medium* confidence and five publications were rated *low* confidence (Figure 3-25). Timmermann et al. (2017a) 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. Two studies from the Genetic and Biomarker Study for Childhood Asthma (GBCA) (Zhou et al., 2017c; 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)), and few statistically significant results were observed. Several studies observed positive associations with ORs greater than 1.2 between PFOA concentration levels and increased "current" or "ever" asthma (Jackson-Browne et al., 2020; Kvalem et al., 2020; Averina et al., 2019; Beck et al., 2019; Zeng et al., 2019a; Timmermann et al., 2017a), 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. Beck et al. (2019) observed statistically significant increased odds of self-reported asthma per PFOA increase in boys, but this was not observed in girls. For doctor-diagnosed asthma in the same study, an inverse association ($p > 0.05$) was observed in boys and a positive association ($p > 0.05$) was observed in girls. Kvalem et al. (2020) reported increased odds of asthma in girls at age 10 ($p < 0.05$) and between 10 and 16 years of age, but null associations at 16 years, while the opposite was true for boys. Zeng et al. (2019a) observed a positive association in girls and an inverse association in boys (both $p > 0.05$). Jackson-Browne et al. (2020) also observed statistically significant increased odds of "ever" asthma from increased PFOA concentrations in children aged 3–5. However, these associations were null in other age groups and in sex and race categories. Gaylord et al. (2019) reported nonsignificant positive associations in youths of 13–22 years in age. The *low* confidence study by Timmermann et al. (2017a) observed positive associations ($p < 0.05$) between increased asthma odds and elevated PFOA concentrations in a small subset of children aged 5 and 13 who did not receive their measles, mumps, and rubella

[13] 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.

(MMR) vaccination before age 5. However, in children of the same ages who had received their MMR vaccination before age 5, an inverse association was observed ($p > 0.05$). *Low* confidence studies from the GBCA study (Zhou et al., 2017c; Zhu et al., 2016) observed elevated PFOA levels ($p < 0.001$) in children with asthma compared with those without (Zhou et al., 2017b), and the odds of current asthma were also found to be elevated among boys and girls with increasing PFOA exposure (Zhu et al., 2016). Two other studies (Impinen et al., 2019; Impinen et al., 2018) observed small positive associations (OR: 1.1); in Impinen et al. (2019), this was only observed for current asthma in boys. Two studies reported nonsignificant inverse associations with asthma (Manzano-Salgado et al., 2019; Smit et al., 2015), and 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 using data from NHANES examined fractional exhaled nitric oxide (FeNO), a measure of airway inflammation, in adults ((Xu et al., 2020a); see Appendix D, (U.S. EPA, 2024a)). Among participants without current asthma, this study found higher FeNO levels with higher PFOA exposure, indicating greater inflammation (percent change (95% CI) for tertiles vs. T1, T2: 5.29 (1.88, 8.81); T3: 6.34 (2.81, 10.01)).

Overall, there is some evidence of an association between PFOA exposure and asthma, but there is considerable uncertainty due to inconsistency across studies and sub-populations. Sex-specific differences were reported in multiple studies, but there was inconsistency in the direction of association within each sex. There is not an obvious pattern of results by analysis of "ever" versus "current" asthma, and no studies beyond the Dong et al. (2013) study described in the 2016 PFOA HESD examined asthma incidence.

Seven studies observed associations between PFOA exposure and allergies, specifically allergic rhinitis or rhinoconjunctivitis, skin prick test, and food or inhaled allergies. Five of these studies were cohorts (Ait Bamai et al., 2020; Kvalem et al., 2020; Impinen et al., 2019; Timmermann et al., 2017a; 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 2005–2010 (Buser and Scinicariello, 2016). One study was considered *high* confidence (Goudarzi et al., 2016) and the rest were considered *medium* confidence for allergy outcomes. PFOA concentrations were measured at a variety of time points: three studies measured PFOA during pregnancy (Ait Bamai et al., 2020; Impinen et al., 2019; Goudarzi et al., 2016); three studies measured PFOA concentrations in children at age 5 years (Timmermann et al., 2017a), age 10 years (Kvalem et al., 2020), age 13 years (Timmermann et al., 2017a) and ages 12–19 years (Buser and Scinicariello, 2016); and one study measured PFOA in cord blood at delivery (Impinen et al., 2018) (see Appendix, (U.S. EPA, 2024a)).

Results were generally inconsistent across studies. 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., 2017a), 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. Kvalem et al. (2020) reported a statistically significant but small association (OR: 1.1) with a positive skin prick test at ages 10 and 16 years. Timmermann et al. (2017a) also reported a positive association ($p > 0.05$) in children who had received an MMR before age 5 years (but an inverse association in those who had not received an MMR) and results in Impinen et al. (2018) were null. 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., 2017a; Goudarzi et al., 2016). Rhinitis was defined as at least one symptom of runny or blocked nose or sneezing. Rhinoconjunctivitis was defined as having symptoms of rhinitis, in addition to itchy and watery eyes. Rhinitis was increased with exposure at age 16 years ($p < 0.05$) but decreased at age 10 years in Kvalem et al. (2020). Nonsignificant increases in rhinitis were also reported in Impinen et al. (2018) and Timmermann et al. (2017a), but results were null in Ait Bamai et al. (2020) and Goudarzi et al. (2016) for rhinoconjunctivitis. Impinen et al. (2019) measured parent-reported, doctor-diagnosed "current" or "ever" allergy symptoms at age 7 years in addition to known food and inhaled allergies and reported higher odds of current food allergies and ever inhaled allergies (both $p > 0.05$), but not ever food allergies or current inhaled allergies. Buser et al. (2016) measured food sensitization (defined as having at least one 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 PFOA 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., 2017a; Goudarzi et al., 2016), and one was a case-control analysis (Impinen et al., 2018). Four studies measured PFOA 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 maternal PFOA concentrations during pregnancy (Manzano-Salgado et al., 2019; Timmermann et al., 2017a; Goudarzi et al., 2016), and one study measured PFOA concentrations in children at age 5 and 13 years (Timmermann et al., 2017a). All of the studies were considered *medium* confidence for eczema (see Appendix D, (U.S. EPA, 2024a)).

Two studies (three publications) observed statistically significant associations between increased odds of eczema within the highest quantiles of PFOA exposure (Wen et al., 2019a; Wen et al., 2019b; Chen et al., 2018); however, the associations were nonmonotonic across categories of exposure. Impinen et al. (2018) also observed a nonsignificant association between higher PFOA concentrations and "ever" eczema at age 2 years; however, results were null for "current" eczema at age 10 years. Results from Goudarzi et al. (2016), Manzano-Salgado et al. (2019) and Timmermann et al. (2017a) were null.

One *medium* confidence nested case-control study examined chronic spontaneous urticaria (Shen et al., 2022). They found no association between PFOA exposure and case status.

3.4.2.1.4 Autoimmune Disease Study Quality Evaluation and Synthesis from the Updated Literature Review

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 that respond 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 PFOA HESD (U.S. EPA, 2016c) identified one *low* confidence occupational study in workers highly exposed to PFOA (part of the C8 Health Project) (Steenland et al., 2015) that reported significant positive trends for rheumatoid arthritis and ulcerative colitis with increasing cumulative PFOA exposure. The C8 Science Panel concluded there was a probable link between PFOA and ulcerative colitis (C8 Science Panel, 2012b).

There are six epidemiological studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and autoimmune disease. Study quality evaluations for these 6 studies are shown in Figure 3-26. *High* and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

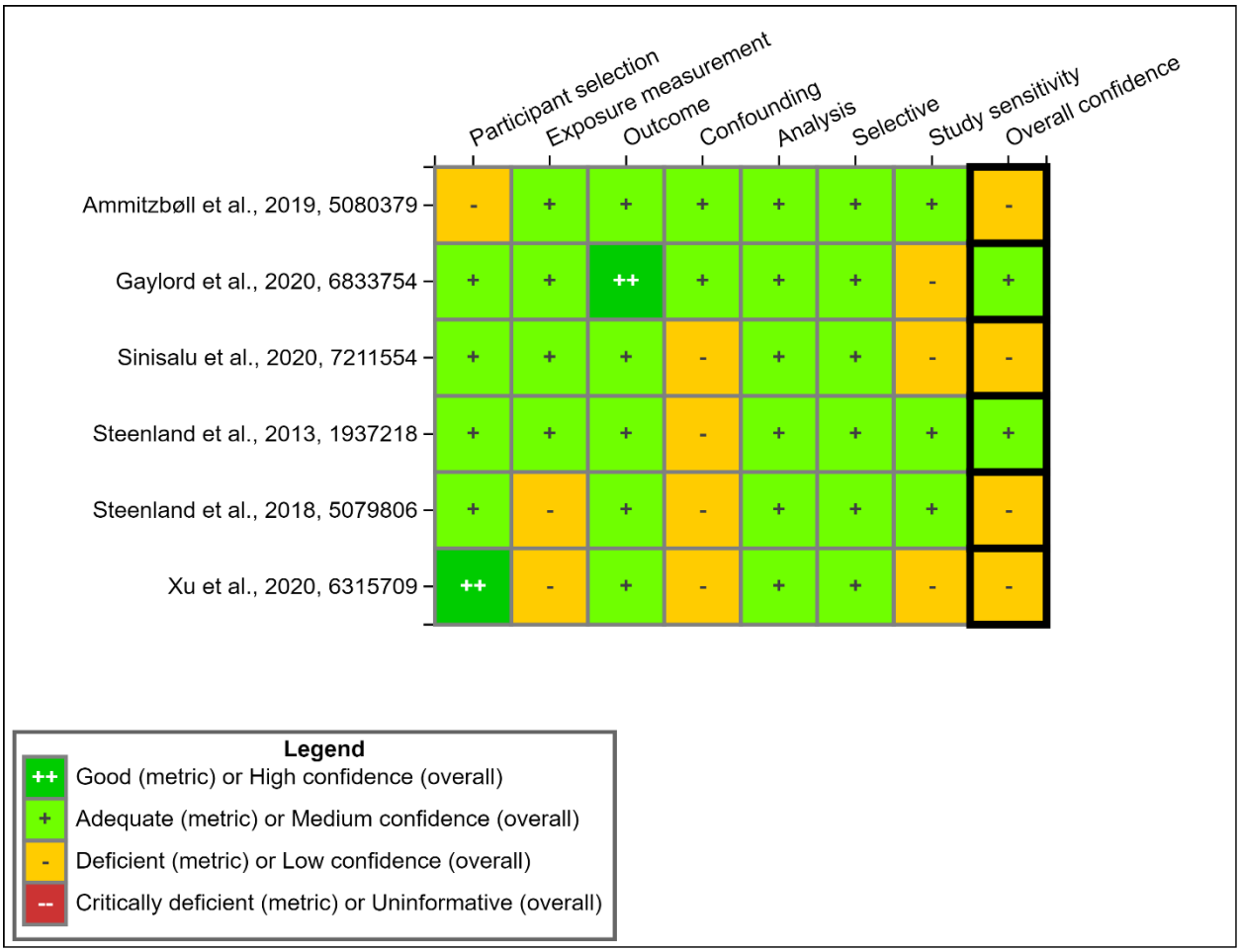


Figure 3-26. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Autoimmune Effects

Interactive figure and additional study details available on HAWC.

One study examined the association between PFOA exposure and multiple autoimmune conditions (rheumatoid arthritis, lupus, MS, ulcerative colitis, and Crohn's disease) in the combined C8 Health Project occupational and community cohort (Steenland et al., 2013). Two case-control studies examined MS (Ammitzbøll et al., 2019) and ulcerative colitis (Steenland et al., 2018b) in adults, and two case-control studies examined celiac disease in children and young adults (Gaylord et al., 2020; Sinisalu et al., 2020). One study was a cohort study that examined ulcerative colitis in children and adults from a high-exposure community in Sweden (Ronneby cohort) (Xu et al., 2020d). The combined occupational and community study used modeled PFOA exposure based on serum concentrations and historical data on residences and drinking water quality (Steenland et al., 2013), and the case-control studies measured PFOA in serum or plasma only (Gaylord et al., 2020; Sinisalu et al., 2020; Ammitzbøll et al., 2019; Steenland et al., 2018b). Two studies were without notable deficiencies and considered *medium* confidence (Gaylord et al., 2020; Steenland et al., 2013). Four studies were considered *low* confidence (Sinisalu et al., 2020; Xu et al., 2020d; Ammitzbøll et al., 2019; Steenland et al., 2018b). 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. Information on participant selection, particularly control selection, was not reported in Ammitzbøll (2019). Additionally, PFOA was evaluated as a dependent rather than independent variable, making no informative determinations about associations between PFOA exposure and risk of MS.

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

Evidence from a case-control study suggested lower PFOA concentrations among healthy controls compared with those with MS (Ammitzbøll et al., 2019). Serum PFOA concentrations were 12% lower (95% CI: −24%, 2%; p = 0.099) in healthy controls compared with cases of relapsing remitting MS and clinically isolated MS. Restricting the analysis to men, serum PFOA levels were 28% lower (95% CI: −42%, −9%; p = 0.006) in healthy controls compared with cases, but this effect was not seen in women. Steenland et al. (2018b) detected significantly increased levels of PFOA in ulcerative colitis cases versus those with Crohn's disease or controls and observed statistically significantly increased odds of ulcerative colitis with increased PFOA exposure among combined children and adults; however, the trend was not consistent across increasing quintiles of PFOA exposure, with a peak in the third quintile. Xu et al. (2020d) observed significant decreases in risk of Crohn's disease in an early exposure period, but not in later exposure periods, or for UC in children and adults from a high-exposure community in Sweden (Ronneby cohort).

The risk of celiac disease was elevated among children and young adults (≤21 years old) in a case-control study (Gaylord et al., 2020), particularly in females ($p < 0.05$), but the association did not reach significance among the whole population.

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 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 PFOA exposure in those children that later developed celiac disease, which may be due to the small sample size, but age at diagnosis of celiac disease was strongly associated with the PFOA exposure.

3.4.2.2 Animal Evidence Study Quality Evaluation and Synthesis

There are four studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and nine studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and immune effects in animal models. Study quality evaluations for these 13 studies are shown in Figure 3-27.

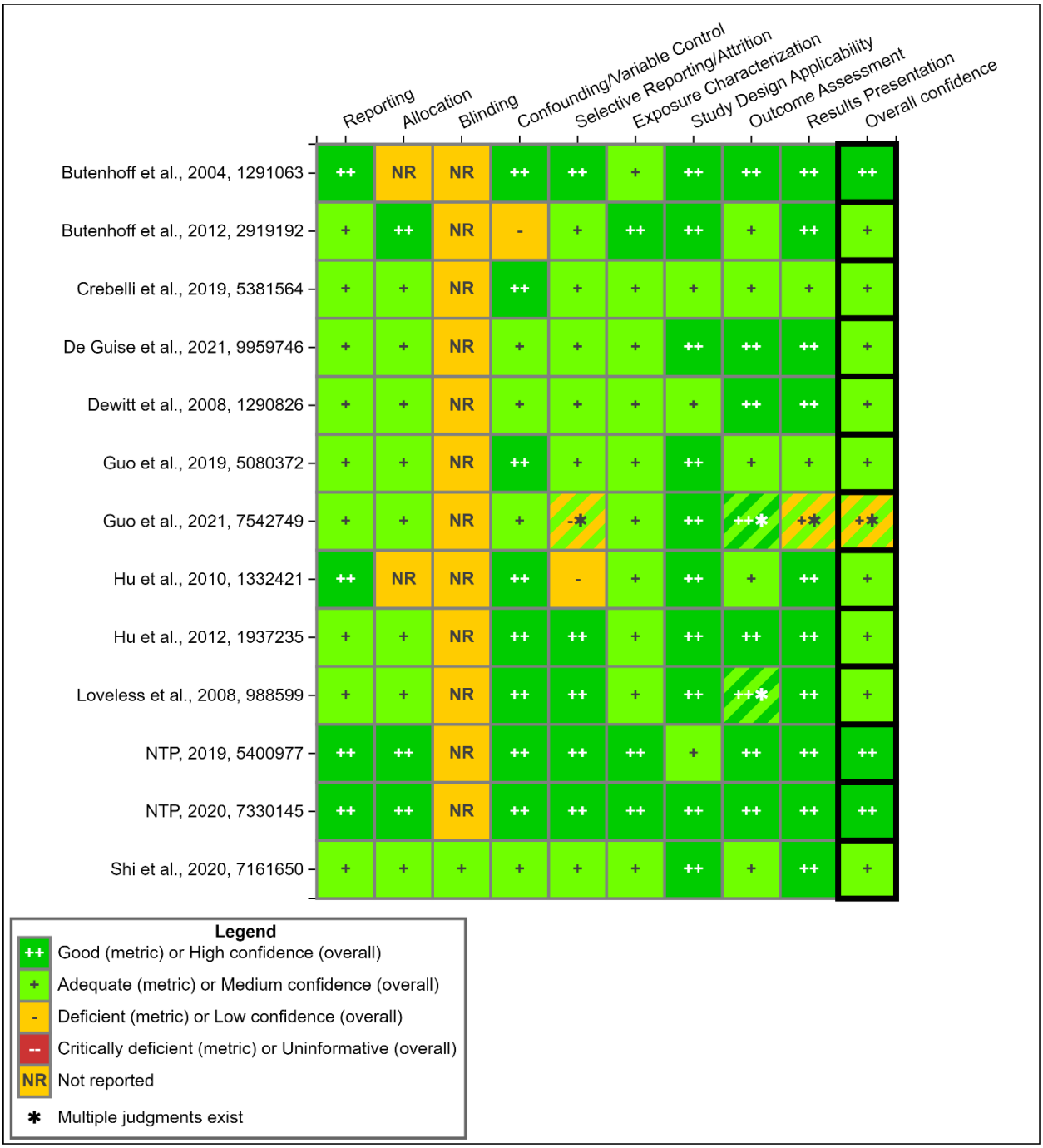


Figure 3-27. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Immune Effects

Interactive figure and additional study details available on HAWC.

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

3.4.2.2.1 Organ Weight/Histopathology

Short-term exposure studies by Yang et al. (2000), Yang et al. (2001), Qazi et al. (2009), and Yang et al. (2002b) using male C57BL/6 mice, by DeWitt et al. (2008) using female C57BL/6 mice, and by DeWitt et al. (2016b) using female C57BL/6Tac mice were conducted using relatively high PFOA doses (up to approximately 40 mg/kg/day). In each study, the PFOA-treated C57BL/6 mice exhibited significant reductions in spleen and thymus weights after 5–16 days of exposure. Yang et al. (2000) and DeWitt et al. (2008) observed up to an approximately 80% reduction in absolute and relative thymus weight and up to a 30%–48% reduction in absolute and relative spleen weight. Similar reductions in absolute thymus and spleen weights were observed in Yang et al. (2002b); relative weights were not reported. In DeWitt et al. (2016b), relative spleen weights were significantly reduced by 30% after exposure to 30 mg/kg/day, and relative thymus weights were significantly reduced by 55.4% after exposure to 7.5 mg/kg/day (but not after exposure to 30 mg/kg/day). Absolute weights were not reported in this study. In male CD-1 mice exposed for 29 days via gavage to 1, 10, or 30 mg/kg/day PFOA, absolute and relative spleen weights were reduced to approximately 90%, 60%, and 50% of controls, respectively (Loveless et al., 2008). Absolute and relative thymus weights were decreased to approximately 50% of controls in the 10 and 30 mg/kg/day groups. Spleen and thymus weights were only reduced by up to 9% (not statistically significant) in male ICR mice administered 47.21 mg/kg/day PFOA in drinking water for 21 days (Son et al., 2009). In male BALB/c mice dosed with 0.4, 2, or 10 mg/kg/day PFOA via gavage for 28 days, absolute spleen weights were significantly reduced to 88% and 50% of the control in the 2 and 10 mg/kg/day groups, respectively (Guo et al., 2021b). Relative spleen weights in these groups were similarly reduced to 84% and 56% of the control. In the same study, however, no significant changes in spleen or thymus weights were observed in male Sprague-Dawley rats. In a separate 28-day study, male Sprague-Dawley rats administered 2.5–10 mg/kg/day displayed significantly lower absolute spleen weights that reached 76% of control at the highest dose (NTP, 2019). Absolute thymus weight was decreased to 74% of control in males administered 10 mg/kg/day compared with those of the vehicle group. Female spleen and thymus weights were not altered.

In one developmental study, pregnant C57BL/6N mice were exposed to 0.5 or 1 mg/kg/day PFOA from GD 6 to GD 17; the relative spleen and thymus weights of the female offspring were unchanged at PND 48 (Hu et al., 2010). The male offspring were not assessed in this study. However, a reduction in spleen and thymus weights has been reported in male rats following developmental PFOA exposure. NTP (2020) exposed pregnant rats to PFOA beginning on GD 6, and exposure was continued in offspring postweaning for a total of 107 weeks. Dose groups for this report are referred to as "[perinatal exposure level (ppm)]/[postweaning exposure level (ppm)]" (see further study design details in Section 3.4.4.2.1.2). Following perinatal and postweaning PFOA exposure (150/150 and 300/300 ppm), significant reductions in absolute and relative spleen weight and absolute thymus weight were observed at 16 weeks in male rats. Reduced absolute and relative spleen weights were also observed in rats following 300/20, 300/40, and 300/80 ppm PFOA exposure. Postweaning exposure alone (0/20, 0/40, 0/150, and 0/300 ppm) significantly reduced absolute and relative spleen weights. Absolute thymus weight was reduced following 0/150 and 0/300 ppm (NTP, 2020). No changes in spleen or thymus weights were reported in females.

Two studies describing effects of subchronic PFOA exposure in adult male mice (Shi et al., 2020; Crebelli et al., 2019) and one chronic study in adult male rats (Butenhoff et al., 2012) did not report reduced spleen weight, and thymus weights were not examined. No changes to spleen weights were observed in C57BL/6 male mice administered ≤5 mg/kg/day for 5 weeks (Shi et al., 2020; Crebelli et al., 2019). Although the changes were not statistically significant, Shi et al. (2020) observed 21%, 32%, and 32% reductions in relative spleen weight (compared with controls) in mice exposed to 0.5, 1, or 3 mg/kg/day, respectively. Body weight gain was also significantly reduced in these groups, and absolute spleen weight was not reported. Similarly, spleen weight was not affected in male Sprague-Dawley rats chronically exposed to 30 or 300 ppm (1.3 or 14.2 mg/kg/day) for 1 or 2 years (Butenhoff et al., 2012). An increase in absolute and relative spleen weight (40% and 30% increase, respectively) was observed only in female rats exposed to 30 ppm (1.6 mg/kg/day) for 2 years.

3.4.2.2.2 Histopathology

Several studies reported on histological evaluations of the spleen and thymus from rodents orally administered PFOA at varying doses and durations. In male Crl:CD-1 (ICR)BR mice administered PFOA for 29 days, decreased spleen weights at 10 and 30 mg/kg/day correlated with the gross observation of small spleens (Loveless et al., 2008). An increased incidence of spleen atrophy was also observed in the 30 mg/kg/day group. The decreased thymus weights at these doses correlated with the microscopic finding of lymphoid depletion and with the gross observation of small thymuses (Loveless et al., 2008). Loveless et al. (2008) also reported increased incidences of granulocytic hyperplasia of the bone marrow in mice in the 10 and 30 mg/kg/day groups.

Other microscopic findings were reported in Son et al. (2009) in the histological evaluation of male ICR mice administered PFOA (0.49–47.21 mg/kg/day) for 21 days. The thymus of mice exposed to 47.21 mg/kg/day PFOA revealed atrophy with decreased thickness of the cortex and medulla compared with control, but increased cellular density of lymphoid cells in the cortex was observed (Son et al., 2009). The authors also reported an enlargement of the spleen with marked hyperplasia of the white pulp in the 47.21 mg/kg/day PFOA-treated group, and an increased area of the lymphoid follicles in the spleen with increased cellular density (Son et al., 2009). In contrast, in a study in male BALB/c mice administered 0.4–10 mg/kg/day PFOA via gavage, the authors noted decreased white pulp content, with the white pulp content in the highest dose group being reduced to nearly in half of that of the control group (quantitative results were not provided) (Guo et al., 2021b).

After 5–6 days of recovery, Loveless et al. (2008) observed increased extramedullary hematopoiesis in the spleens of male Crl:CD(SD)IGS BR rats and Crl:CD-1 (ICR)BR mice exposed to 30 mg/kg/day PFOA for 23–24 days. However, these changes were not observed in rats and mice after a continuous 29-day exposure (Loveless et al., 2008). Likewise, splenic hematopoiesis was not affected in male or female Sprague-Dawley rats administered 0.625–10 or 6.25–50 mg/kg/day PFOA, respectively (NTP, 2019).

Two studies in male Sprague-Dawley rats exposed to up to 30 mg/kg/day PFOA for 28–29 days reported no histopathological changes in the spleen, thymus, and/or lymph nodes (NTP, 2019; Loveless et al., 2008). However, a significant increase in bone marrow hypocellularity of

minimal severity was reported in male rats exposed to 10 mg/kg/day (6/10 compared with 1/10 in controls) but not in female rats (NTP, 2019).

Histological evaluation of the spleen following chronic PFOA exposure was only reported in one study, which administered 30 or 300 ppm PFOA to male and female Sprague-Dawley rats for 2 years. Hemosiderin, an iron-rich pigment, was found in greater amounts in the spleens of males dosed with 300 ppm (approximately 15 mg/kg/day), though this change was not significant, but was significantly reduced in the 30 ppm groups (approximately 1.5 mg/kg/day) and in the 300 ppm females (Butenhoff et al., 2012). However, no histopathological changes in the thymus, spleen, bone marrow, or lymph nodes were reported in a study that exposed Sprague-Dawley rats to up to 300 ppm PFOA for 16 weeks (males and females) or up to 80 ppm PFOA (males) or 300 ppm (females) for 2 years (NTP, 2020).

Histological evaluation of the spleen and thymus following reproductive PFOA exposure was only reported in one study (Butenhoff et al., 2004a). P_0 males and females were administered 1–30 mg/kg/day PFOA from premating until the end of lactation and the F_1 generation was exposed throughout their life. The authors note that no histopathological changes were reported, though quantitative results were not provided.

3.4.2.2.3 Immune Cellularity

3.4.2.2.3.1 White Blood Cells and Differentials

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

In a similar study, male Crl:CD-1(ICR)BR mice were exposed to PFOA (10 or 30 mg/kg/day) by oral gavage for 29 days. At both doses tested, increases in total serum neutrophils and monocytes (reaching 296% and 254% of control, respectively, at the highest dose), and a decrease in total number of eosinophils (approximately 60% of control, data not statistically significant) were observed (Loveless et al., 2008). Loveless et al. (2008) also reported a decrease in lymphocytes in male mice dosed with 30 mg/kg/day, but these data were not provided in the study. In a second short-term study, white blood cell count was significantly decreased to 71% and 36% of the control in male BALB/c mice exposed to 2 and 10 mg/kg/day PFOA, respectively, for 28 days (Guo et al., 2021b). White blood cell differentials were not measured in this study.

In a short-term study in male and female Sprague-Dawley exposed to 0.625–10 or 6.25–100 mg/kg/day PFOA, respectively, no changes in white blood cell counts or differentials were reported (NTP, 2019).

In male and female Sprague-Dawley rats chronically exposed to 30 or 300 ppm PFOA (approximately 1.5 or 15 mg/kg/day) for 2 years, PFOA did not affect total white blood cell count, blood lymphocytes, or neutrophils (Butenhoff et al., 2012). However, white blood cell counts were increased in males through the first year of the study. The authors suggest that these

changes were due to increases in absolute counts of lymphocytes at 3 and 6 months and in neutrophils at 12 months (Butenhoff et al., 2012).

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

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

No changes in splenocyte and thymocyte cell populations were observed in one study of male Sprague-Dawley rats exposed to 0.3–30 mg/kg/day PFOA for 29 days (Loveless et al., 2008).

Developmental PFOA exposure may also impact cellularity of the spleen. In one study by Hu et al. (2012), an approximate 22% reduction in splenic regulatory T cells (CD4 + CD25 + $Foxp3^+$) was observed in PND 42 male and female offspring from C57BL/6N dams exposed to 2 mg/kg/day PFOA from gestation through lactation. Thymic cellularity was not examined in this study (Hu et al., 2012).

3.4.2.2.4 Ability to Generate an Immune Response

The ability to generate an immune response following PFOA has been investigated in rodent models. Male Crl:CD-1 (ICR)BR mice were exposed to PFOA (0, 0.3, 1, 10, or 30 mg/kg/day) by oral gavage for 29 days and received an injection of serum sheep red blood cells (SRBC) on day 24 (Loveless et al., 2008). The induced immunoglobulin M (IgM) response was significantly reduced to 80% and 72% of controls in mice exposed to 10 and 30 mg/kg/day, respectively. The same study found no changes in IgM in rats. After an injection with keyhole limpet hemocyanin (KLH), a similar reduction in anti-KLH IgM response was observed in female B6C3F1 mice administered 1.88 and 7.5 mg/kg/day PFOA in drinking water for 28 days (De Guise and Levin, 2021). The IgM response in the mice exposed to 1.88 or 7.5 mg/kg/day was significantly reduced to 29% and 8% of the control's response, respectively. The ability to respond to an immunological challenge was also reduced in female C57BL/6N mice exposed to 3.75 to 30 mg/kg/day PFOA in drinking water for 15 days (Dewitt et al., 2008). The mice showed a dose-dependent reduction in IgM levels (between 11% and 30% decrease) after injection with SRBC to induce an immune response. The IgG response to SRBC significantly increased by approximately 15% following 3.75 and 7.5 mg/kg/day PFOA exposure, but no change was observed at higher doses (Dewitt et al., 2008). In a separate study, female C57BL/6Tac mice were exposed to 0, 7.5, or 30 mg/kg/day PFOA in drinking water for 15 days and injected with SRBC on day 11 (Dewitt et al., 2016b). Exposure to 30 mg/kg/day PFOA reduced SRBC-specific IgM antibody responses by 16%. Similarly, male C57BL/6 mice were fed approximately 40 mg/kg/day PFOA for 10 days and then evaluated for their immune response to horse red blood cells (Yang et al., 2002a). PFOA-exposed mice had no increase in plaque-forming cells in

response to the immune challenge, compared with unimmunized control mice, suggesting a suppression of the humoral immune response.

One developmental study assessed the ability to generate an immune response following gestational exposure to PFOA (Hu et al., 2010). In this study, pregnant C57BL/6N mice were exposed to 0.5 or 1 mg/kg/day PFOA from GD 6 to GD 17. The adult female offspring were immunized with SRBC on PND 44. No change in the immune response was observed, as measured through IgM titers (PND 48) and IgG titers 2 weeks later (PND 63) following an SRBC booster.

Alterations in the serum levels of globulin can be associated with decreases in antibody production (FDA, 2002). PFOA exposure at 12.5 mg/kg/day and up to 100 mg/kg/day for 28 days decreased globulin concentrations in female Sprague-Dawley rats by up to 79% of control. In males, a decrease in globulin concentrations was observed at 0.625 mg/kg/day (74% of control) and up to 10 mg/kg/day (61% of control), highlighting greater PFOA tolerance in females compared with males (Figure 3-28) (NTP, 2019). In contrast, an increase in globulin concentrations, by approximately 7%, was observed in male BALB/c mice exposed to 0.4 or 2 mg/kg/day PFOA (but not 10 mg/kg/day) for 4 weeks (Figure 3-28) (Guo et al., 2019). In a similar study by the same group, immunoglobulins were measured, and IgA concentrations were found to be significantly increased by 12%, 16%, and 33% in male BALB/c mice exposed to 0.4, 2, or 10 mg/kg/day, respectively, PFOA for 4 weeks (Guo et al., 2021b). IgM was increased by 3% and 6% in mice exposed to 2 or 10 mg/kg/day, respectively, and IgG was increased by 6% in mice exposed to 10 mg/kg/day.

Globulin levels were also decreased in pregnant ICR dams on GD 18 following 5 or 10 mg/kg/day PFOA from GD 0 to GD 18 (Yahia et al., 2010). Globulin levels were decreased to 78 and 68% of control, respectively. Globulin levels in offspring were not measured. In a developmental study conducted by NTP (2020), Sprague-Dawley rats were exposed perinatally and/or postweaning for a total of 107 weeks to varying doses of PFOA ((perinatal exposure level (ppm))/(postweaning exposure level (ppm)); see further study design details in Section 3.4.4.2.1.2). In male Sprague-Dawley rats at the 16-week interim timepoint, perinatal exposure to 300 ppm (300/0) and/or postweaning exposure to doses ranging from 20 to 300 ppm (0/150, 0/300, 150/150, 300/300, 0/20, 0/40, 0/80, 300/20, 300/40, or 300/80 ppm) significantly decreased globulin levels. Female rats displayed decreased globulin levels following exposure to 0/300, 0/1,000, 150/300, or 300/1,000 ppm PFOA (NTP, 2020) (Figure 3-28).

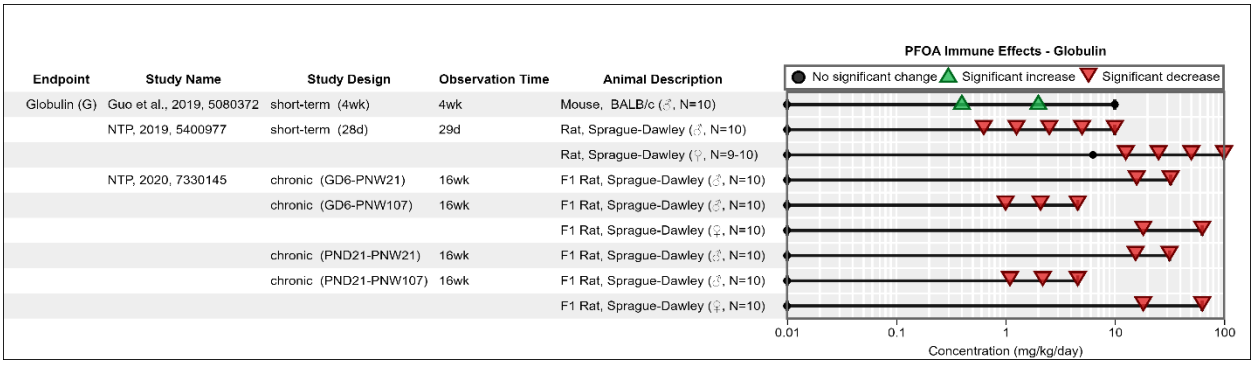


Figure 3-28. Globulin Levels in Rodents Following Exposure to PFOA (logarithmic scale)

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

3.4.2.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse immune outcomes is discussed in Sections 3.3.2 and 3.4.1 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 22 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA 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 3-29.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Cell Growth, Differentiation, Proliferation, Or Viability	3	0	3	6
Cell Signaling Or Signal Transduction	3	0	1	4
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	1	2
Inflammation And Immune Response	11	6	5	20
Oxidative Stress	1	0	2	3
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	12	6	7	22

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

Interactive figure and additional study details available on HAWC.

A consistent pattern of findings from human (Section 3.4.2.1) and animal (Section 3.4.2.2) studies support that higher serum concentrations of PFOA are associated with immunosuppression. Additional findings included reduced spleen and thymus weights, reduced cellularity of white blood cells and differentials in circulation, reduced immune cellularity in primary and secondary lymphoid organs, and altered globulin levels. Mechanistic data available from in vitro, in vivo, and epidemiological studies were used to evaluate the mode of action of PFOA-associated immunosuppression and other effects on the immune system.

3.4.2.3.1 Mechanistic Evidence for PFOA-Mediated Effects on Immune System Development and Physiology

Reductions in lymphocyte numbers have been consistently reported in animal toxicological studies (Section 3.4.2.2), with parallel observations of reduced antibody responses in human studies (Section 3.4.2.1). PFOA can alter the number of various B and T cell subsets in primary and secondary lymphoid organs, which may reflect effects on immune system development including effects on proliferation, differentiation, and/or apoptosis of immune cells.

Two in vivo studies were identified that evaluated PFOA-mediated effects on immune system development, reflected in numbers of B and T cell populations. In female BALB/c mice dermally exposed to PFOA for 14 days, the total numbers of splenic CD4+ T cells were reduced, as were the total numbers and percent of CD4+ T cells in the lymph nodes. The percent of splenic CD4+ T cells was increased (Shane et al., 2020). The authors also observed that the absolute number

and percent of splenic B cells were reduced, an observation which could be explained by increased apoptosis of B cells in the spleen or diminished proliferation in the bone marrow, where B cells develop. Effects on B cell differentiation may also reflect reduced cellularity of bone marrow, thymus, and spleen. Qazi et al. (2012) reported reduced percentages of the relatively undifferentiated pro/pre-B cells (CD19+/CD138+/IgM-) in the bone marrow of male C57BL/6 mice fed diets containing 0.02% PFOA for 10 days. Morphological assessment of the bone marrow was consistent with the reduced cell populations; mice treated with 0.02% PFOA displayed hypocellularity in the bone marrow. The authors note that food consumption by the mice exposed to 0.02% PFOA can be reduced up to 35%. Moreover, although experimentally restricting food consumption by 35% in the absence of PFOA exposure affects pro/pre-B cell populations in a similar manner to PFOA, the effect is not identical, which may support that PFOA exposure is associated with decreased pro/pre-B cells in the bone marrow independent of reduced food consumption. The study also demonstrated that the number of myeloid cells (Gr1+/CD11b+) is reduced by 0.02% PFOA but to a lesser magnitude than that of B-lymphoid cells (CD19+), suggesting that the B-lymphoid cell lineage is more sensitive than the myeloid cell lineage.

Several in vitro studies have reported reductions in immune cell viability or increases in cytotoxicity following exposure to PFOA (Sørli et al., 2020; Rainieri et al., 2017), which could also contribute to reduced lymphocyte cellularity or reduced immune organ weight observed in the animal literature (Section 3.4.2.2).

Reductions in immune cellularity of B and T cell populations in the thymus and spleen (Section 3.4.2.2) as well as the bone marrow may reflect perturbations in cellular and/or molecular events including cell proliferation, apoptosis, and oxidative stress. An in vitro study by Rainieri et al. (2017) evaluated the effects of PFOA on cell proliferation by quantifying the distribution of cells in different stages of the cell cycle in a human macrophage cell line (TLT cells). Significantly more cells were in G2/M phase of mitosis following exposure to PFOA in parallel with a lower proportion of cells in the G0/G1 phase, suggesting increased cell proliferation. However, increased cell proliferation is inconsistent with the immune organ atrophy reported in animal toxicological studies (Section 3.4.2.2) and findings of other mechanistic studies in immune organs. Yang et al. (2002b) reported significant reductions in the proportion of thymocytes in the S and G2/M phases and significant increases in the G0/G1 phases of mice treated with PFOA, which were attenuated in PPARα-null mice. These results imply that reductions in cell numbers in the S and G2/M phases of the cell cycle are partially mediated by PPARα.

Two studies (Rainieri et al., 2017; Wang et al., 2014) have reported increased apoptosis in immune cells following PFOA exposure in vivo and in vitro. Increased apoptosis may contribute to the reductions in immune organ weight observed in the animal literature and/or reduced populations of immune cells (Section 3.4.2.2). Wang et al. (2014) exposed BALB/c mice to 0, 5, 10, or 20 mg/kg/day PFOA via gavage for 14 days and reported that the percent of apoptotic cells increased in the spleen at 10 and 20 mg/kg/day and increased in the thymus at 20 mg/kg/day. Increased apoptosis was associated with atrophy of these immune system organs, suggesting that PFOA-induced apoptosis may contribute to organ atrophy. In parallel, the authors explored the association between lipid metabolism and immunotoxicity of PFOA by including a high-fat diet (HFD) group in addition to the regular diet (RD) group; there was a higher percentage of apoptosis in the HFD vehicle control group than the RD vehicle control group,

indicating that HFD could cause or exacerbate apoptosis. Given these diet-related results along with gene expression data showing that PPARα and PPARγ were also upregulated in the thymus and the spleen, the authors concluded that immunomodulation by PFOA occurs via the PPAR pathway and the induction of mitochondrial damage and lymphocyte apoptosis pathway. Rainieri et al. (2017) evaluated apoptosis in TLT cells exposed to 0, 50, 250, or 500 mg/L PFOA for 12 hours. The percentage of apoptotic cells was significantly elevated only at the highest concentration.

Generation of oxidative stress is a potential underlying mechanism linking PFOA to the aforementioned effects on proliferation, differentiation, and/or apoptosis of immune cells. Oxidative stress has been implicated in PFOA immunotoxicity by one in vivo study and several in vitro studies (Rainieri et al., 2017; Yahia et al., 2016; Wang et al., 2014). Wang et al. (2014) observed that the spleens of mice treated with PFOA had mitochondrial swelling and cavitation as well as swollen and ruptured cristae, which suggests impaired oxidative processes. However, there were no significant changes in H_2O_2 concentrations or superoxide dismutase (SOD) activity in spleens of mice exposed to PFOA versus controls. There were no differences in mitochondrial ultrastructure between the HFD group and the RD group, implying that although PFOA-related mitochondrial damage may contribute to apoptosis in lymphocytes, the mechanism may not involve perturbed lipid metabolism. Rainieri et al. (2017) reported increased lipid peroxidation in zebrafish embryos that coincided with a dose-dependent increase in gene expression of glutathione S-transferase pi 1.2 (gstp1) and heat shock cognate 70-kd protein, like (hsp70l), which is typically observed in response to oxidative stress. However, it is important to note that lipid peroxidation and gene expression analyses were evaluated in whole zebrafish embryos and therefore may not necessarily be specific to effects in immune organs. Oxidative DNA damage was reported by Yahia et al. (2016) in a human lymphoblast cell line (TK6 cells) exposed to PFOA at concentrations of 0, 125, 250, and 500 ppm, including a dose-dependent increase in 8-OHdG levels that coincided with increases in tail moment, Olive Tail moment, and tail length in the comet assay at 250 and 500 ppm, which is indicative of DNA damage. Altogether, the evidence suggests that PFOA can induce oxidative stress in immune cells, including oxidation of lipids and DNA, potentially leading to DNA damage.

3.4.2.3.2 Mechanistic Evidence for PFOA-Mediated Effects on Adaptive Immune Responses

3.4.2.3.2.1 Mechanistic Data Informing Suppression of Immune Responses to Vaccines and Infectious Diseases

PFOA-associated immunosuppressive effects are described in Section 3.4.2.2.1. Adaptive immune responses include B and T cell-mediated responses to infection and vaccination, as well as allergic responses related to allergens or autoimmune responses. Mechanistic studies suggest that chemicals, such as PFOA, can perturb the function of mature B or T lymphocytes by acting at several stages of leukocyte function, including antigen recognition, antigen signaling through the antigen receptor, activation, proliferation, and differentiation (Klaassen, 2013). In mice, PFOA has been shown to diminish the immune response to sheep red blood cells (SRBC), a T cell-dependent antibody response (Section 3.4.2.2), indicating that B and/or T cells can be impacted by PFOA. A review of antigen-specific IgM antibody responses by NTP (2016) indicated that both T cell-independent responses (e.g., immunized with dinitrophenyl (DNP) or trinitrophenyl (TNP)) and T cell-dependent responses were reduced by PFOA.

One study provided evidence that antibody glycosylation patterns could be perturbed by PFOA: Liu et al. (2020b) reported that children with higher levels of serum PFOA had altered levels of N-glycosylation of IgG antibodies, which could perturb normal cell-cell interactions through protein receptors involved in antigen recognition and presentation.

Activation of T cells can be demonstrated by transcriptional changes in the genes that encode cytokines (e.g., IL-2) and cell surface proteins (e.g., IL-2 receptor); however, none of the transcriptomic studies reported significant associations with IL-2 levels and PFOA. Although not significant, one study by Zhu et al. (2016) reported trending reductions in the levels of IL-2 and increased serum PFOA concentrations in male and female asthmatic children.

The effect of PFOA on immunoglobulin classes was evaluated in a study by Zhang et al. (2014a), in which zebrafish were exposed to 0, 0.05, 0.1, 0.5, or 1 mg/L PFOA and immunoglobulin gene expression was quantified in spleens. In contrast to mammals, which have five different classes of immunoglobulin (i.e., IgM, IgA, IgD, IgE, and IgG), zebrafish have three (IgM, IgD, and IgZ). The authors reported a dose-dependent reduction in IgM and nonmonotonic dose responses in IgD and IgZ, where the greatest increases in expression were observed at the middle doses. Another zebrafish study by Zhong et al. (2020) reported a similar inverse U-shaped dose-response curve for IgD after 7 or 14 days of exposure to 0, 0.05, 0.1, 0.5, or 1 mg/L PFOA, but reported that IgZ and IgM were elevated in groups exposed to 0.1 or 0.5 mg/L PFOA. Additionally, the effect of PFOA on gene expression of B cell activating factor (baff) paralleled that of IgD, suggesting that PFOA disrupts immunoglobulin levels by interfering with baff mRNA expression.

Differentiation of B and T cells into mature effector cells can also be affected by PFOA exposure. The cytokine milieu surrounding the T cell and antigen presenting cell (APC) influences the fate of the T cell. In addition to the cytokines mentioned above, fluctuations have been reported in IL-10, IL-5, and IL-4 levels. Associations between PFOA exposure and IL-4 or IL-5 are discussed in relation to allergic and asthmatic responses below. The data on IL-10 is limited to a single developmental study by Hu et al. (2012), which exposed pregnant C57BL/6N mice to 0, 0.02, 0.2, or 2 mg/kg PFOA via gavage and examined cytokine levels in the spleens of male and female PND 21 offspring. In males, IL-10 was reduced by approximately 70% relative to IL-10 released from control animals at every exposure level. In contrast, IL-10 was unaffected in females at every exposure level except for an elevation at 0.02%. IL-10 is released by regulatory T (TReg) cells and function to inhibit macrophage responses, therefore the aforementioned impacts of PFOA on macrophages may be downstream of an effect on TRegs.

The impacts of PFOA on the adaptive immune system may reflect dysregulation of cell-signaling pathways involved in adaptive immune responses. The predominant cell-signaling pathways implicated in PFOA-mediated immunotoxicity include the PPAR and NF-κB signaling pathways, which are both involved in the generation of adaptive immune responses. PPARγ activation is involved in the differentiation and development of TH1, TH2, and NK cells, and inhibits the production of inflammatory cytokines in monocytes (Liang et al., 2021).

Multiple in vitro and in vivo studies have investigated the involvement of the PPAR pathway in PFOA immunotoxicity (Dewitt et al., 2016b; Wang et al., 2014; Yang et al., 2002b). Wang et al. evaluated the effects of PFOA in thymocytes of mice exposed to PFOA (0, 5, 10, or 20 mg/kg/day) via gavage and fed RD or HFD. PFOA upregulated gene expression of PPARα

and PPARγ in the thymus of RD animals at the highest dose and elicited a dose-dependent elevation in PPARγ in the thymus for HFD animals that reached significance at 10 mg/kg group. An additional study using PPARα knockout mice suggested the immunosuppressive effects of PFOA are independent of PPARα (Dewitt et al., 2016b). In this study, female C57BL/6Tac PPARα knockout mice and C57BL/6Tac wild-type mice were exposed to 0, 7.5, or 30 mg/kg/day PFOA in drinking water for 14 days and then injected with SRBC on day 11 (Dewitt et al., 2016b). Exposure to 30 mg/kg/day PFOA for 15 days reduced SRBC-specific IgM antibody responses in both wild-type and PPARα knockout mice by 16% and 14%, respectively. There was no significant difference between genotypes, suggesting that PPARα may not be responsible for the suppression of the immune system induced by PFOA exposure. Interestingly, this study also reported reductions in relative spleen weights (30% reduction after exposure to 30 mg/kg/day PFOA) and thymus weights (55.4% after exposure to 7.5 mg/kg/day PFOA) in the wild-type mice, but not in the knockout mice. Similarly, absolute spleen weights of male Sv/129 PPARα-null mice fed approximately 40 mg/kg/day for 7 days were unaffected by PFOA exposure, whereas in male C57BL/6 wild-type mice, absolute spleen weights were significantly reduced by 39% (Yang et al., 2002b). A significant decrease in absolute thymus weight was observed in PFOA-exposed PPARα-null mice, to a lesser degree compared with the reduction observed in PFOA-exposed wild-type mice (39% reduction in PPARα-null mice and 79% reduction in wild-type mice).

One transcriptomics study in humans reported significant associations between maternal blood levels of PFAS (including PFOA), enrichment of genes in neonatal cord blood samples, and episodes of the common cold and antibody titers against the rubella vaccine in children (Pennings et al., 2016). Enrichment of PPARD in neonatal cord blood samples was correlated with maternal PFAS exposure and later common cold episodes in the children. The NF-κB pathway was proposed to be involved in this phenomenon; a comparison of the transcriptomics to the number of common cold episodes revealed that several genes in the NF-κB pathway were altered.

The NF-κB signaling pathway is essential for many parts and functions of the immune system, including a pro-survival role during lymphopoiesis and regulation of T cell differentiation. Wang et al. (2014) provided indirect evidence that NF-κB pathway stimulation may be involved in PFOA immunotoxicity. Gene expression of the glucocorticoid receptor (GR), which stimulates the NF-κB pathway, was increased in the thymus of PFOA-treated animals at the highest exposure level (20 mg/kg), suggesting mechanisms involving NF-κB pathway stimulation may be involved in PFOA immunotoxicity. Additionally, the authors observed that IL-1B gene expression was elevated in the thymus, suggesting that the NF-κB pathway is not suppressed.

3.4.2.3.2.2 Mechanistic Data Informing Allergic or Asthmatic Responses

Several studies evaluated potential associations between PFOA exposure and allergic responses or asthma. An epidemiological study by Zhu et al. (2016) explored the associations between PFOA exposure and TH1/ TH2 polarization in asthmatic children. Male asthmatic children with higher serum levels of PFOA tended to have higher serum IL-4 and IL-5, evident of a TH2 skew. This association was not observed in females, suggesting that the exacerbation of asthma by PFOA involving TH2 cytokines may be male-specific (Table 3-7).

More detailed mechanistic evidence on the relationship between PFOA and allergic responses is available from animal toxicological studies. A dermal exposure study by Shane et al. (2020) applied 0.5–2 % (w/v; equivalent to 12.5–50 mg/kg) PFOA to the skin of BALB/C mice and evaluated allergic sensitization and IgM response. PFOA did not elicit an irritancy response, suggesting that PFOA is not an allergic sensitizer or dermal irritant. However, the splenic IgM response to SRBC was suppressed after 4 days of exposure to 2% PFOA, implying that T cell-dependent immune responses to dermal allergens may be affected by PFOA. Moreover, mice exposed to PFOA had increased expression of Tslp, which is associated with a polarization toward a TH2 response (Shane et al., 2020). In adult zebrafish, the effect of PFOA exposure on mRNA expression of IL-4 was mixed: it was elevated at most doses tested, but reduced at the highest dose (Zhang et al., 2014a). More data from mammalian models on the associations between IL-4 or IL-10 and PFOA are needed to better understand the potential impacts of PFOA on adaptive immune responses involving T cell subsets.

An in vitro study conducted by Lee et al. (2017a) demonstrated that PFOA increased IL-1β gene and protein expression in a dose-related manner in IgE-stimulated RBL-2H3 cells (a rat basophil cell line). Elevated IL-1β was also observed in a study of human bronchial epithelial cells (HBEC3-KT cells) stimulated with a pro-inflammatory agent, Poly I:C, and then treated with 0.13, 0.4, 1.1, 3.3, or 10 μM PFOA (Sørli et al., 2020).

Several studies have evaluated molecular signaling pathways to better understand the mechanistic underpinnings of allergic or asthmatic responses related to exposure to PFOA. At least four mechanistic studies have evaluated the involvement of the NF-κB signaling pathway, which plays an important role in the regulation of inflammation and immune responses, including expression of pro-inflammatory cytokines (Shane et al., 2020; Zhong et al., 2020; Lee et al., 2017a; Zhang et al., 2014a). Histamine release and mast cell degradation were increased in parallel with increased nuclear localization of NF-κB and concomitant reduction in IκB in IgE-stimulated mast cells, suggesting that allergic immune responses and inflammation are exacerbated by PFOA through a mechanism involving the NF-κB pathway (Lee et al., 2017a). Zhang et al. (2014a) reported that PFOA exposure for 21 days can disrupt the NF-κB pathway to mediate inflammatory cytokines in zebrafish. The authors reported a nonmonotonic dose response in gene expression of the p65 transcription factor in RNA isolated from zebrafish splenocytes. In a more recent study, zebrafish were exposed to PFOA for a shorter period (7 or 14 days) and the authors reported that splenic p65 gene expression was increased in all exposed groups (Zhong et al., 2020). Shane et al. (2020) showed that gene expression of NF-κB (Nfkb1) was reduced in the skin of female BALB/c mice dermally exposed to 1 or 2% PFOA after 14 days. However, the study design did not quantify nuclear NF-κB, so it is difficult to discern whether the NF-κB pathway was activated. The authors also reported that gene expression of PPARα was reduced by more than 50% in female mice dermally exposed to 1% or 2% PFOA for 14 days. Mechanistically, PPARα is known to block the NF-κB pathway and thereby modulate immune responses. These data suggest that the NF-κB pathway activity can be reduced independent of action by PPARα in PFOA-mediated immunotoxicity with respect to allergic responses in the skin.

Table 3-7. Effects of PFOA Exposure on Cytokines Impacting Adaptive Immune Responses

Study	Species or Cell Type	Study Type	Cytokine	Measurement	Significant Change in Cytokine	Relevant Immune response
(Zhu et al., 2016)	Human males and females, GBCA study	Epi	IL-2	serum protein (ELISA)	None	Allergy
			IL-4	serum protein (ELISA)	↑[a]	Allergy
			IL-5	serum protein (ELISA)	↑[a]	Allergy
(Hu et al., 2012)	C57BL/6N mice	Ex vivo	IL-10	IL-10 production assay in CD4 + CD25+ T cells[b]		T_{Reg} responses

Notes: ELISA = enzyme-linked immunosorbent assay; GBCA = Genetic and Biomarkers study for Childhood Asthma; IL-2 = Interleukin 2; IL-4 = Interleukin 4; IL-5 = Interleukin 5; IL-10 = Interleukin 10; T_{Reg} = regulatory T cells.
[a] Males only
[b] Purity of CD4 + CD25+ T cells derived by cell estimate to be 84%–95% based on manufacturer specification for the cell isolation kit.

3.4.2.3.2.3 Mechanistic Data Informing Autoimmune Diseases

Select data on PFOA and autoimmune diseases in humans have been summarized by NTP (2016). NTP's conclusion that PFOA was presumed to be an immune hazard to humans was partially based on the positive associations that exist between PFOA exposure and rheumatoid arthritis, ulcerative colitis, and auto-antibodies specific to neural and non-neural antigens. However, the association was considered *low* confidence by the NTP. No animal or in vitro studies have been identified to inform the potential associations between PFOA and autoimmunity.

3.4.2.3.3 Mechanistic Evidence for PFOA-Mediated Effects on Innate Immune Responses

Neutrophils are important cells of the innate immune system that contribute to inflammation and are the first cells to arrive at the site of injury or infection. Reductions in neutrophil migration to the site of injury have been noted in zebrafish exposed to PFOA (Pecquet et al., 2020), suggesting diminished innate immune responses.

Neutrophil migration occurs in response to inflammation and in response to effector cytokines such as IL-8 released from macrophages, which may also be sensitive to PFOA. Qazi et al. (2010) evaluated liver homogenates from male C57BL/6 mice and found that ex vivo production of TNF-α was significantly decreased in animals treated with 0.002% or 0.005% PFOA. Because macrophages are the major producers of TNF-α, the authors propose that PFOA may directly or indirectly affect specialized hepatic macrophages (e.g., Kupffer cells). The decrease in TNF-α release from macrophages could also be related to PFOA effects on the adaptive immune system, given that macrophage responses are inhibited by IL-10 released by TReg cells. Indeed, Hu et al. (2012) demonstrated that ex vivo release of IL-10 from splenocytes was reduced in male mice. Furthermore, cells of the monocyte/macrophage lineage express PPARα and PPARγ (Zhu et al.,

2016; Braissant and Wahli, 1998), which supports a mechanism for immunosuppression involving macrophages and PPAR pathways.

Rainieri et al. (2017) also conducted an in vitro assessment using TLT cells and found that PFOA led to an increase in relative reactive oxygen species (ROS) production measured via the dichlorodihydrofluorescein diacetate (DCF-DA) assay, indicating that PFOA can induce ROS in macrophages.

Although the innate immune system also includes natural killer (NK) cells, no mechanistic studies were identified that evaluated associations with PFOA. One study by Qazi et al. (2010) reported that there were no significant differences in number or percent of NK cells in isolated hepatic immune cells (IHICs) of mice exposed to 0.002% (w/w) PFOA in the diet for 10 days.

3.4.2.3.4 Mechanistic Evidence for PFOA-Mediated Effects on Intrinsic Cellular Defense Pathways

Zhang et al. (2014a) exposed zebrafish to PFOA (0.05, 0.1, 0.5, and 1 mg/L) for 21 days. After exposure, spleens were analyzed for expression patterns of myeloid differentiation 88 (MyD88) and toll-like receptor 2 (TLR2) as well as several cytokines. In addition to the above-mentioned effects on gene expression of *IL-4*, PFOA exerted dose-dependent effects on IL-1β and IL-21 that were stimulated at a low exposure concentration (0.05 mg/L) and inhibited at higher exposure concentrations (≥0.1 mg/L). The Myd88/NF-κB pathway was found to mediate inflammatory cytokine (IL-1 and IL-21) gene expression in zebrafish spleen. Interestingly, exposure of zebrafish to 1 mg/L PFOA reduced TLR2 mRNA expression in spleen by 56% compared with controls. These findings suggest that exposure to PFOA in zebrafish can activate the NF-κB pathway and interfere with TLR2 expression in a dose-dependent manner to enhance pro-inflammatory cytokine gene expression.

3.4.2.3.4.1 Mechanistic Evidence for PFOA-Mediated Effects on Inflammation

The observed increases in circulating leukocytes (neutrophils and monocytes) of experimental animals (Section 3.4.2.2) are consistent with an inflammatory response. Inflammation is a physiological response to tissue damage or infection that can induce components of the innate and adaptive immune system (Klaassen, 2013). Processes that contribute to inflammation and are affected by PFOA include the complement cascade, release and/or upregulation of pro-inflammatory cytokines, and neutrophil migration.

3.4.2.3.4.1.1 Pro-Inflammatory Responses Including Cytokines

The available mechanistic data support that pro-inflammatory cytokines such as IL-1β, TNF-α, and possibly IL-6 are elevated by PFOA exposure (Table 3-8). However, the effect of PFOA (or lack thereof) for some cytokines varies between model organisms and exposure levels. Altered production and/or release of these cytokines may represent an underlying mechanism of the reductions in innate and/or adaptive immune function that has been reported in the human (Section 3.4.2.1) and animal (Section 3.4.2.2) literature.

Elevation of IL-1β is consistent across study designs in mammalian models in vivo and in vitro. Wang et al. (2014) exposed 4–5-week-old male BALB/C mice to 0, 5, 10, or 20 mg/kg/day PFOA via gavage for 14 days in combination with HFD or RD and measured gene expression of cytokines in the thymus and spleen. In the thymus, IL-1β was elevated in mice exposed to

20 mg/kg/day and fed RD. There were no significant effects in the spleen for mice fed RD at any PFOA concentration. In HFD-fed mice, there was an increase in IL-1β in the spleen for the 10 mg/kg/day PFOA group, but no significant changes at any exposure level in the thymus. Likewise, Lee et al. (2017a) and Sørli et al. (2020) have demonstrated that PFOA elevates IL-1β gene and/or protein expression in various cell lines. In contrast to the consistent increases in IL-1β reported in mammalian models, one study in adult zebrafish reported decreased IL-1β mRNA in the spleen following exposure to 0.1, 0.5, or 1 mg/L PFOA for 21 days (Zhang et al., 2014a). More research is needed to determine whether interspecies differences exist in immunomodulation by PFOA. Elevated production of IL-1β is triggered by activation of the inflammasome, which is an innate immune response known to be activated by xenobiotics, and this mechanism may deserve further investigation (Mills et al., 2013).

Several studies have reported elevated levels of TNF-α during immune responses following exposure to PFOA. Qazi et al. (2010) reported decreased levels of TNF-α in liver homogenates of male C57BL/6 mice orally exposed to 0.002% PFOA for 10 days. Lee et al. (2017a) quantified TNF-α levels in blood from male ICR mice following an active systemic anaphylaxis experiment. Mice were sensitized to ovalbumin on day 0 and day 7 via intraperitoneal (i.p.) injection, and PFOA was orally administered on day 9, 11, and 13. Following ovalbumin challenge (i.p.) on day 14, a dose-dependent increase in TNF-α levels in blood was observed, suggesting PFOA aggravates allergic inflammation. In the same study, in vitro experiments using three independent methods (Western blot, RT-PCR, and ELISA) demonstrated a dose-dependent elevation in TNF-α in RBL-2H3 cells sensitized with anti-DNP IgE, then treated with PFOA for 24 hours. Likewise, an in vitro study by Brieger et al. (2011) observed a slight increase in TNF-α released from peripheral blood mononuclear cells (PBMCs) obtained from the blood of 11 human donors. Not all studies reported positive associations of PFOA and TNF-α. Although Bassler et al. (2019) reported positive associations between serum PFOA levels and IFN-γ, the authors found inverse associations with TNF-α.

A few of the studies that observed increases in IL-1β and TNF-α also evaluated other pro-inflammatory cytokines such as IL-8 and IL-6. The in vitro studies by Lee et al. (2017a) did not find significant effects of PFOA on IL-8 expression. This finding was consistent with those of Sørli et al. (2020) and Bassler et al. (2019). IL6 gene and protein expression were elevated in the study by Lee et al. (2017a), which was consistent with results of Brieger et al. (2011) in human PBMCs stimulated with LPS. Most other studies reported either no effect or inverse associations with IL-6 (Mitro et al., 2020; Shane et al., 2020). Giménez-Bastida et al. (2015) reported that PFOA attenuated the elevation in IL-6 levels that normally follows IL-1β-induction in a human colon cell line (CCD-18Co).

IFN-γ is released from activated T cells and NK cells and induces macrophages to produce a variety of inflammatory mediators and reactive oxygen and nitrogen intermediates that contribute to inflammation (Klaassen, 2013). In general, studies did not find associations between PFOA and changes in IFN-γ. The sole exception by Zhong et al. (2020) reported elevations in IFN gene expression in splenocytes of adult zebrafish exposed to 0.05, 0.1, 0.5, or 1 mg/L PFOA for 7 days. Zhu et al. (2016) reported that children with asthma generally had higher serum PFOA concentrations and lower levels of IFN-γ than non-asthmatic children, but there was not a significant association between IFN-γ and PFOA. Qazi et al. (2010) measured IFN-γ levels secreted from IHICs of 6–8-week-old male C57BL/6 (H-2b) mice that were

exposed to 0 or 0.002% (w/w) PFOA in feed for 10 days. A subgroup of IHIC were stimulated with Concanavalin A, which activates T cells to produce IFN-γ. No PFOA-related differences in IFN-γ production were observed in any group in IHICs. The authors also reported a 37% reduction in hepatic levels of IFN-γ, in parallel with reductions in hepatic levels of IL-4 and TNF-α.

Inflammatory responses can be accompanied by increased levels of the activated pro-inflammatory transcription factor, NF-κB. Sirtuins (SIRTs) have been shown to deacetylate NF-κB, which suppresses its transcriptional activation, thereby inhibiting the production of pro-inflammatory cytokines. Park et al. (2019b) exposed a macrophage cell line (RAW 264.7 cells) to 0, 0.5, 5 or 50 μM PFOA and observed significant increases in expression for SIRT3 and SIRT6 at 5 μM exposure, which is inconsistent with a model where PFOA induces inflammation. Interestingly, SIRT4 and SIRT7 expression was more sensitive to PFOA and exhibited non-linear dose-response curves; SIRT4 was significantly reduced at 0.5 μM and significantly elevated at 5 μM, whereas SIRT7 was significantly elevated at 0.5 μM and significantly reduced at 5 and 50 μM. Altogether, the results support that a pro-inflammatory response of PFOA may not follow a linear dose response.

3.4.2.3.4.1.2 Complement Pathways

PFOA can affect both the innate and adaptive immune system to perturb activation of one of the three main pathways of the complement cascade. A study conducted in the C8 Health Project cohort found that serum biomarkers of PFOA were positively associated with serum C3a levels in men, but negatively associated in women, supporting sex-specific perturbations in immune function (Bassler et al., 2019). Also using data from the C8 Health Project, another group of researchers, Genser et al. (2015) found evidence that PFOA blood levels were negatively associated with blood levels of C-reactive Protein (CRP), which is essential for the classical pathway of complement activation (Klaassen, 2013). However, another human study, that measured CRP as one among several blood biomarkers of cardiometabolic disruption reported that serum PFOA was "generally weakly" (i.e., not significantly) associated with CRP and other biomarkers in women 3 years postpartum (Mitro et al., 2020). In contrast to the human evidence, serum C3 levels were reduced in male C57BL/6 (H-2b) mice exposed to 0.02% w/w PFOA in feed for 10 consecutive days (Botelho et al., 2015). Female mice were not studied. Reduced activities of the classical and alternative complement pathways (reflected by CH50 and AH50 response, respectively) were also reported, supporting that PFOA can disrupt the classical (IgM/IgG dependent) and alternative pathways of complement activation, which both require C3.

Table 3-8. Effects of PFOA Exposure on Pro-Inflammatory Cytokines and Markers of Inflammation

Study	Species or Cell Type	Study Type	Cytokine or Inflammatory Marker	Measurement	Direction of Change Following PFOA Exposure
Mitro et al. (2020)		In vivo	IL-6	blood protein (ELISA)	↑

Study	Species or Cell Type	Study Type	Cytokine or Inflammatory Marker	Measurement	Direction of Change Following PFOA Exposure
	Human females, 3 years post-partum		CRP	blood protein (immunoturbidimetric high-sensitivity assay)	↓
Bassler et al. (2019)	Human males and females, C8 Health Project	In vivo	IL-6	serum protein (Multispot Immunoassay)	None
			TNF-α	serum protein (Multispot Immunoassay)	↓
			IL-8	serum protein (Multispot Immunoassay)	None
			IFNγ	serum protein (Multispot Immunoassay)	↑
			C3a	serum protein (ELISA)	None
Sørli et al. (2020)	Human bronchial epithelial cell line	In vitro	Il-6	culture supernatant protein (ELISA)	None
			IL-1α	culture supernatant protein (ELISA)	None
			IL-1β	culture supernatant protein (ELISA)	↑
			CXCL8	culture supernatant protein (ELISA)	None
Wang et al. (2014)	BALB/c mice	In vivo	IL-1β	Gene expression	↑
Shane et al. (2020)	BALB/c mice	In vivo	IL-1β	Gene expression	↑
			IL-6	Gene expression	None
Qazi et al. (2010)	C57BL/6 mice	Ex vivo	IFN-γ	culture supernatant protein (ELISA)	None

Notes: IL-6 = Interleukin 6; CRP = C-Reactive Protein; TNF-α = Tumor Necrosis Factor α; IL-8 = Interleukin 8; IFNγ = Interferon γ; C3a = cleavage product of Complement 3

3.4.2.3.5 Conclusions

Overall, the available evidence supports that PFOA affects the innate and adaptive immune system as well as immune organ physiology at multiple levels including immune system development, survival, proliferation, and differentiation of B and T cells, inflammatory responses, neutrophil migration, and complement activation. One study provided evidence that antibody glycosylation patterns could be perturbed. Mechanistic data available from in vitro, in vivo, and epidemiological studies were used to evaluate the etiology and mode of action of PFOA-associated immunosuppression and other effects on the immune system. The pleotropic immunomodulatory effects of PFOA, including impaired vaccine responses, may reflect perturbed function of B and/or T cells. At the molecular level, dysregulation of the NF-κB pathway may contribute to the immunosuppressive effects of PFOA. The NF-κB pathway facilitates initial T cell responses by supporting proliferation and regulating apoptosis, participates in the regulation of CD4+ T cell differentiation, and is involved in mediating inflammatory responses. Dysregulation of the NF-κB pathway by PFOA, potentially consequent to the induction of oxidative stress, may be a key component of the mechanism underlying

PFOA-mediated immunosuppression. Reduced NF-κB activation and consequent elevation of apoptosis is consistent with increased apoptosis in multiple cell types, the reduction of pre/pro-B cell numbers, and dysregulation of pro-inflammatory cytokines and mediators of inflammation.

NF-κB activation also facilitates the induction of apoptosis during negative selection of T cells in the thymus, which is essential for the deletion of T cells that recognize self. In contrast, NF-κB acts as a pro-survival factor during the negative selection of B cells. In human studies, PFOA exposure has been associated with autoimmune diseases including ulcerative colitis. Further mechanistic evidence is needed to determine the directionality of the effect of PFOA on NF-κB, which will inform the cell types that predominantly contribute to the etiology of autoimmune diseases associated with PFOA exposure.

3.4.2.4 Evidence Integration

There is *moderate* evidence for an association between PFOA exposure and immunosuppressive effects in human studies based on largely consistent decreases in antibody response following vaccinations (against two different infectious agents: tetanus and diphtheria) in multiple *medium* confidence studies in children (Timmermann et al., 2021; Abraham et al., 2020; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012). Reduced antibody response is an indication of immunosuppression and may result in increased susceptibility to infectious disease. The antibody response results present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFOA were associated with suppression of at least one measure of the anti-vaccine antibody response to common vaccines in two well-conducted (though overlapping) birth cohorts in the Faroe Islands, supported by a *low* confidence study in adults.

The results in human epidemiological studies measuring PFOA concentrations and hypersensitivity were mixed. Significant associations between PFOA exposure and "ever" or "current" asthma were seen primarily in sex- or age-specific subgroups but were null or insignificant in whole study analyses. For allergy and eczema outcomes, results were inconsistent across studies.

The associations between PFOA exposure and human autoimmune disease were also mixed. Two studies (Steenland et al., 2018b; Steenland et al., 2013) found significant associations indicating increased risk of autoimmune disease. Also, PFOA levels were found to be lower in healthy controls compared with cases with MS (Ammitzbøll et al., 2019). Results were most consistent for ulcerative colitis, with significant associations indicating increased risk with increasing PFOA exposure in one *medium* confidence study (Steenland et al., 2013) and one *low* confidence study (Steenland et al., 2018b).

The animal evidence for an association between PFOA exposure and immunosuppressive responses is *moderate* based on 13 *high* or *medium* confidence animal toxicological studies. Short-term and developmental PFOA exposure in rodents resulted in reduced spleen and thymus weights, altered immune cell populations, and decreased splenic and thymic cellularity. In functional assessment of the immune response, PFOA exposure was associated with reduced globulin and immunoglobulin levels (Dewitt et al., 2008; Loveless et al., 2008). Suppression of the immunoglobulin response in these animals is consistent with decreased antibody response seen in human subpopulations.

Mechanistic data related to the human immunomodulatory effects were similarly inconsistent compared with the human epidemiological data. The available mechanistic data indicate that pro-inflammatory cytokines such as IL-1β, TNF-α, and possibly IL-6 are elevated by PFOA exposure. However, the specific effects vary across model organisms and exposure levels. Altered production and/or release of these cytokines may reflect reductions in innate and/or adaptive immune function that has been reported in the human and animal literature.

While evidence exists for reduced antibody response, such as diminished immune response to sheep red blood cells in mice treated with PFOA (a T cell-dependent antibody response), data are limited. Both T cell-dependent and T cell-independent responses are reduced by PFOA, according to a systematic review conducted by the NTP (NTP, 2016). Alterations to these responses could explain the decreased antibody response in humans. Although the evidence is not consistent across studies or between sexes and/or model systems, several studies have reported that PFOA appears to exacerbate allergic immune and inflammatory response, likely through disruption to the NF-κB pathway, increased TNFα, and/or TH2 response.

One proposed mechanism of immunotoxicity involves apoptosis of immune cells, which appears to be a high-dose phenomenon, as evidenced by in vivo and in vitro studies in which the effects were only seen at ≥10 mg/kg/day in mice or 500 mg/L in the human macrophage TLT cell line. Relatedly, NF-κB activation also facilitates the induction of apoptosis during negative selection of T cells in the thymus, which is essential for the deletion of T cells that recognize host cells (i.e., "self"). In contrast, NF-κB acts as a pro-survival factor during the negative selection of B cells. PFOA has been shown to disrupt the NF-κB pathway. At the molecular level, dysregulation of the NF-κB pathway may contribute to the immunosuppressive effects of PFOA. The NF-κB pathway facilitates initial T cell responses by supporting proliferation and regulating apoptosis, participating in the regulation of CD4+ T cell differentiation, and participating in mediating inflammatory responses. Dysregulation of the NF-κB pathway by PFOA, potentially consequent to the induction of oxidative stress, may be a key component of the mechanism underlying PFOA-mediated immunosuppression. Reduced NF-κB activation and consequent elevation of apoptosis is consistent with increased apoptosis in multiple cell types, the reduction of pre/pro B cell numbers, and dysregulation of pro-inflammatory cytokines and mediators of inflammation.

There is conflicting evidence regarding the involvement of PPAR signaling in immunotoxic effects of PFOA: there is evidence of PPAR-independent alterations to adaptive immunity, while suppressive effects of innate immunity appear to involve macrophages and PPAR signaling.

3.4.2.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the *evidence indicates* that PFOA exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans under relevant exposure circumstances (Table 3-9). The hazard judgment is driven primarily by consistent evidence of reduced antibody response from epidemiological studies at median levels as low as 1.1 ng/mL PFOA. The evidence in animals showed coherent immunomodulatory responses at doses as low as 1 mg/kg/day PFOA 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 PFOA 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 PFOA. 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 source of uncertainty in the immunotoxicity database of PFOA.

Table 3-9. Evidence Profile Table for PFOA 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 19 *Medium* confidence studies 8 *Low* confidence studies 3 *Mixed*[a] confidence study	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 mo, age 5 yr (pre-and post-booster), and at age 7 yr. Similarly, antibody levels against rubella (2/2) were significantly decreased in *medium* confidence studies of children. Findings in the five studies examining adults and adolescents were less consistent than in children. Three studies reported inverse associations, one for rubella, one for hepatitis B antibodies and one for influenza A/H3N2, but other antibody responses were inconsistent across	• *High* and *medium* confidence studies the reported effects • *Consistent direction* of effect • *Coherence* of findings across antibody response and increased infectious disease	• *Low* confidence studies • *Imprecision* of findings	⊕⊕⊙ *Moderate* Evidence for immune effects is based on decreases in childhood antibody responses to pathogens such as diphtheria and tetanus. Reductions in antibody response were observed at multiple timepoints in childhood, using both prenatal and childhood exposure levels. Similar decreases in antibody response to other pathogens, such as rubella, were observed, although the number of studies analyzing these antibody responses to these pathogens was limited. 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	*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 PFOA 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 has only 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**	
	all exposure windows. Infectious disease was examined in 14 studies of children. Studies examining infections of the respiratory system observed some positive associations (5/14), although many findings from other studies were not precise. Findings for infectious disease in adults were mixed, with two studies reporting inconsistent results for COVID-19 infections.			evidence of increased risk of asthma, and autoimmune disease, however, the number of studies examining the same type of autoimmune disease was limited.	*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 PFOA. The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental life stages represents a source of uncertainty in the immunotoxicity database of PFOA.
Immune hypersensitivity 1 *High* confidence study 20 *Medium* confidence studies 6 *Low* confidence studies 2 *Mixed*[a] confidence studies	Examination of immune hypersensitivity includes outcomes such as asthma, allergies, and eczema. Increased odds of asthma were reported in most *medium* confidence studies (8/12), 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. Eight studies	• *High* and *medium* confidence studies • *Consistent direction* of effect for asthma across *medium* confidence studies	• *Low* confidence studies • *Inconsistent direction* of effect between subpopulations		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	examined allergies, rhinitis, or rhinoconjunctivitis. Some positive associations (3/8) were observed, although this varied by outcome timing and were at times inconsistent. Significantly increased odds of eczema or atopic dermatitis were observed in several studies (4/13), although these associations were sometimes limited to subgroups (2/4).				
Autoimmune disease 2 *Medium* confidence studies 4 *Low* confidence studies	Increased risk of autoimmune disease was reported in several studies (4/6). One study reported a significantly increased risk of rheumatoid arthritis, and two studies reported a significantly increased risk of ulcerative colitis. Two studies reported positive associations for multiple sclerosis, with one reaching significance in men. One study (1/2) observed increased risk of celiac disease among female children and young adults. Findings	• *Medium* confidence studies	• *Low* confidence studies • *Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	for Crohn's disease were less consistent.				
Evidence from In Vivo Animal Toxicological Studies (Section 3.4.2.2)					
Organ weights 3 *High* confidence studies 7 *Medium* confidence studies	Decreases in absolute (6/8) and relative (4/8) spleen weights and in absolute (5/5) and relative (3/5) thymus weights were observed across studies regardless of study design. Overall, decreases in spleen and thymus weights were more frequently observed in males than females and tended to coincide with reductions in body weight.	• *High* and *medium* confidence studies • *Dose-response* relationship seen within multiple studies • *Coherence* of findings of other immunological endpoints	• *Inconsistent direction* of effects across sex • *Confounding variables* such as decreases in body weights	⊕⊕⊙ *Moderate* Evidence is based on 13 *high* or *moderate* confidence animal toxicological studies. Short-term and developmental PFOA exposure in rodents resulted in reduced spleen and thymus weights, altered immune cell populations, and decreased splenic and thymic cellularity. In functional assessments of the immune response, PFOA exposure was associated with reduced globulin and immunoglobulin levels. Suppression of the immunoglobulin response in these animals is consistent with decreased antibody response seen in human subpopulations.	
Immune cellularity 1 *High* confidence study 4 *Medium* confidence studies	Of the studies that measured circulating WBCs and differentials, one short-term study in male mice found decreases in WBC counts, while a chronic rat study observed transient increases in males that were attributed to increased counts of lymphocytes and neutrophils. One short-term study in male rats and mice reported increased neutrophils and monocytes, decreased	• *High* and *medium* confidence studies • *Dose-response* relationship seen within multiple studies • *Coherence* of findings	• *Inconsistent direction* of effects across species, sex, and study design • *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	eosinophils, as well as reduced splenocytes and thymocytes in mice but no changes in rats. One developmental study in mice observed decreases in splenic regulatory T cells in males and females.				
Globulins and immunoglobulins 2 *High* confidence studies 2 *Medium* confidence studies	Mixed results were reported for concentrations of globulins and immunoglobulins. Decreased globulin levels (2/3) were observed in male and female rats, in a dose-dependent manner (1/3), following short-term and chronic exposure to PFOA. One short-term study reported increased globulins (1/3) in male mice. Additional findings, including increases in IgA, IgG, and IgM, were found in male mice.	• *High* and *medium* confidence studies • *Dose-response* relationship	• *Inconsistent direction* of effects between species • *Limited number* of studies examining specific outcomes		
Immune response 4 *Medium* confidence studies	Dose-dependent decreases in IgM following a SRBC or KLH challenge was seen in three short-term studies in mice (3/4).	• *Medium* confidence studies • *Dose-response* relationship seen within multiple studies	• *Inconsistent direction* of effects across study design and species • *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	No changes in IgM were observed in chronically exposed male rats nor developmentally exposed female mice (2/4). In a short-term study that assessed female mice, increased IgG levels were observed after a SRBC challenge (1/2), but a developmental study in female mice found no changes in IgG levels (1/2).				
Histopathology 3 *High* confidence studies 2 *Medium* confidence studies	A short-term study in male mice and rats reported increased incidence of granulocytic hyperplasia of the bone marrow and increased incidence of splenic and thymic atrophy in mice but not rats. One *high* confidence short-term study in male and female rats observed no changes in the spleen, thymus, or lymph nodes but found increased bone marrow hypocellularity in male rats. One chronic study found decreased incidence of splenic hemosiderosis in male and female rats. One	• *High* and *medium* confidence studies • *Coherence* of findings	• *Limited number* of studies examining specific outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	chronic and one developmental study observed histopathological changes in the spleen, thymus, bone marrow, and/or lymph nodes of male and female rats.				

Mechanistic Evidence and Supplemental Information (Section 3.4.2.3.4)	
Summary of Key Findings, Interpretation, and Limitations	**Evidence Stream Judgment**
Key findings and interpretation: • Apoptosis of immune cells is a high dose immunotoxic phenomenon that has been observed in both in vivo and in vitro studies of PFOA. • Disruption of the NF-κB signaling pathway, which is involved in T cell responses, regulation of apoptosis, and inflammatory response, has been demonstrated both directly and indirectly in in vivo human and animal data, as well as in vitro. • Inconsistent evidence of exacerbation of allergic immune and inflammatory responses via NF-κB pathway, increased TNFα, and/or T_H2 response. **Limitations:** • Inconsistent findings between sexes, model systems, and studies regarding allergic immune response. • Limited database for immune response data. • While PPARα is mechanistically linked to immune signaling (blocking the NF-κB pathway), it is not clear if PFOA-induced alterations to PPARα are involved in immunomodulatory effects: some PPARα-knockout mouse studies have suggested that immunomodulation occurs independent of PPARα.	Findings support plausibility that PFOA exposure can lead to dysregulation of signaling pathways related to immune response; however, data have inconsistencies.

Notes: HFMD = hand, foot, and mouth disease; A/H3N2 = influenza A virus subtype H3N2; COVID-19 = coronavirus disease 2019; WBC = white blood cells; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; SRBC = sheep red blood cells; KLH = keyhole limpet hemocyanin; NF-κB = nuclear factor kappa B; TNFα = tumor necrosis factor alpha; T_H2 = T helper 2; PPARα = peroxisome proliferator-activated receptor alpha.

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

3.4.3 Cardiovascular

EPA identified 112 epidemiological and 10 animal toxicological studies that investigated the association between PFOA and cardiovascular effects. Of the 54 epidemiological studies addressing cardiovascular endpoints, 3 were classified as *high* confidence, 28 as *medium* confidence, 14 as *low* confidence, 5 as *mixed* (1 *high/medium* and 4 *medium/low*) confidence, and 4 were considered *uninformative* (Section 3.4.3.1). Of the 89 epidemiological studies addressing serum lipid endpoints, 1 was classified as *high* confidence, 29 as *medium* confidence, 32 as *low* confidence, 19 as *mixed* (1 *high/medium* and 18 *medium/low*) confidence, and 8 were considered *uninformative* (Section 3.4.3.1). Of the animal toxicological studies, three were classified as *high* confidence, five as *medium* confidence, and two were considered *low* confidence (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 build-up inside arteries and hardening and narrowing of their walls), microvascular disease, markers of inflammation (e.g., C-reactive protein), and mortality. These health outcomes are interrelated – IHD is caused by decreased blood flow through coronary arteries due to atherosclerosis resulting in myocardial ischemia. 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).

There are seven epidemiological studies from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and cardiovascular effects. Study quality evaluations for these seven studies are shown in Figure 3-30. Results from studies summarized in the 2016 PFOA HESD are described in Table 3-10 and below.

The 2016 PFOA HESD (U.S. EPA, 2016c) did not identify strong evidence for an association between CVD and PFOA, based on five occupational studies. Several occupational studies examined cardiovascular-related cause of death among PFOA-exposed workers at the West Virginia Washington Works plant (Steenland and Woskie, 2012; Sakr et al., 2009; Leonard et al., 2008) and the 3M Cottage Grove plant in Minnesota (Raleigh et al., 2014; Lundin et al., 2009; Gilliland and Mandel, 1993). This type of mortality is of interest because of the relation between lipid profiles (e.g., LDL) and the risk of CVD. A study in West Virginia did not find an association between cumulative PFOA levels and IHD mortality across four quartiles of

cumulative exposure (Steenland and Woskie, 2012). On the basis of these data from the worker cohorts (part of the C8 Health Project), the C8 Science Panel (2012b) concluded that there is no probable link between PFOA and stroke and CAD. A later study of community residents from the C8 Health Project reported an elevated risk of stroke in quintiles 2 through 4 of PFOA concentrations compared with quintile 1 (HRs ranging 1.36 to 1.45); however, the association was null in continuous analyses (HR, linear = 1.00, 95% CI: 0.99, 1.01) (Simpson et al., 2013). Study authors reported a significant increased risk (HR, linear = 1.10, 95% CI: 1.02, 1.18) after excluding the highest quintile of exposure. The analysis of the workers at the Minnesota plant also did not observe an association between cumulative PFOA exposure and IHD risk, but an increased risk of cerebrovascular disease mortality was seen in the highest exposure category (Lundin et al., 2009). These studies are limited by the reliance on mortality (rather than incidence) data, which can result in a substantial degree of under ascertainment and misclassification. Evidence was limited in studies on the general population, with only one high-exposure community study and two NHANES studies examining the association between PFOA and hypertension risk. Increased risk of hypertension was observed in a C8 community study (Winquist and Steenland, 2014); however, the association was imprecise for estimates comparing the highest two quintiles to the lowest quintile of exposure. One NHANES study identified in the 2021 ATSDR *Toxicological Profile for Perfluoroalkyls* (ATSDR, 2021) observed a large increased risk of hypertension for adults not using hypertensive medication in the highest exposure quartile (Min et al., 2012). The other NHANES study reported a decreased risk of hypertension in children (Geiger et al., 2014b).

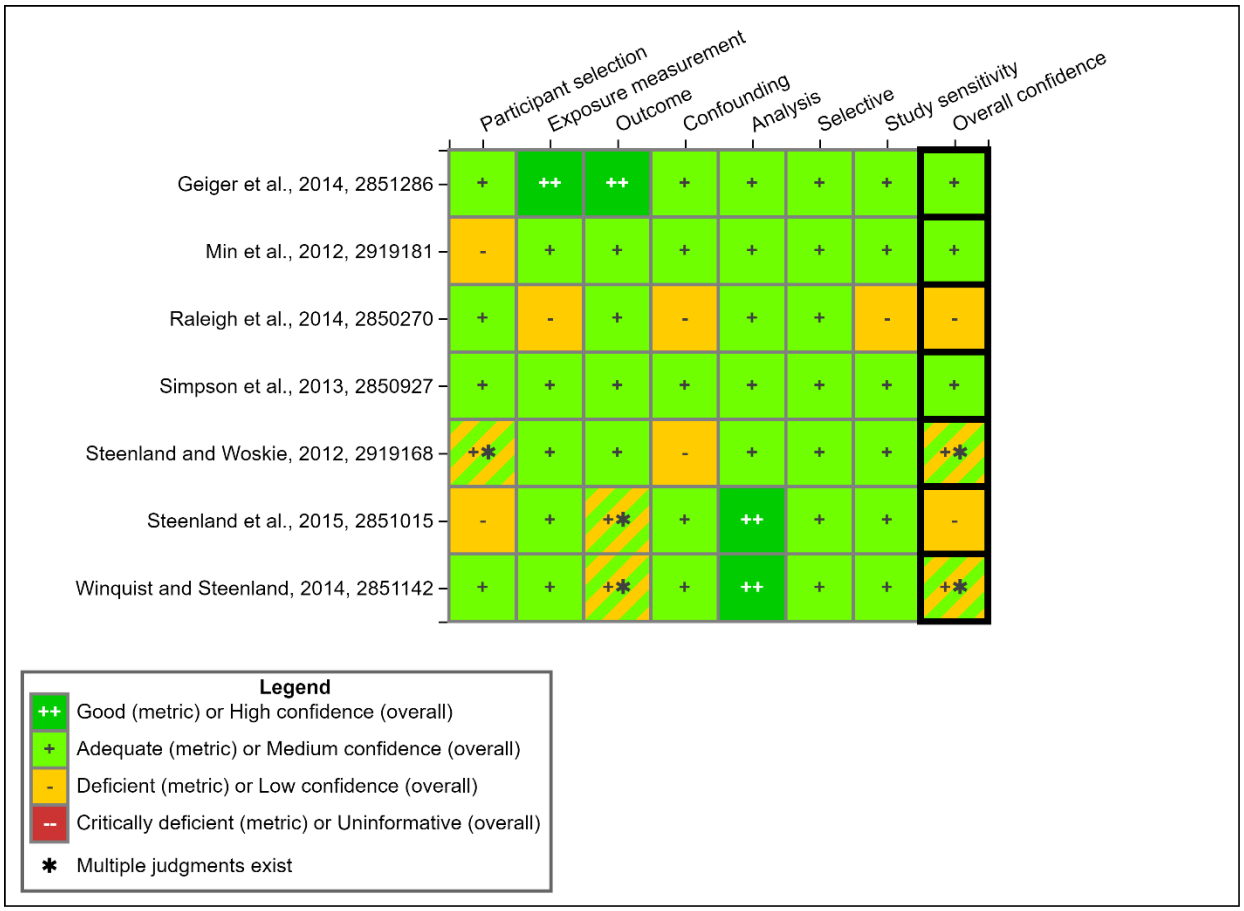


Figure 3-30. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cardiovascular Effects Published Before 2016 (References from 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Table 3-10. Associations Between Elevated Exposure to PFOA and Cardiovascular Outcomes from Studies Identified in the 2016 PFOA HESD

Reference, Confidence	Study Design	Population	SBP[a]	DBP[a]	Hypertension[b]	Stroke[b]	CHD, IHD, CAD[b]
Geiger et al., 2014, 2851286 *Medium*	Cross-sectional	Children	↓	–	–	NA	NA
Min, 2012, 2919181	Cross-sectional	Adults	NA	NA	↑↑	NA	NA
Raleigh et al., 2014, 2850270[c]	Cohort	Occupational	NA	NA	NA	NA	–
Steenland and Woskie, 2012, 2919168[d] *Mixed*[e]	Cohort	Occupational	NA	NA	NA	–	–
Simpson, 2013, 2850927 *Medium*	Cohort	Adults and Occupational	NA	NA	NA	↑	NA
Steenland, 2015, 2851015 *Low*	Cohort	Occupational	NA	NA	–	↑	–
Winquist and Steenland, 2014, 2851142 *Mixed*[f]	Cohort	Occupational	NA	NA	↑	NA	–

Notes: SBP = systolic blood pressure; DBP = diastolic blood pressure; CHD = coronary heart disease; IHD = ischemic heart disease; CAD = coronary heart disease; ↑ = nonsignificant positive association; ↑↑ = significant positive association; ↓ = nonsignificant inverse association; ↓↓ = significant inverse association; – = no (null) association; NA = no analysis was for this outcome was performed.

[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] Gilliland, 1993, 1290858 and Lundin, 2009, 1291108 report overlapping data with Raleigh, 2014, 2850270, which was considered the most updated data.

[d] Leonard, 2008, 1291100 and Sakr, 2009, 2593135 report overlapping data with Steenland and Woskie, 2012, 2919168, which was considered the most updated data.

[e] Steenland and Woskie, 2012, 2919168 was rated *medium* confidence for comparisons with the DuPont referent population and *low* confidence for comparisons with the U.S. population.

[f] Winquist and Steenland, 2014, 2851142 was rated *medium* confidence for hypertension and *low* confidence for coronary heart disease.

Since publication of the 2016 PFOA HESD (U.S. EPA, 2016c), 48 new epidemiological studies report on the association between PFOA and CVD, including outcomes such as hypertension, CAD, congestive heart failure (CHF), microvascular diseases, and mortality. Of these, 21 examined blood pressure or hypertension in adults. Pregnancy-related hypertension is discussed in the Appendix (U.S. EPA, 2024a). Two of the publications (Girardi and Merler, 2019; Steenland et al., 2015) were occupational studies and the remainder were conducted on the general population. Six general population studies (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) were 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., 2021; Papadopoulou et al., 2021; Lin et al., 2020c; Mitro et al., 2020; Donat-Vargas et al., 2019; Girardi and Merler, 2019; Warembourg et al., 2019; Fry and Power, 2017; Manzano-Salgado et al., 2017b; Matilla-Santander et al., 2017; Steenland et al., 2015), one case-control study (Mattsson et al., 2015), and 35 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., 2020e; 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; He et al., 2018; 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; Shankar et al., 2012). 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 the Diabetes Prevention Program (DPP) and Outcomes Study (DPPOS) (Osorio-Yáñez et al., 2021; Cardenas et al., 2019) and weight loss in Prevention of Obesity Using Novel Dietary Strategies Lost (POUNDS-Lost) Study (Liu et al., 2018b). Thus, these studies can be interpreted as cohort studies for evaluating cardiovascular risk purposes.

The studies were conducted in different study populations with the majority of studies conducted in the United States (Koskela et al., 2022; Li et al., 2021; 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; He et al., 2018; 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; Steenland et al., 2015; Shankar et al., 2012). 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., 2020e; 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), Italy (Canova et al., 2021; Zare Jeddi et al., 2021; Pitter et al., 2020; Girardi and Merler, 2019), 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 PFOA in blood components (i.e., serum or plasma) with three studies measuring levels in maternal serum (Li et al., 2021; 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 48 epidemiological studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and cardiovascular effects. Study quality evaluations for these 48 studies are shown in Figure 3-31, Figure 3-32, and Figure 3-33.

Of the 48 studies identified since the 2016 assessment, 3 studies were *high* confidence, 26 were *medium* confidence, 12 were considered *low* confidence, 3 were considered *mixed* confidence, and 4 studies were 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 potential for residual confounding or selection bias (e.g., unequal recruitment and participation among subjects with outcome of interest, lack of consideration and potential exclusion due to medication usage). Residual confounding was possible due to SES, which can be associated with both exposure and the cardiovascular outcome. Although PFOA has a long half-life in the blood, concurrent measurements may not be appropriate for cardiovascular effects with long latencies. Further, temporality of PFOA exposure could not be established for several *low* confidence studies due to their cross-sectional design. Several of the *low* confidence studies also had sensitivity issues due to limited sample sizes (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 PFOA as the dependent variable, resulting in both studies being considered *uninformative* (Jain, 2020a, b). Analyses treating PFOA as a dependent variable support inferences for characteristics (e.g., kidney function, disease status, race/ethnicity) that affect PFOA levels in the body, but it does not inform the association between exposure to PFOA and incidence of cardiovascular disease. Small sample size (n = 45) and missing details on exposure measurements were the primary concerns about the remaining *uninformative* study (Leary et al., 2020).

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

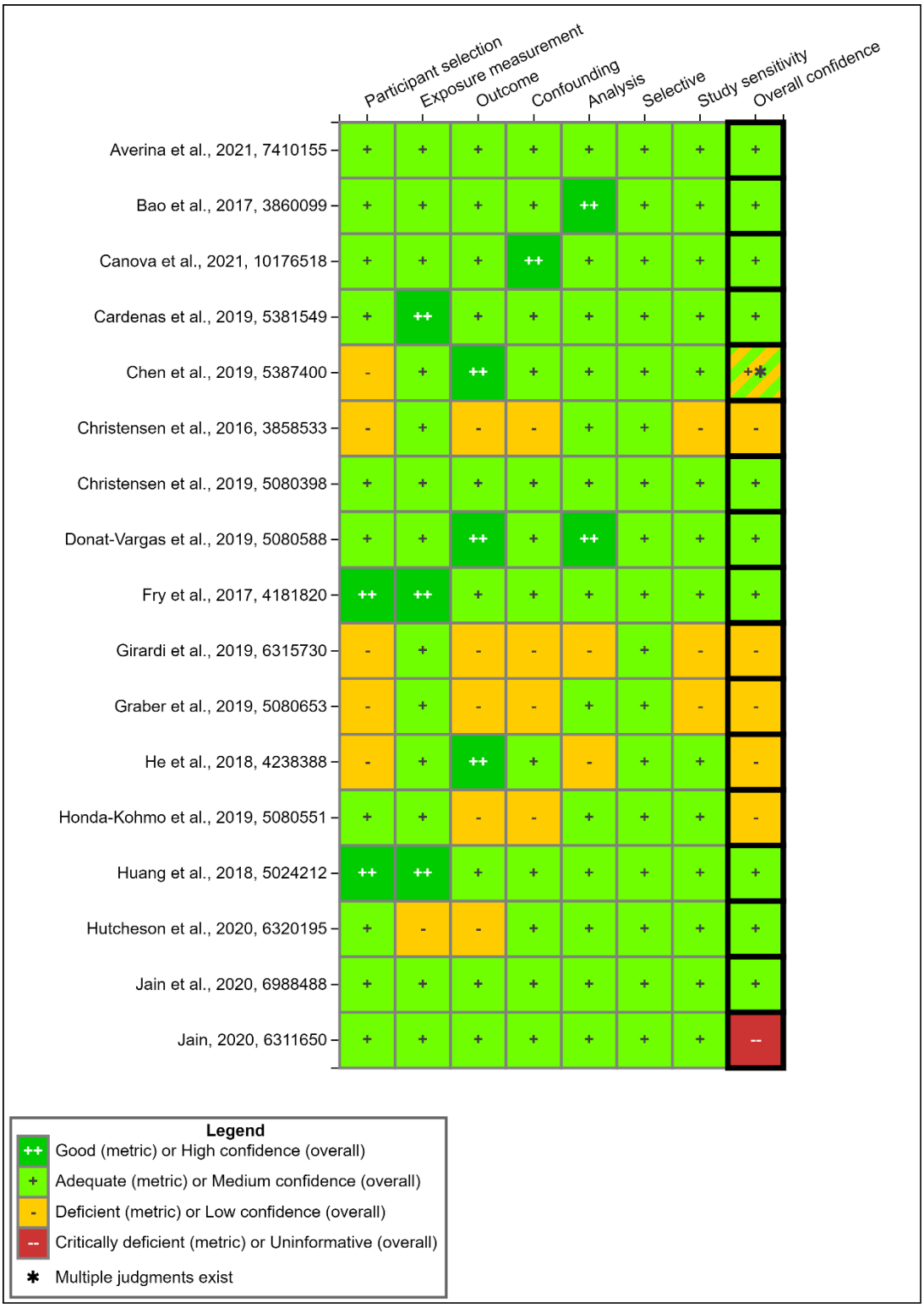


Figure 3-31. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

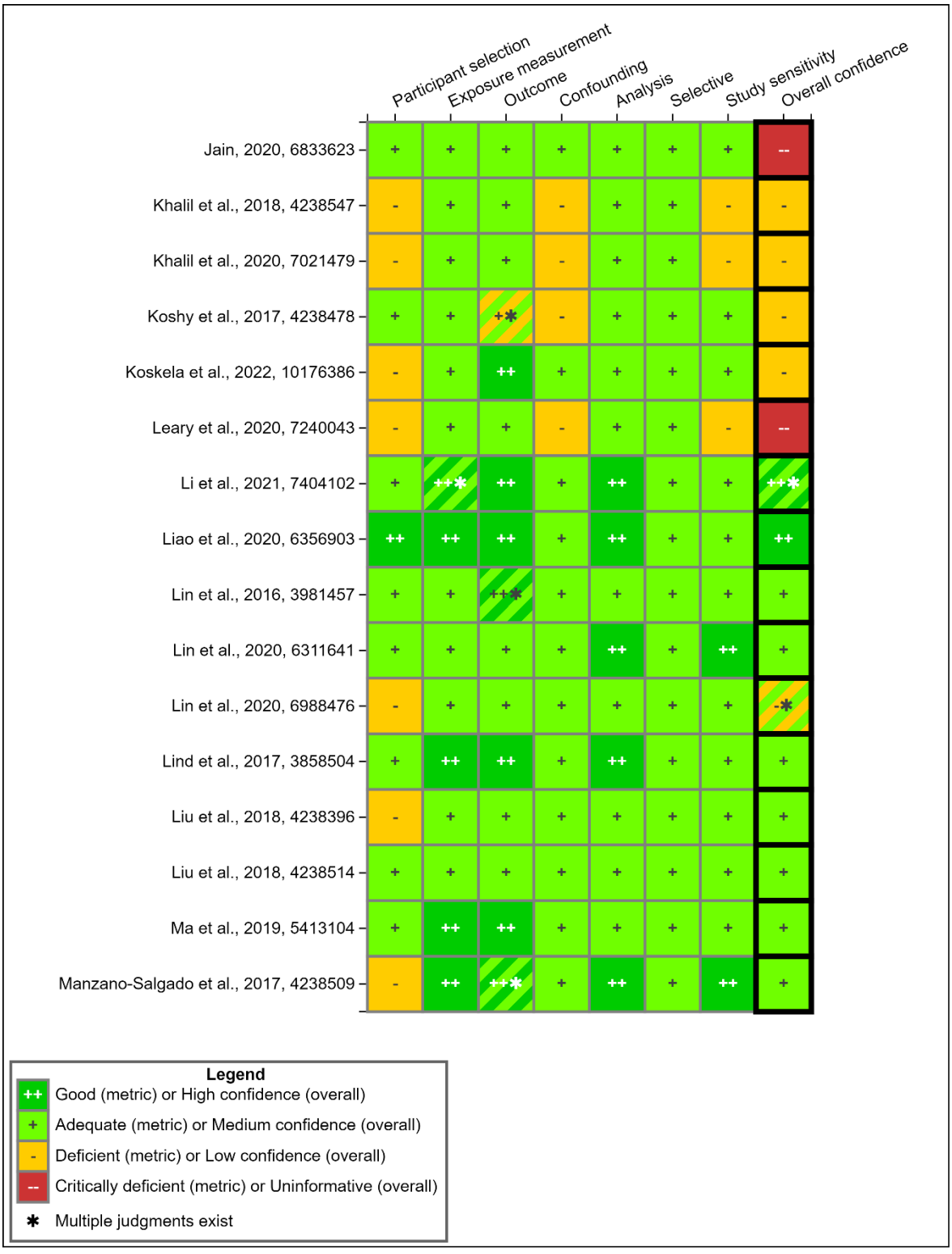


Figure 3-32. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cardiovascular Effects (Continued)

Interactive figure and additional study details available on HAWC.

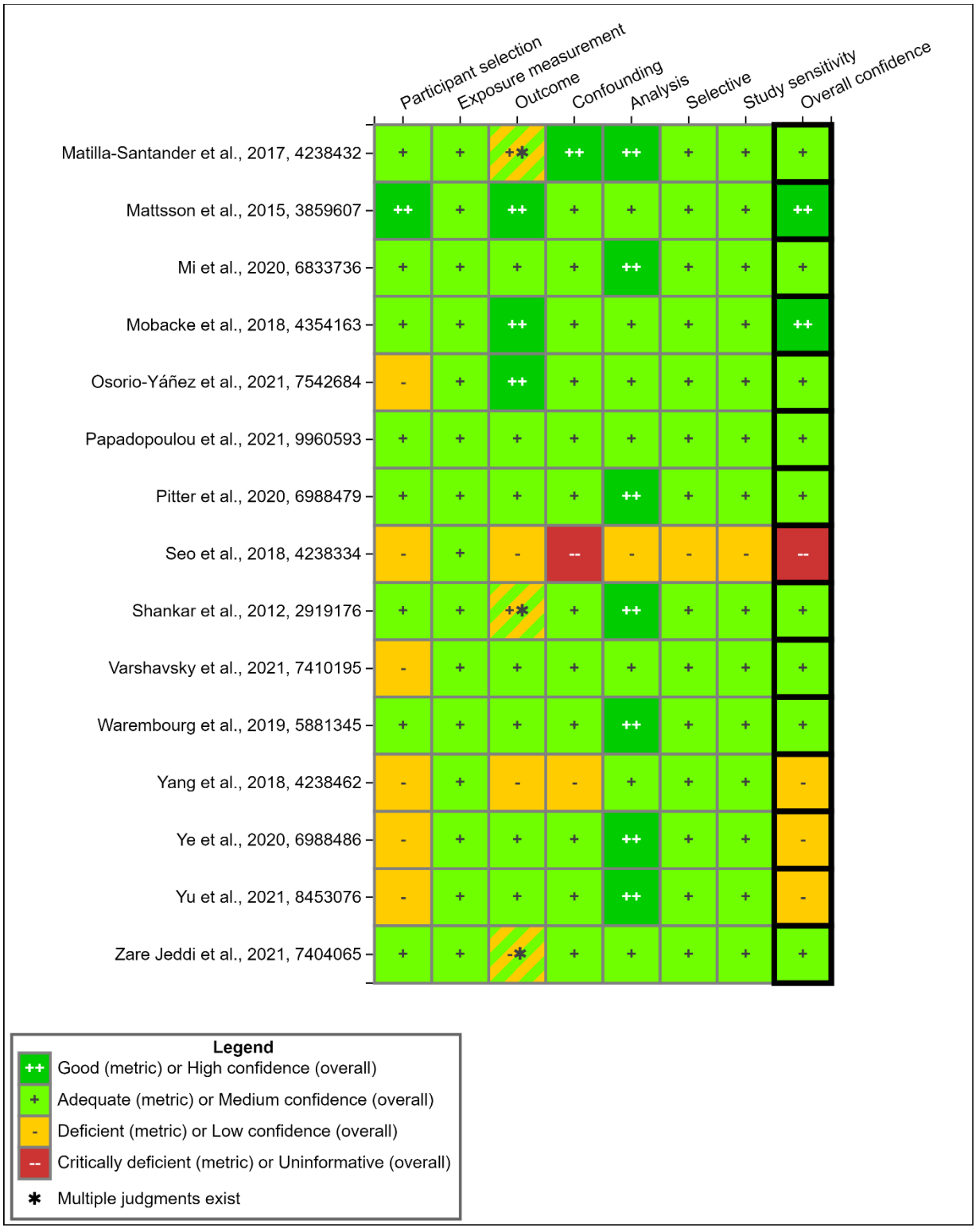


Figure 3-33. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Cardiovascular Effects (Continued)

Interactive figure and additional study details available on HAWC.

3.4.3.1.1.3 Findings From Children and Adolescents

One *high* confidence study (Li et al., 2021) and six *medium* confidence studies (Averina et al., 2021; Canova et al., 2021; Papadopoulou et al., 2021; Ma et al., 2019; Warembourg et al., 2019; Manzano-Salgado et al., 2017b) examined blood pressure in children and adolescents and reported no associations (see Appendix, (U.S. EPA, 2024a)). No association was observed in a *high* confidence study in infants from the Health Outcomes and Measures of the Environment (HOME) Study (Li et al., 2021) between PFOA in maternal serum and child blood pressure measured at 12 years of age. In a cross-sectional analysis, Ma et al. (2019) did not observe an association between serum PFOA and blood pressure among 2,251 NHANES (2003–2012) participants (mean age 15.5 years). Similarly, Manzano-Salgado et al. (2017b) did not observe an association between maternal PFOA and child blood pressure in combined or in gender-stratified analyses at age 4 and 7 years.

In a cohort of 1,277 children (age 6–11 years), PFOA measured both 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 (Warembourg et al., 2019). However, the association was significantly positive for systolic blood pressure (SBP) after co-adjustment for organochlorine compounds (i.e., dichlorodiphenyldichloroethane (DDE) and hexachlorobenzene (0.9; 95% CI: 0.1, 1.6; p = 0.021)). An overlapping study (Papadopoulou et al., 2021) examined the association for z-scores of blood pressure in children in a model mutually adjusted for other PFAS and did not find an association. In a cross-sectional study of children and adolescents in a high-exposure community (Canova et al., 2021), blood pressure was lower among adolescents with increasing serum PFOA, but none of the associations reached significance. An increased risk of hypertension (SBP ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg) was observed in a *medium* confidence cross-sectional study (Averina et al., 2021) on Norwegian adolescents taking part in the Fit Futures. The magnitude of the association was larger among increasing quartiles of PFOA exposure, reaching significance for those in the fourth quartile of exposure (OR: 2.08; 95% CI: 1.17, 3.69, p = 0.013). Two *low* confidence studies did not observe associations between serum PFOA and blood pressure (Khalil et al., 2018; Lin et al., 2013).

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

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, (U.S. EPA, 2024a)). A total of 15 studies examined PFOA in association with SBP, diastolic blood pressure (DBP), hypertension, and elevated blood pressure (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; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Yang et al., 2018; Bao et al., 2017; Christensen et al., 2016).

Of the 10 studies that examined blood pressure as a continuous measure, six reported statistically significant positive associations (Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Pitter et al., 2020; Liu et al., 2018b; Yang et al., 2018; Bao et al., 2017). However, the results were not always consistent between SBP and DBP.

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

A *high* confidence study (Mitro et al., 2020) conducted among 761 women that examined associations between PFOA concentrations measured during pregnancy and blood pressure assessed at 3 years post-partum reported a positive but nonsignificant association with SBP (β per doubling of PFOA: 0.8; 95% CI: −0.3, 1.8). No association was observed with DBP.

Two *medium* confidence cross-sectional studies with overlapping data from the "Isomers of C8 Health Project," a highly exposed population of Shenyang, China (Mi et al., 2020; Bao et al., 2017), also reported positive associations for blood pressure. In 1,612 participants with elevated PFOA levels (median 6.19 ng/mL), Bao et al. (2017) reported large increases in DBP (β: 2.18; 95% CI: 1.38, 2.98) and SBP (β: 1.69; 95% CI: 0.25, 3.13). After stratification by sex, a positive association was observed in men only for DBP (β: 1.48; 95% CI: 0.58, 2.37) and in women only for SBP (β: 6.65; 95% CI: 4.32, 8.99). In participants with high PFOA levels (median 4.8 ng/mL), Mi et al. (2020) observed statistically significant increases in DBP (β: 1.49; 95% CI: 0.34, 2.64). No association was observed for SBP.

Similar findings were observed in another *medium* confidence study in a high-exposure community in Italy (Pitter et al., 2020). Adults (20–39 years old) included in a regional (i.e., Vento Region) surveillance program were included in a cross-sectional analysis of blood pressure and PFOA exposure. Significant positive associations were reported for DBP (β: 0.34; 95% CI: 0.21, 0.47) and SBP (β: 0.37; 95% CI: 0.19, 0.54) in the overall (n = 15,380) population. Results were generally consistent after stratification by sex. Minor sex differences were observed, such as slightly larger increases in SBP among men (β: 0.46; 95% CI: 0.19, 0.73) and larger increases in DBP among women (β: 0.39; 95% CI: 0.21, 0.57). Monotonic trends were observed in all quartile analyses, although significance was not reported.

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

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

for SBP (Chen et al., 2019; He et al., 2018), and three studies reported no associations for DBP (Chen et al., 2019; He et al., 2018; Yang et al., 2018).

Of the 11 studies that examined risk of elevated blood pressure (hypertension), six reported statistically significant associations (Ye et al., 2021; Liao et al., 2020; Lin et al., 2020c; Mi et al., 2020; Pitter 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. Using a generalized additive model and restricted cubic splines, Liao et al. (2020) reported a non-linear (J-shaped) relationship with hypertension, with the inflection point of PFOA at 1.80 ng/mL. Each 10-fold increase in PFOA was associated with a 44% decrease (OR: 0.56; 95% CI: 0.32, 0.99) in the risk of hypertension on the left side of the inflection point, and an 85% increase (OR: 1.85; 95% CI: 1.34, 2.54) on the right side of the inflection point. A significant association with hypertension was observed for the highest (>4.4 ng/mL) versus lowest (≤2.5 ng/mL) tertile (OR: 1.32; 95% CI: 1.13, 1.54), and the test for trend was significant ($p < 0.001$). Additionally, positive associations were observed among women (OR: 1.42; 95% CI: 1.12, 1.79) and in participants 60 years and older (OR: 1.32; 95% CI: 1.03, 1.68). The studies (Ye et al., 2021; Mi et al., 2020; Bao et al., 2017) with overlapping data on highly exposed Isomers of C8 Health Project participants reported significant associations. An overlapping *low* confidence study (Ye et al., 2021) on metabolic syndrome observed a moderate increase (OR: 1.31; 95% CI: 1.11, 1.56) in the risk of elevated blood pressure (SBP ≥130 and/or DBP ≥85; or medication use). Mi et al. (2020) reported higher risk of hypertension overall (OR: 1.72; 95% CI: 1.27, 2.31) and among women (OR: 2.32; 95% CI: 1.38, 3.91), but not in men. Bao et al. (2017) did not observe an association between total PFOA and hypertension. However, in isomer-specific analysis, a natural-log unit (ng/mL) increase of 6-m-PFOA was significantly associated with higher risk of hypertension among all participants (OR: 1.24; 95% CI: 1.05, 1.47) and among women (OR: 1.86; 95% CI: 1.25, 2.78). These results suggest branched PFOA isomers have a stronger association with increased risk of hypertension compared with linear isomers (n-PFOA).

Increased risk of hypertension was observed in a pair of overlapping studies on another high exposure community located in Italy (Zare Jeddi et al., 2021; Pitter et al., 2020). Pitter et al. (2020), a *medium* confidence study, observed a significant association (OR: 1.06; 95% CI: 1.01, 1.12) between PFOA exposure and hypertension in a large cross-sectional sample of adults (n = 15,786). The association remained significant in men (OR: 1.08; 95% CI: 1.02, 1.15), but was not significant in women (OR: 1.06; 95% CI: 0.97, 1.15). A similar increased risk of hypertension was observed among all participants in the overlapping study (Zare Jeddi et al., 2021).

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

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

Among the four studies that examined CHD, the findings were mixed. A *high* confidence study (Mattsson et al., 2015), a *medium* confidence study of 10,850 NHANES participants from 1999–2014 (Huang et al., 2018), and a *low* confidence study (Christensen et al., 2016) all reported no associations with CHD. A *low* confidence study from the C8 Health Project (Honda-Kohmo et al., 2019) reported a significant inverse association between PFOA and CHD among adults with and without diabetes. However, study limitations that may have influenced these findings include the reliance on self-reporting of a clinician-based diagnosis for CHD outcome classification and residual confounding by SES.

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

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

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

In addition, a *low* confidence study of adults and children did not observe an association between serum PFOA and self-reported cardiovascular conditions, including high blood pressure, CAD, and stroke (Graber et al., 2019). However, potential selection bias is a major concern for this study owing to the recruitment of volunteers who already knew their PFAS exposure levels and were motivated to participate in a lawsuit.

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

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

One *medium* confidence study (Osorio-Yáñez et al., 2021) examined changes in atherosclerotic plaque in a sample of participants enrolled in the Diabetes Prevention Program. A nonsignificant

positive association (OR: 1.17; 95% CI: 0.91, 1.50) was observed for the odds of having a mild to moderate coronary artery calcium Agatston score (11– 400). Two studies examined changes in heart structure (Mobacke et al., 2018) and carotid atherosclerosis (Lind et al., 2017b) in participants 70 years and older. Mobacke et al. (2018) examined alterations of left ventricular geometry, a risk factor for CVD, and reported that serum PFOA was significantly associated with a decrease in relative wall thickness (β: −0.12; 95% CI: −0.22, −0.001; p = 0.03), but PFOA was not observed to be associated with left ventricular mass or left ventricular end diastolic diameter. Lind et al. (2017b) examined markers of carotid artery atherosclerosis including atherosclerotic plaque, the intima-media complex, and the CIMT (a measure used to diagnose the extent of carotid atherosclerotic vascular disease) and observed no associations.

The association between exposure to PFOA and apolipoprotein B, a protein associated with LDL and increased risk of arthrosclerosis, was examined in a *medium* confidence study (Jain, 2020b) on NHANES participants (2007–2014). Serum apolipoprotein B was significantly increased (β per log10-unit increase PFOA: 0.03878; $p < 0.01$) with increasing PFOA exposure in non-diabetic participants who did not take lipid-lowering medication. No significant associations were observed among lipid-lowering medication users and participants with diabetes. No association between PFOA and C-reactive protein levels (a risk factor for CVD) were observed in two studies, one in women from Project Viva (Mitro et al., 2020) and the other in pregnant women from the Spanish Environment and Childhood (Infancia y Medio Ambiente, INMA) study (Matilla-Santander et al., 2017). One *medium* confidence study examined mortality due to heart/cerebrovascular diseases in 1,043 NHANES (2003–2006) participants 60 years and older and observed no associations (Fry and Power, 2017).

Overall, the findings from one *high* confidence study and several *medium* confidence studies conducted among the general population provide consistent evidence for an association between PFOA and blood pressure. The evidence for an association between PFOA and increased risk of hypertension/elevated blood pressure, overall and in gender-stratified analyses was inconsistent. Evidence for other CVD-related outcomes was more limited, and similarly inconsistent.

3.4.3.1.1.5 Findings From Occupational Studies

Two *low* confidence studies examined occupational PFOA exposure and cardiovascular effects (see Appendix, (U.S. EPA, 2024a)). Steenland et al. (2015) examined 1,881 workers with high serum PFOA levels (median 113 ng/mL) from a subset of two prior studies conducted by the C8 Science Panel. No trend was observed in the exposure-response gradient for stroke, CHD, and hypertension and. In analysis of PFOA levels by quartiles, a significantly higher risk of stroke (no lag) was observed for the 2nd quartile versus the 1st quartile (Rate Ratio (RR): 2.63; 95% CI: 1.06, 6.56). No association was observed with 10-year lag stroke, CHD, and hypertension, respectively. For the assessment of stroke, this study had *low* confidence because of concerns for selection bias, specifically survival bias. For other chronic diseases examined, this study is of *low* confidence due to concerns about outcome misclassification, particularly for hypertension due to lack of medical record validation. In another occupational study of 120 male workers with very high PFOA serum levels (GM: 4,048 ng/mL), Girardi et al. (2019) reported no association with increased risk of mortality due to cardiovascular causes, including hypertensive disease, ischemic heart disease, stroke, and circulatory diseases. However, the potential for selection bias, outcome misclassification, and limited control for confounding may have influenced the reported results.

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

3.4.3.1.2 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. Elevated levels of TC, LDL, and triglycerides are associated with increased cardiovascular risks, while 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 22 epidemiological studies (24 publications)[14] from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and serum lipid effects. Study quality evaluations for these 23 studies are shown in Figure 3-34. Results from studies summarized in the 2016 PFOA HESD are described in Table 3-11 and below.

In the 2016 Health Assessment (U.S. EPA, 2016c) for PFOA, there was relatively consistent and strong evidence of positive associations between PFOA and TC and LDL in occupational (Costa et al., 2009; Sakr et al., 2007a; Sakr et al., 2007b; Olsen et al., 2003) and high-exposure community settings (Winquist and Steenland, 2014; Fitz-Simon et al., 2013; Frisbee et al., 2010; Steenland et al., 2009). Two of the studies were cross-sectional, however, Fitz-Simon (2013) reported positive associations for LDL and TC in a longitudinal analysis of the change in lipids seen in relation to a change in serum PFOA. General population studies (Geiger et al., 2014a; Nelson et al., 2010; Lin et al., 2009) in children and adults using NHANES reported positive associations for TC and increased risk of elevated TC. Other general population studies were generally consistent, reporting positive associations for TC in adults (Eriksen et al., 2013; Fisher et al., 2013) and pregnant women (Starling et al., 2014). Positive associations between PFOA and HDL were also observed in most studies in the general population (Fisher et al., 2013; Frisbee et al., 2010; Lin et al., 2009; Steenland et al., 2009). Positive associations were observed for triglycerides and LDL in high-exposure community studies (Frisbee et al., 2010; Steenland et al., 2009), but associations for triglycerides and LDL were less consistent in other general population studies (Geiger et al., 2014a; Fisher et al., 2013; Lin et al., 2009).

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

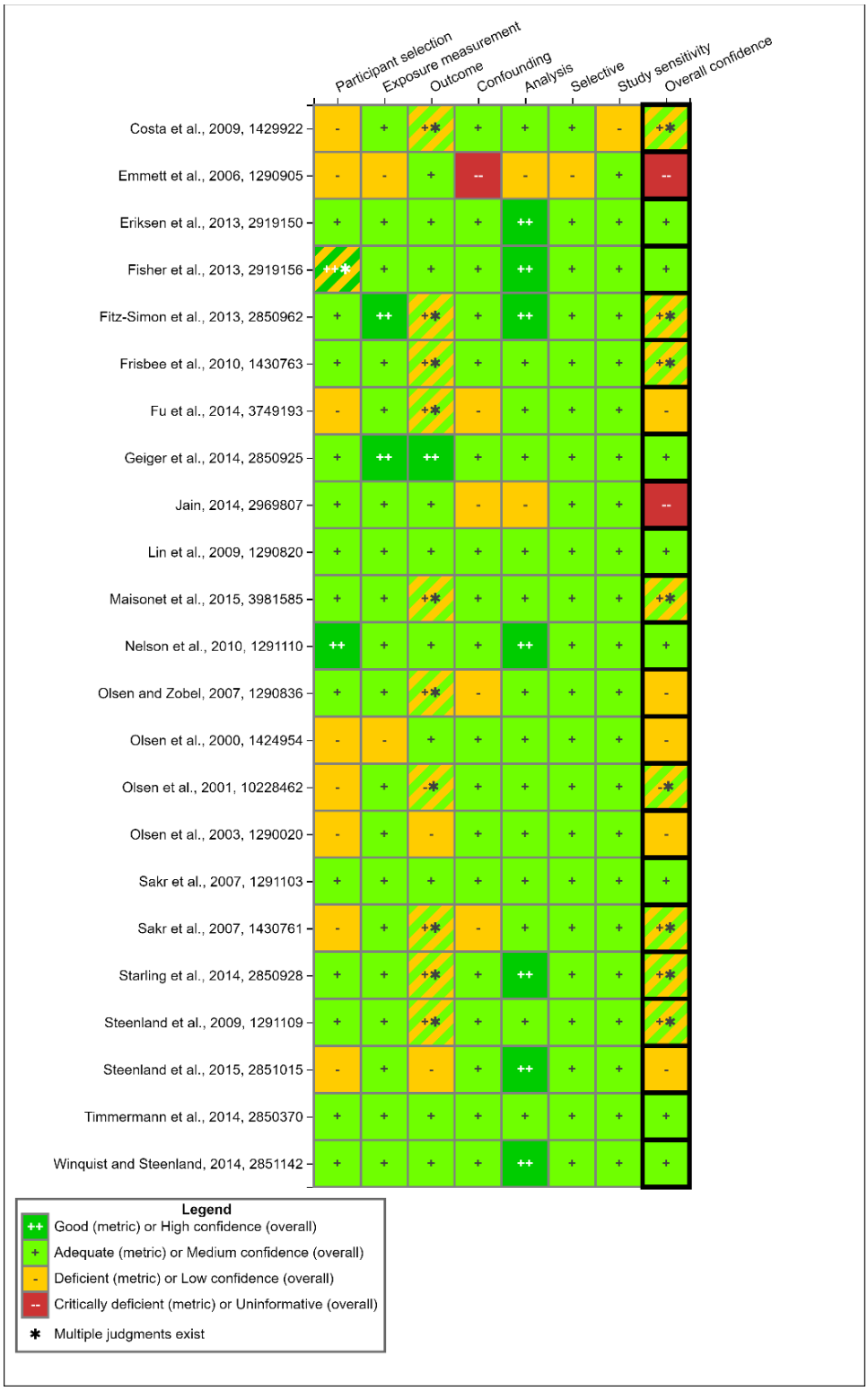


Figure 3-34. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Serum Lipids Published Before 2016 (References from 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Table 3-11. Associations Between Elevated Exposure to PFOA and Serum Lipids from Studies Identified in the 2016 PFOA HESD

Reference, Confidence	Study Design	Population	TC[a]	HDL[a]	LDL[a]	TG[a]	High Cholesterol[b]
Costa, 2009, 1429922 *Mixed*[c]	Cohort	Occupational	↑↑	↓	NA	↑	NA
Eriksen, 2013, 2919150 *Medium*	Cross-sectional	Adults	↑↑	NA	NA	NA	NA
Fisher, 2013, 2919156 *Medium*	Cross-sectional	Adults	–	–	–	–	NA
Fitz-Simon, 2013, 2850962 *Mixed*[c]	Cohort	Adults	↑	↓	↑	–	NA
Frisbee, 2010, 1430763 *Mixed*[c]	Cross-sectional	Children	↑↑	–	↑↑	↑↑	NA
Fu, 2014, 3749193 *Low*	Cross-sectional	Adults and children	↑↑	–	↑	↑	NA
Geiger, 2014, 2850925 *Medium*	Cross-sectional	Adolescents	↑↑	↓↓	↑↑	–	NA
Lin, 2009, 1290820 *Medium*	Cross-sectional	Adults	NA	↑	NA	–	NA
Maisonet, 2015, 3981585 *Mixed*[c]	Cohort	Children	↓	–	–	↓	NA
Nelson, 2010, 1291110 *Medium*	Cross-sectional	Adults	↑↑	↓	↑	NA	NA
Olsen, 2000, 1424954 *Low*	Cross-sectional	Occupational	↑	↓↓	–	NA	NA
Olsen, 2003, 1290020 *Low*[c]	Cohort	Occupational	↑↑	NA	NA	↑↑	NA
Olsen and Zobel, 2007, 1290836 *Low*	Cross-sectional	Occupational	↑	↓↓	↑	↑↑	NA

Reference, Confidence	Study Design	Population	TC[a]	HDL[a]	LDL[a]	TG[a]	High Cholesterol[b]
Sakr, 2007, 1291103 *Medium*	Cross-sectional	Occupational	↑↑	↓	↑↑	↑	NA
Sakr, 2007, 1430761 *Mixed*[c]	Cohort	Occupational	↑↑	↓↓	↑	–	NA
Starling, 2014, 2850928 *Mixed*[c]	Cohort	Children	↑	↑	↑	–	NA
Steenland, 2009, 1291109 *Mixed*[c]	Cross-sectional	Adults	↑↑	↑	↑	↑	NA
Steenland, 2015, 2851015 *Low*	Cohort	Occupational	NA	NA	NA	NA	–
Timmerman, 2014, 2850370 *Medium*	Cohort	Children	NA	NA	NA	↑	NA
Winquist and Steenland, 2014, 2851142 *Mixed*[c]	Cohort	Occupational	NA	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.

[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] Olsen (2001a) and Olsen (2001b) report data overlapping with Olsen (2003), which was considered the most updated information.

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

3.4.3.1.2.2 Study Quality

All studies were evaluated for risk of bias, selective reporting, and sensitivity following the EPA IRIS protocol. Three considerations were specific to evaluating the quality of studies on serum lipids. First, because lipid-lowering medications strongly affect serum lipid levels, unless the prevalence of medication use is assumed to be low in the study population (e.g., children), studies that did not account for the use of lipid-lowering medications by restriction, stratification, or adjustment were rated as *deficient* in the *participant selection* domain. Second, because triglyceride levels are sensitive to recent food intake (Mora, 2016), outcome measurement error is likely substantial when triglyceride is measured without fasting. Thus, studies that did not measure triglycerides in fasting blood samples were rated *deficient* in the *outcome measures* domain for triglycerides. The o*utcome 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 PFOA and serum lipids concurrently was considered *adequate* in terms of exposure assessment timing. Given the long half-life of PFOA (median half-life = 2.7 years) (Li et al., 2018c), 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 not yet clear evidence to support these reverse causal pathways.

Since publication of the 2016 PFOA HESD (U.S. EPA, 2016c), 64 new epidemiological studies (65 publications)[15] report on the association between PFOA exposure and serum lipids. Except for 10 studies (Blomberg et al., 2021; Li et al., 2021; Liu et al., 2020a; 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., 2021; Sinisalu et al., 2020). Most studies assessed exposure to PFOA using biomarkers in blood, and measured serum lipids with standard clinical biochemistry methods. Serum lipids were frequently analyzed as continuous outcomes, but a few studies examined the prevalence or incidence of hypercholesterolemia, hypertriglyceridemia, and low HDL based on clinical cut-points, medication use, doctor's diagnosis, or criteria for metabolic syndrome. Study quality evaluations for these 65 studies are shown in Figure 3-35, Figure 3-36, Figure 3-37.

On the basis of the considerations mentioned, one study was classified as *high* confidence*,* one study was classified as *high* confidence for prospective analyses and *medium* confidence for cross-sectional analyses, 21 studies were classified *medium* confidence for all lipid outcomes, nine studies were rated *medium* confidence for TC or HDL, but *low* confidence for triglycerides or LDL, 26 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; Sinisalu et al., 2020; Huang et al., 2018; Seo et al., 2018; Predieri et al., 2015). Notably, 10 studies (Blomberg et al., 2021; Canova et al., 2021; Dalla Zuanna et al., 2021; Canova et al., 2020; Lin et al., 2020e; 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

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

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., 2020b; Lin et al., 2020a; Chen et al., 2019; Fassler 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; 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., 2020b; Lin et al., 2020a; Sinisalu et al., 2020; Fassler et al., 2019; Graber et al., 2019; Convertino et al., 2018; Khalil et al., 2018; Yang et al., 2018; Koshy 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 (Sinisalu et al., 2020; Graber et al., 2019; Khalil et al., 2018; Christensen et al., 2016; Rotander et al., 2015; Olsen et al., 2012; Wang et al., 2012), or selective reporting (adolescent portion) (Dong et al., 2019).

The most common reason for a *low* confidence rating was potential for selection bias, including 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., 2020b; Lin et al., 2020a; Chen et al., 2019; He et al., 2018; Liu et al., 2018b; Sun et al., 2018; Yang et al., 2018; Wang et al., 2012), potential for self-selection (Li et al., 2020b; Graber et al., 2019; Christensen et al., 2016; Rotander et al., 2015), highly unequal recruitment efforts in sampling frames with potentially different joint distributions of PFOA and lipids (Lin et al., 2013), and missing key information on the recruitment process (Khalil et al., 2020; Fassler et al., 2019; 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., 2020b; Lin et al., 2020a; Sinisalu et al., 2020; Fassler et al., 2019; Graber et al., 2019; Khalil et al., 2018; Yang et al., 2018; Koshy 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 PFOA as an outcome of all lipids instead of an exposure, which limits the ability to make causal inference for the purpose of hazard determination (Predieri 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).

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

Figure 3-35. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Serum Lipids

Interactive figure and additional study details available on HAWC.

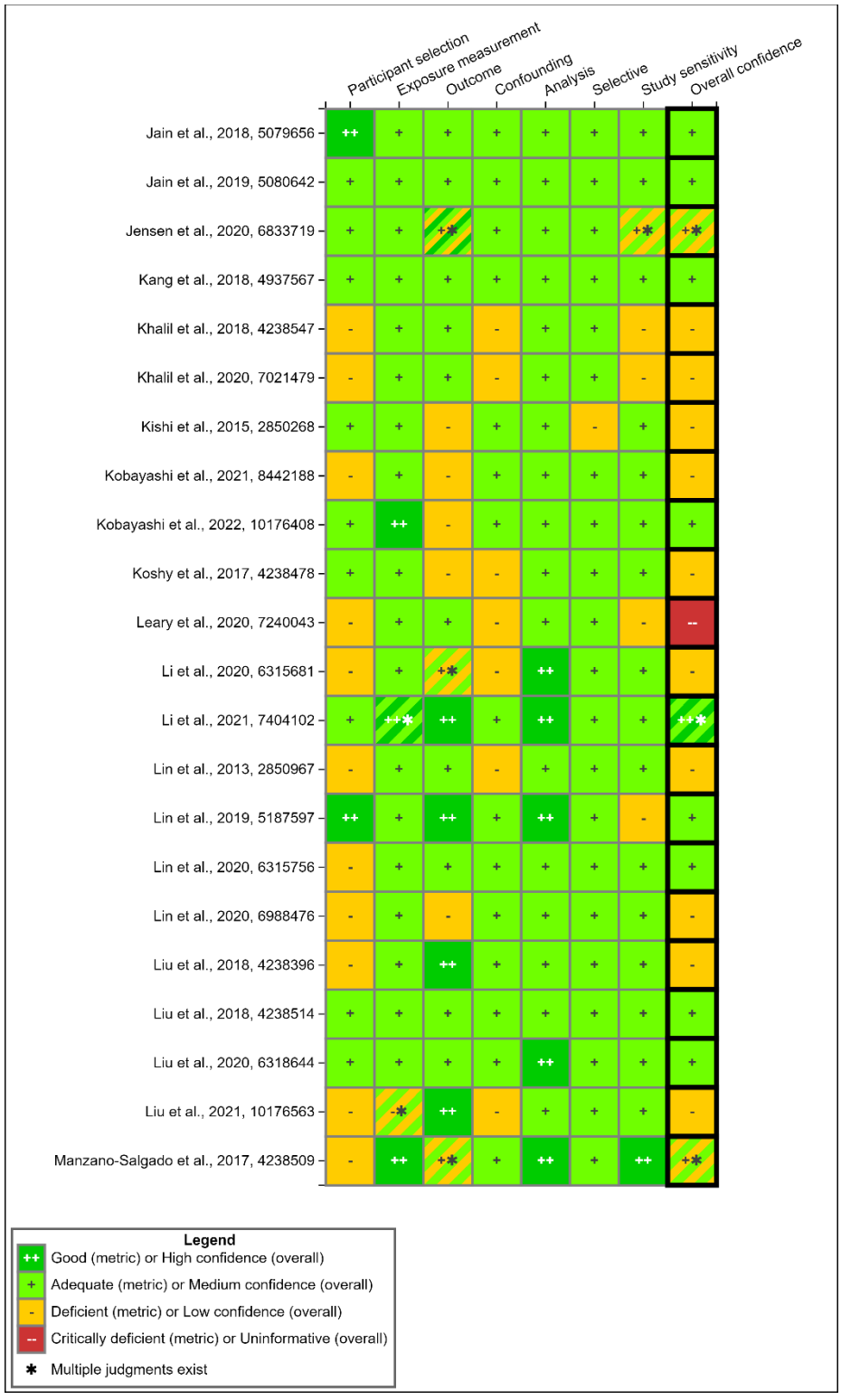


Figure 3-36. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

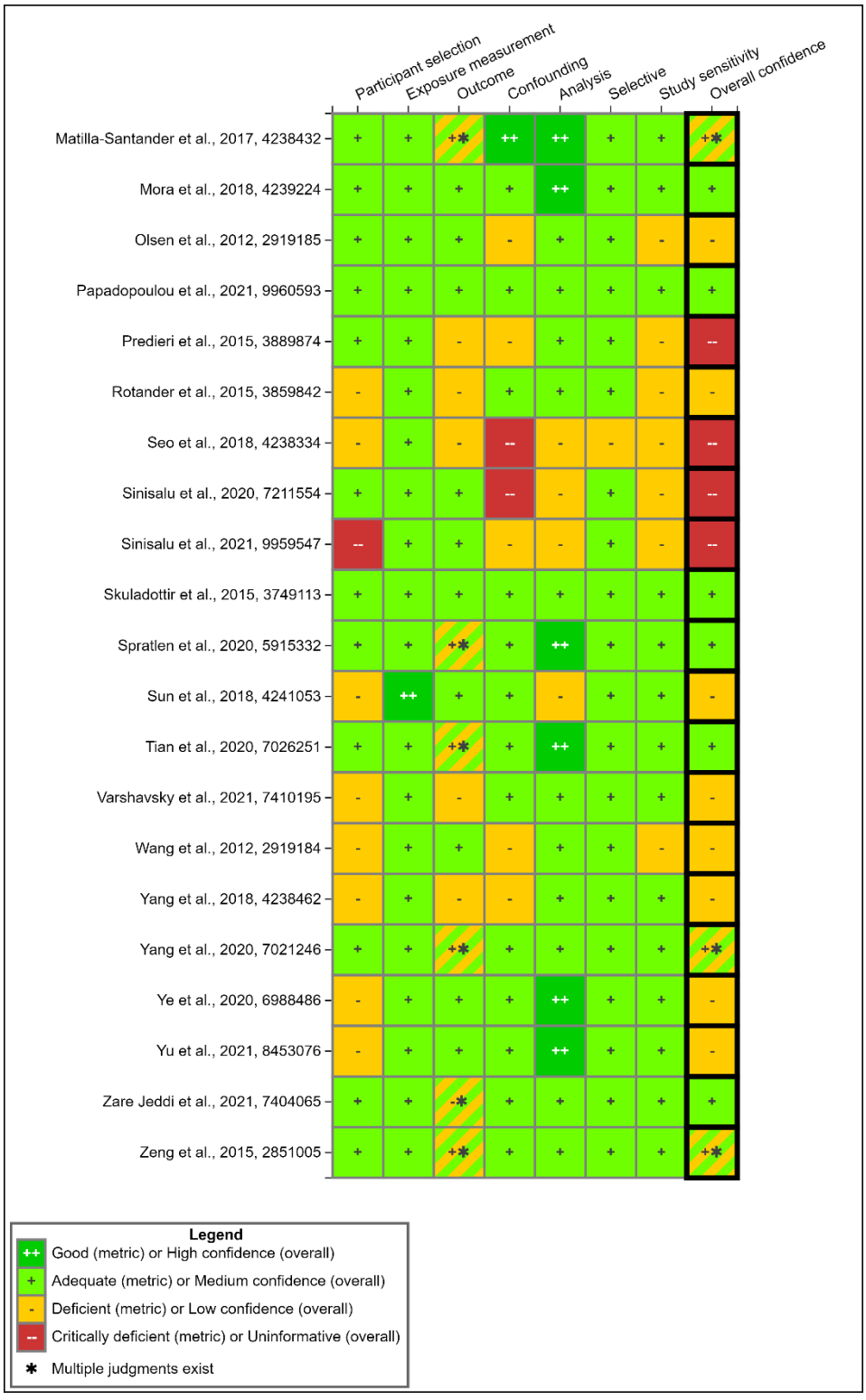


Figure 3-37. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Serum Lipids (Continued)

Interactive figure and additional study details available on HAWC.

3.4.3.1.2.3 Findings From Children

Results for the studies that examined TC in children are presented in the Appendix (U.S. EPA, 2024a). Eleven *medium* confidence and four *low* confidence studies examined the association between PFOA and TC in children. Of these, five studies examined the association between prenatal PFOA exposure and TC in childhood (Averina et al., 2021; Jensen et al., 2020; Spratlen et al., 2020; Tian et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and 10 examined the association between childhood PFOA exposure and concurrent TC (Blomberg et al., 2021; Canova et al., 2021; Dong et al., 2019; Fassler 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). Positive associations between PFOA and TC were reported in seven *medium* confidence studies (Blomberg et al., 2021; Canova et al., 2021; Jensen et al., 2020; Spratlen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b; Zeng et al., 2015), but the direction of association sometimes differed by age and sex (Blomberg et al., 2021; Jensen et al., 2020; Manzano-Salgado et al., 2017b). Of all the positive associations observed in *medium* confidence studies, only three were significant, including: all children (age 12–15 years) in Zeng (2015), among girls in mid-childhood in Mora (2018), and children and adolescents in the highest quartile of exposure from Canova (2021).

In three out of four *low* confidence studies, PFOA was positively associated with TC (Fassler et al., 2019; Khalil et al., 2018; Koshy et al., 2017). However, residual confounding by SES may have positively biased these findings. Taken together, these studies suggest a positive association between PFOA and TC in children. However, the true association between PFOA and TC remains uncertain given the heterogeneity by age and sex and the imprecise findings in most *medium* confidence studies.

Seven *medium* confidence and five *low* confidence studies examined the association between PFOA and LDL in children. Of these, five examined prenatal exposure (Papadopoulou et al., 2021; Jensen et al., 2020; Tian et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and eight examined childhood exposure (Averina et al., 2021; Canova et al., 2021; Dong et al., 2019 adolescent portion; Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). The *medium* studies generally reported small, positive associations between PFOA and LDL, but most of the associations were not statistically significant (see Appendix, (U.S. EPA, 2024a)) (Jensen et al., 2020; Kang et al., 2018; Mora et al., 2018). In one *medium* study, the association was inverse among 3-month old infants and 18-month old boys (Jensen et al., 2020).

One *low* confidence study (Canova et al., 2021) on children and adolescents in a high-exposure community located in Italy observed significantly increased LDL among adolescents (beta per ln-unit increase in PFOA: 1.03; 95% CI: 0.39, 1.66). Most *low* confidence studies reported a positive association between PFOA and LDL (Canova et al., 2021; Khalil et al., 2018; Koshy et al., 2017; Manzano-Salgado et al., 2017b; Zeng et al., 2015), but 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 PFOA were observed in children, though less consistently.

One *high* confidence, nine *medium* confidence and four *low* confidence studies examined the association between PFOA and HDL in children. Of these, six examined prenatal exposure

(Blomberg et al., 2021; Li et al., 2021; Papadopoulou et al., 2021; Jensen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and 12 examined childhood exposure (Averina et al., 2021; Blomberg et al., 2021; Canova et al., 2021; Li et al., 2021; Papadopoulou et al., 2021; Dong et al., 2019 adolescent portion; Fassler et al., 2019; Jain and Ducatman, 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Zeng et al., 2015). Prenatal PFOA exposure was inversely associated with HDL, but most associations were not statistically significant (Blomberg et al., 2021; Li et al., 2021; Papadopoulou et al., 2021; Jensen et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) (see Appendix, (U.S. EPA, 2024a)). Sex-stratified analyses showed that the inverse association occurred mainly in boys (Mora et al., 2018; Manzano-Salgado et al., 2017b). Results on childhood exposure were less consistent (see Appendix, (U.S. EPA, 2024a)). One *medium* study reported a statistically significant, positive association between PFOA and HDL in mid-childhood (Mora et al., 2018), but another *medium* study reported an inverse, though statistically nonsignificant association (Zeng et al., 2015). One *medium* confidence study (Canova et al., 2021) in a high-exposure community observed a significant increase in HDL in children, but results were less consistent in adolescents. Most *low* confidence studies reported a positive association between childhood PFOA exposure and HDL (Fassler et al., 2019; Khalil et al., 2018; Koshy et al., 2017). In summary, PFOA was not consistently associated with lower HDL in children. Effect modification by exposure window may explain this inconsistency.

One *high* confidence, nine *medium* confidence and five *low* confidence studies examined the association between PFOA and triglycerides in children. Of these, seven examined prenatal exposure (Li et al., 2021; Papadopoulou et al., 2021; Jensen et al., 2020; Spratlen et al., 2020; Tian et al., 2020; Mora et al., 2018; Manzano-Salgado et al., 2017b) and 11 examined childhood exposure (Averina et al., 2021; Canova et al., 2021; Li et al., 2021; Papadopoulou et al., 2021; Fassler et al., 2019; Kang et al., 2018; Khalil et al., 2018; Mora et al., 2018; Koshy et al., 2017; Domazet et al., 2016; Zeng et al., 2015). No association was observed in the only *high* confidence study (Li et al., 2021). PFOA was significantly associated with increased triglycerides in newborns in one *medium* study (Spratlen et al., 2020) (see Appendix, (U.S. EPA, 2024a)). Some *medium* studies also reported positive associations, but they were not statistically significant (Jensen et al., 2020; Kang et al., 2018; Mora et al., 2018). Results from other *medium* confidence studies were imprecise (Li et al., 2021; Papadopoulou et al., 2021). In one *medium* study that examined the association between PFOA and triglycerides longitudinally, PFOA at age 9 years was associated with lower triglycerides at age 15 years and 21 years, while PFOA at age 15 years was associated with higher triglycerides at age 21 years (Domazet et al., 2016). None of the associations were statistically significant. In most *low* confidence studies, PFOA was positively associated with triglycerides (Khalil et al., 2018; Koshy et al., 2017; Manzano-Salgado et al., 2017b; Zeng et al., 2015), but the use of non-fasting samples and residual confounding by SES may have biased these results upwards. Overall, increased triglycerides with increasing PFOA were observed in children, but results were less consistent and not always statistically significant.

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

lower HDL, especially in boys, although childhood exposure was associated with higher HDL. Few findings were statistically significant, however, suggesting caution in interpreting these results.

3.4.3.1.2.4 Findings From Pregnant Women

One *high* confidence study (Gardener et al., 2021) and four *medium* confidence studies examined the association between PFOA and TC in pregnant women (Dalla Zuanna et al., 2021; Yang et al., 2020b; Matilla-Santander et al., 2017; Skuladottir et al., 2015) and two reported significantly positive associations between PFOA and TC (see Appendix, (U.S. EPA, 2024a)) (Matilla-Santander et al., 2017; Skuladottir et al., 2015). One *medium* confidence study in a high-exposure community in Italy (Dalla Zuanna et al., 2021) considered PFOA exposure concentrations across trimesters using a generalized additive model (GAM). Authors reported significantly decreased TC with an increasingly inverse trend across all sampled trimesters. Results were consistent for second and third trimester samples in sensitivity analyses, but the direction of effect was positive for first trimester samples (see Appendix, (U.S. EPA, 2024a)). No association between PFOA and TC was observed in a cohort of pregnant women in the United States (Gardener et al., 2021) or 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 PFOA and TC in pregnant women.

Two studies examined PFOA and LDL in pregnant women (Dalla Zuanna et al., 2021; Yang et al., 2020b) and were considered *low* confidence due to lack of fasting blood samples for LDL measurement. In a high-exposure community (Dalla Zuanna et al., 2021), a decrease in LDL was reported with increasing PFOA concentrations when considering exposure concentrations sampled across trimesters. In individual trimester sensitivity analyses, results were consistently inverse for second and third trimester samples, including a significant finding for the third trimester. However, nonsignificant positive associations were observed for first trimester samples. No associations were observed for LDL in the other *low* confidence study, but a significant decrease was reported for the LDL:HDL ratio (see Appendix, (U.S. EPA, 2024a)).

Three *medium* confidence studies examined PFOA and HDL in pregnant women (Starling, 2017, 3858473; Dalla Zuanna, 2021, 7277682; Yang, 2020, 7021246;) and two observed positive statistically significant associations (see Appendix, (U.S. EPA, 2024a)) (Dalla Zuanna et al., 2021; Starling et al., 2017). Starling et al. (2017) reported a positive association between maternal PFOA serum concentrations (collected during 20 to 34 weeks of pregnancy with a median of 27 weeks) and HDL in a United States cohort. Dalla Zuanna (2021) observed significant positive associations when considering blood samples across all trimesters of pregnancy in a high-exposure community in Italy. The association was consistent, but no longer significant, when trimesters were modeled individually. (Yang et al., 2020b) observed a null association between PFOA exposures and HDL levels measured in early pregnancy.

One *high* confidence, one *medium* confidence, and three *low* confidence studies examined the association between PFOA and triglycerides in pregnant women. The *high* confidence study reported a significant increasing trend for triglycerides with increasing PFOA exposure quartile in a cohort of pregnant women from the United States (Gardener et al., 2021). The *medium* confidence study reported an inverse association between PFOA and triglycerides, but the

association was small and not statistically significant (Starling et al., 2017). The *low* confidence studies each reported inverse (Yang et al., 2020b; Matilla-Santander et al., 2017) or positive associations (Kishi et al., 2015) that were not statistically significant. Each study was limited by their use of non-fasting blood samples. Kishi et al. (2015) additionally examined the association between PFOA and select fatty acids in serum. PFOA was not significantly associated with any fatty acids, but the associations were generally positive except for arachidonic acid, docosahexaenoic acid, and omega 3. Together, these studies suggest PFOA was not associated with triglycerides or fatty acids in pregnancy.

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

3.4.3.1.2.5 Findings From the General Adult Population

Ten *medium* confidence and 13 *low* confidence studies examined PFOA and TC or hypercholesterolemia in adults (Figure 3-35, Figure 3-36, and Figure 3-37). All studies examined cross-sectional associations (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., 2020b; Lin et al., 2020e; Liu et al., 2020a; 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; Convertino et al., 2018; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Sun et al., 2018; Christensen et al., 2016; Wang et al., 2012) and two studies additionally examined the association between baseline PFOA and changes in TC or incident hypercholesterolemia (Liu et al., 2020a; Lin et al., 2019).

Of the 10 *medium* confidence studies, eight reported positive associations (Figure 3-39, Figure 3-40, Figure 3-41, Figure 3-42). 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 significant, positive associations with TC, including an increased risk of high cholesterol (Figure 3-38). Canova et al. (2020) also reported a concentration-response curve when PFOA was categorized in quartiles or deciles, with a higher slope at higher PFOA concentrations, which tended to flatten above around 20–30 ng/mL. Results from another *medium* confidence study (Lin et al., 2020e) on older adults in a high-exposure community in Taiwan also reported positive associations for TC, which was consistent across quartiles of PFOA exposure.

Four of the *medium* confidence studies used overlapping data from NHANES 2003–2014. All four studies reported significant positive associations between PFOA and TC in adults (Fan et al., 2020; Dong et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d) (see Appendix, (U.S. EPA, 2024a)). Stratified analyses in Jain et al. (2019b) suggested that the positive association occurred mainly in obese men. A significantly positive association between PFOA and TC also was observed at baseline in the DPPOS (Lin et al., 2019). This study reported positive associations between PFOA and prevalent, as well as incident, hypercholesterolemia. However, the HR for incident hypercholesterolemia was relatively small and not statistically significant (HR = 1.06, 95% CI: 0.94, 1.19). In contrast to these findings, Liu et al. (2020a) reported no association between PFOA and TC. Further, Donat-Vargas et al. (2019) reported generally inverse associations between PFOA and TC, regardless of whether PFOA was measured

concurrently or averaged between baseline and follow-up. It is noteworthy that all participants in Lin et al. (2019) were prediabetic, all participants in Liu et al. (2020a) 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 PFOA and TC or hypercholesterolemia were reported in nine of 13 studies (Cong et al., 2021; Khalil et al., 2020; Li et al., 2020b; 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 PFOA exposure and health problems was a concern in three of these studies (Li et al., 2020b; Graber et al., 2019; Christensen et al., 2016). Selection bias concerns, including lack of consideration of lipid-lowering medication and convenience sampling, were issues in two of the studies (Cong et al., 2021; Khalil et al., 2020). Further, He et al. (2018) used similar data as the four *medium* NHANES studies and thus added little information.

Contrary to these findings, in one *low* confidence study, participants treated with extremely high levels of ammonium perfluorooctanoate (APFO) in an open-label, nonrandomized, phase 1 trial, were found to have reduced levels of TC with increasing plasma PFOA concentrations (Convertino et al., 2018). This study differed from the other studies in several ways. First, all participants were solid-tumor cancer patients who failed standard therapy and may have distinct metabolic profiles compared with the general population. Second, participants ingested high dose levels of APFO rather than being exposed to PFOA. Third, participants' plasma PFOA concentrations were several orders of magnitude higher than those reported in the general population. Participant serum concentrations were of similar magnitude as serum concentrations resulting in decreased TC serum in rodent studies (see Section 3.4.3.2). It is unclear whether these factors explained the inverse association between PFOA and TC.

Considering *medium* and *low* confidence studies together, increased TC with increasing PFOA was observed in adults. Some inconsistencies in the direction of association across studies were found. Further studies are needed to determine if these inconsistencies reflect effect modification by subject characteristics or PFOA dose levels.

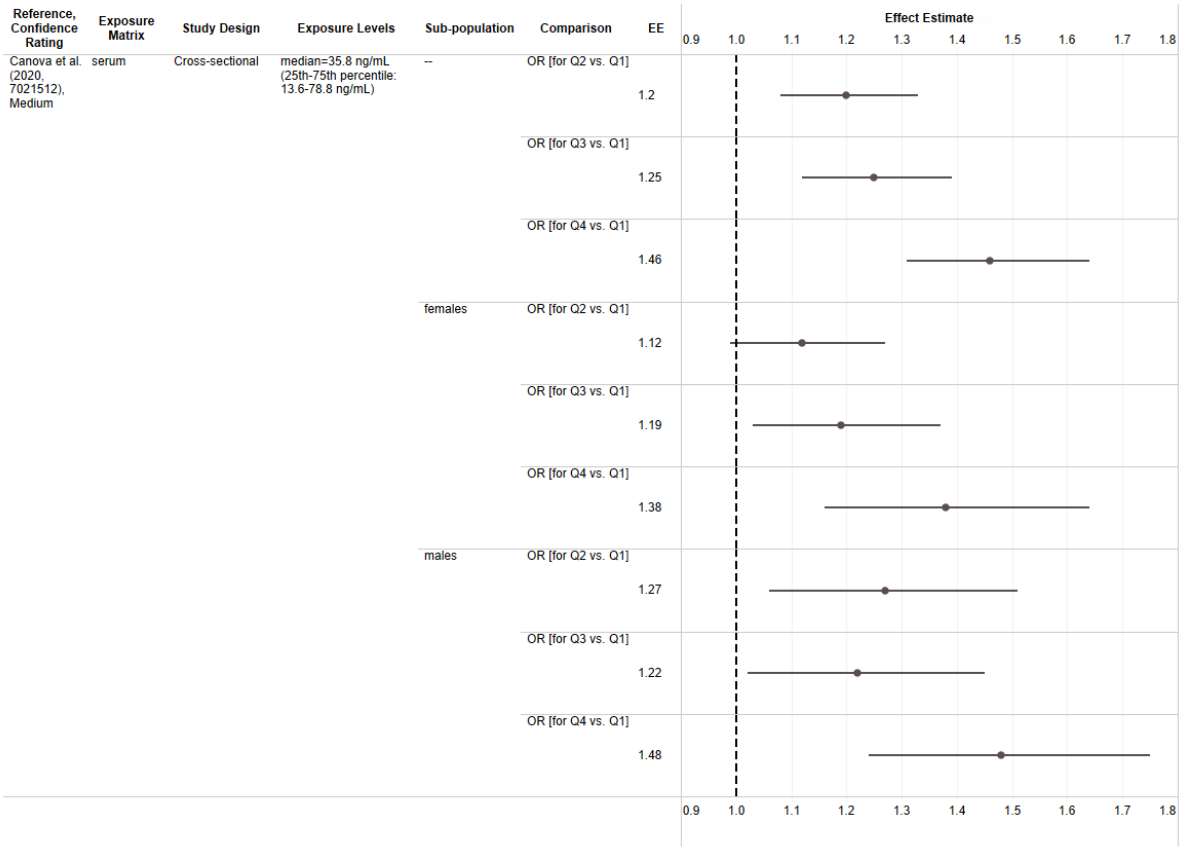


Figure 3-38. Odds of High Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

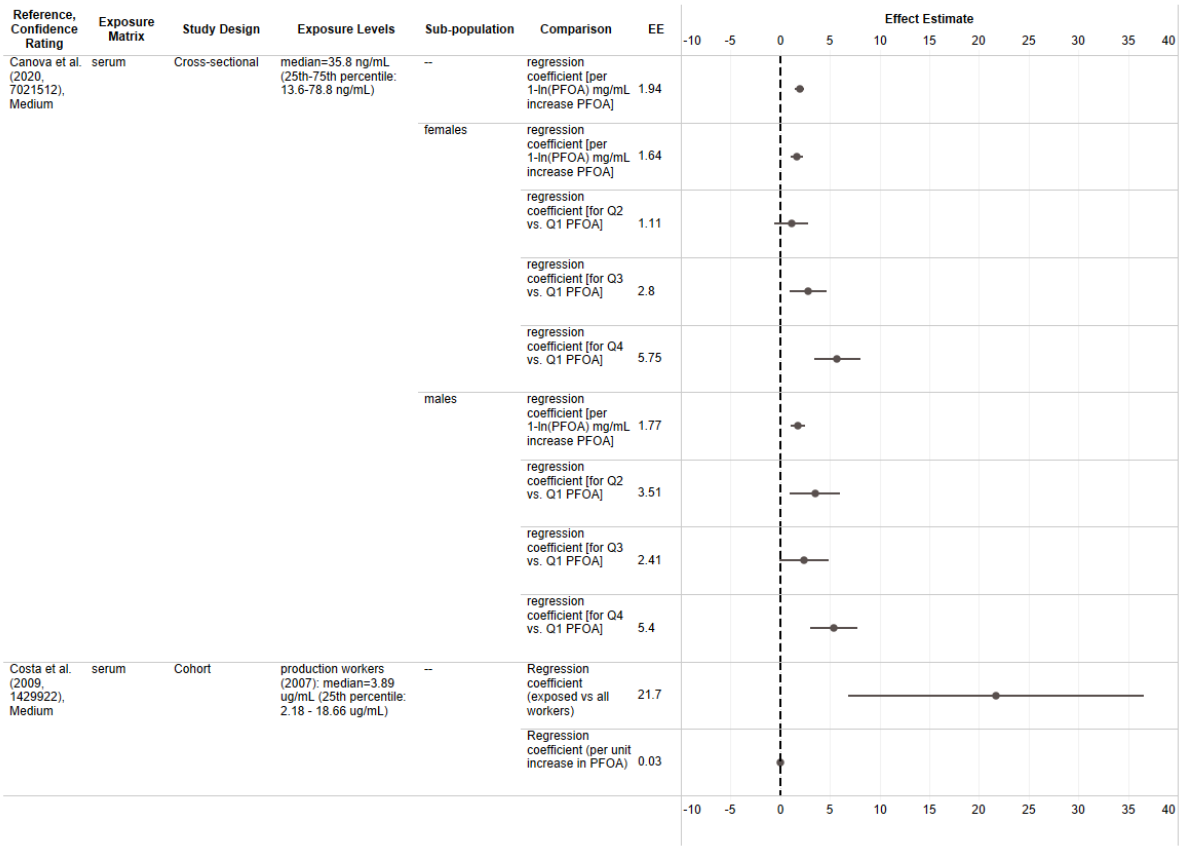


Figure 3-39. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

Reference, Confidence Rating	Exposure Matrix	Study Design	Exposure Levels	Sub-population	Comparison	EE
Donat-Vargas et al. (2019, 5080588), Medium	plasma	Cohort	Baseline median: 2.9 (ng/ml) (25th-75th percentile:2.2-4.2 ng/ml) Follow-up median: 2.7 (ng/ml) (25th-75th percentile: 1.9-3.6 ng/ml)	--	prospective regression coefficient (per 1-SD 1.19 ng/mL PFOA)	-0.04
					prospective regression coefficient (mean change) for tertile 2 vs tertile 1 PFOA	0
					prospective regression coefficient (mean change) for tertile 3 vs tertile 1 PFOA	0.16
					regression coefficient [per 1-SD 1.38 ng/mL PFOA	-0.12
					regression coefficient (mean change) for tertile 2 vs tertile 1 PFOA	0.03
					regression coefficient (mean change) for tertile 3 vs tertile 1 PFOA	-0.11
			median: 2.9 (ng/ml) (25th-75th percentile:2.2-4.2 ng/ml)	baseline	regression coefficient (per 1-SD 1.45 ng/mL PFOA)	-0.19
					regression coefficient (mean change) for tertile 2 vs tertile 1 PFOA	0.01
					regression coefficient (mean change) for tertile 3 vs tertile 1 PFOA	-0.29
			median: 2.7 (ng/ml) (25th-75th percentile: 1.9-3.6 ng/ml)	follow-up	regression coefficient (per 1-SD 1.27 ng/mL PFOA)	-0.03
					regression coefficient (mean change) for tertile 2 vs tertile 1 PFOA	0.1
					regression coefficient (mean change) for tertile 3 vs tertile 1 PFOA	0.1
Dong et al. (2019, 5080195), Medium	serum	Cross-sectional	Not reported	Adults (20-80 years) in NHANES 2003-2004	Regression Coefficient (change in TC per unit increase in serum PFOA)	1.6
				Adults (20-80 years) in NHANES 2005-2006	Regression Coefficient (change in TC per unit increase in serum PFOA)	0.7
				Adults (20-80 years) in NHANES 2007-2008	Regression Coefficient (change in TC per unit increase in serum PFOA)	1.5
				Adults (20-80 years) in NHANES 2009-2010	Regression Coefficient (change in TC per unit increase in serum PFOA)	0.9
				Adults (20-80 years) in NHANES 2011-2012	Regression Coefficient (change in TC per unit increase in serum PFOA)	4.1
				Adults (20-80 years) in NHANES 2013-2014	Regression Coefficient (change in TC per unit increase in serum PFOA)	2.9
			Median: 3.0 ng/ml; Mean ± SD: 3.7 ± 3.4 ng/ml	Adults (20-80 years)	Regression Coefficient (change in TC per unit increase in serum PFOA)	1.5

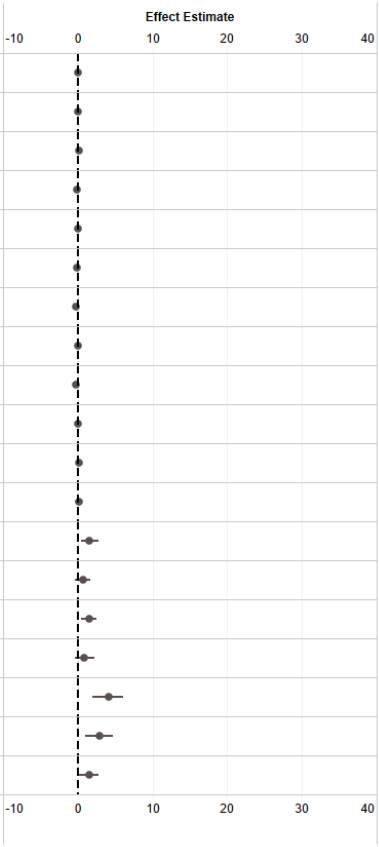


Figure 3-40. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

Reference, Confidence Rating	Exposure Matrix	Study Design	Exposure Levels	Sub-population	Comparison	EE
Fan et al. (2020, 7102734), Medium	serum	Cross-sectional	median=2.05 ug/L (25th-75th percentile: 1.31-3.10 ug/L)	--	regression coefficient [per 1-log(10) increase in PFOA]	6.74
Fisher et al. (2013, 2919156), Medium	plasma	Cross-sectional	geometric mean (SD) = 2.46 ug/L (1.83)	--	regression coefficient (per ln unit increase PFOA)	0.03
Han et al. (2021, 7762348), Medium	serum	Case-control	Cases: median=10.05 ng/mL (25th - 75th percentile: 6.75 - 17.05 ng/mL); Controls: median=11.40 ng/mL (25th - 75th percentile: 9.20 - 17.40 ng/mL)	--	regression coefficient (per log10 ng/mL increase in PFOA)	0.01
Jain et al. (2019, 5080642), Medium	serum	Cross-sectional	Geometric mean=2.5 ng/ml; 25th - 75th percentiles: 1.5 - 4.1; SD 2.1	Non-obese females	regression coefficient (per 1-log10 unit change in PFOA)	0.01
				Obese females	regression coefficient (per 1-log10 unit change in PFOA)	0
			Geometric mean=3.4 ng/ml; 25th - 75th percentiles: 2.3 - 5.3; SD 1.9	Non-obese males	regression coefficient (per 1-log10 unit change in PFOA)	-0.01
				Obese males	regression coefficient (per 1-log10 unit change in PFOA)	0.05
Lin et al. (2019, 5187597), Medium	plasma	Cohort and cross-sectional	median=4.9 ng/ml (25th-75th percentile: 3.5 - 6.7)	participants who were not on lipid-lowering medication	Regression coefficient (mean difference) (per doubling of baseline plasma PFOA)	6.09
					Regression coefficient (mean difference) (for Q2 vs Q1)	2
					Regression coefficient (mean difference) (for Q3 vs Q1)	10.13
					Regression coefficient (mean difference) (for Q4 vs Q1)	13.36
Lin et al. (2020, 6988476), Medium	serum	Cross-sectional	median=8.6 ng/mL (25th-75th percentile: 6.2-11.6)	Participants not taking lipid lowering medication	Regression Coefficient (for Q2 vs Q1)	2.48
					Regression Coefficient (for Q3 vs Q1)	2.88
					Regression Coefficient (for Q4 vs Q1)	4.04
Nelson et al. (2010, 1291110), Medium	serum	Cross-sectional	median: 3.9 ug/L (range: 0.1-37.3 ug/L)	20- to 80-year-olds	regression coefficient (per ug/L increase in PFOA)	1.22
					regression coefficient [for Q4 (5.5-37.3 ug/L) vs. Q1 (0.1-2.7 ug/L) PFOA]	9.8

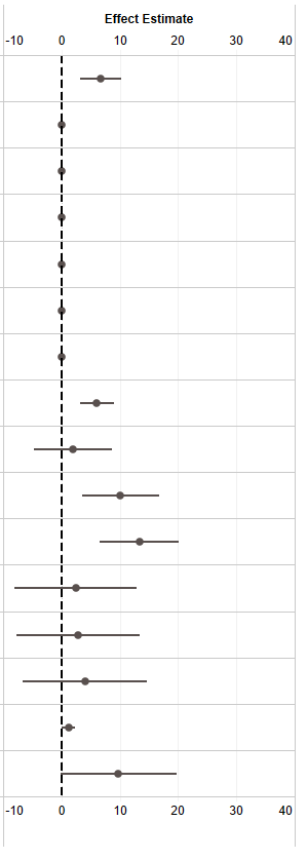


Figure 3-41. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

Reference, Confidence Rating	Exposure Matrix	Study Design	Exposure Levels	Sub-population	Comparison	EE
Olsen et al. (2003, 1290020), Medium	serum	Cohort	Antwerp Mean (SD) = 1.03 ppm (1.09); Decatur=1.90 ppm (1.59)	Males	Regression coefficient (per unit increase in PFOA)	0.03
Sakr et al. (2007, 1291103), Medium	serum	Cross-sectional	Mean (SD): 0.428 ppm (0.86); Min-max: 0.005-9.550 ppm	--	Regression coefficient (per ppm increase PFOA)	4.04
				Workers not on lipid-lowering medications	Regression coefficient (per ppm increase PFOA)	5.52
Steenland et al. (2009, 1291109), Medium	serum	Cross-sectional	median=26.6 ng/mL (min - max: 0.25-17556.6 ng/mL)	--	regression coefficient (for decile 2 vs. decile 1)	0.01
					regression coefficient (for decile 3 vs. decile 1)	0.02
					regression coefficient (for decile 4 vs. decile 1)	0.03
					regression coefficient (for decile 5 vs. decile 1)	0.04
					regression coefficient (for decile 6 vs. decile 1)	0.03
					regression coefficient (for decile 7 vs. decile 1)	0.04
					regression coefficient (for decile 8 vs. decile 1)	0.04
					regression coefficient (for decile 9 vs. decile 1)	0.04
					regression coefficient (per 1-ln ng/mL increase in PFOA)	0.01
					Regression coefficient for PFOA Decile 10 vs Decile 1	0.05

Figure 3-42. Overall Levels of Total Cholesterol in Adults from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

Six *medium* confidence studies examined PFOA and LDL in adults, and all reported positive associations (Figure 3-35, Figure 3-36, and Figure 3-37). Higher PFOA was significantly associated with higher LDL at baseline in the DPPOS (Lin et al., 2019) (see Appendix, (U.S. EPA, 2024a)). This study also reported statistically significant, positive associations between PFOA and cholesterol in non-HDL and VLDL, which are lipoprotein fractions related to LDL and associated with increased cardiovascular risks (Lin et al., 2019). A positive association was observed in a cross-sectional analysis of cases and controls in a study on type 2 diabetes (Han et al., 2021). Positive associations between PFOA and LDL were also reported in the four NHANES studies (Fan et al., 2020; Dong et al., 2019; Jain and Ducatman, 2019b; Liu et al., 2018d), but statistical significance was observed in obese men only (Jain and Ducatman, 2019b) and in participants from NHANES cycle 2011–2012 (Fan et al., 2020; Dong et al., 2019). Liu et al. (2020a) reported that PFOA was positively associated with cholesterol and apolipoprotein C-III (ApoC-III) in combined fractions of intermediate-density (IDL) and LDL that contained ApoC-III; the association with ApoC-III was statistically significant. IDL and LDL containing ApoC-III and ApoC-III itself are strongly associated with increased cardiovascular risks. Thus, the positive associations with cholesterol and ApoC-III in ApoC-III-containing fractions of IDL and LDL were consistent with the positive associations reported for LDL.

Consistent with these findings, nine of the 13 *low* confidence studies report positive associations between PFOA and LDL (Cong et al., 2021; Liu et al., 2021; Canova et al., 2020; Khalil et al., 2020; Li et al., 2020b; Lin et al., 2020e; Lin et al., 2020a; Chen et al., 2019; He et al., 2018; Liu et al., 2018b). Altogether, the available evidence supports a relatively consistent positive association between PFOA and LDL in adults, especially those who are obese or prediabetic. Associations with other lipoprotein cholesterol known to increase cardiovascular risks were also positive, which increased confidence in the findings for LDL.

Eleven *medium* confidence and 13 *low* confidence studies examined PFOA and HDL or clinically defined low HDL in adults (). All studies examined cross-sectional associations (Cong et al., 2021; Han et al., 2021; Liu et al., 2021; Yu 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., 2020b; Lin et al., 2020e; Lin et al., 2020a; Liu et al., 2020a; Chen et al., 2019; Christensen et al., 2019; Dong et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Convertino et al., 2018; He et al., 2018; Liu et al., 2018d; Liu et al., 2018b; Yang et al., 2018; Wang et al., 2012). Two studies also examined the association between baseline PFOA and changes in HDL (Liu et al., 2020a; Liu et al., 2018b). In a population of young adults aged 20 to 39 years in the Veneto region, Italy, an area with water contamination by PFAS, Canova et al. (2020) reported statistically significant, positive associations with HDL. Canova et al. (2020) also reported a concentration-response curve when PFOA was categorized in deciles. PFOA was inversely associated with HDL at baseline in the DPPOS, but the association was not statistically significant (Lin et al., 2019) (see Appendix, (U.S. EPA, 2024a)). Four studies used overlapping data from NHANES 2003–2014 and reported associations with HDL that were sometimes positive (Fan et al., 2020; Christensen et al., 2019; Liu et al., 2018d) and sometimes inverse (Dong et al., 2019). The direction of association differed by survey cycles. Few associations in this set of NHANES analyses were statistically significant. In an additional *medium* confidence study, PFOA was not associated with HDL at baseline or changes in HDL over two years (Liu et al., 2020a). Similarly, *low* confidence studies also reported a mix of positive (Li et al., 2020b; Lin et al., 2020a; He et al., 2018; Liu et al., 2018b; Yang et al., 2018) associations with changes in HDL in the 6–24 months of the study), inverse (Chen et al., 2019; Liu et al., 2018b) associations with concurrent HDL or changes in HDL in the first 6 months of the study (Ye et al., 2021 positive finding for reduced HDL), or essentially null (Cong et al., 2021; Liu et al., 2021; Bjorke-Monsen et al., 2020; Khalil et al., 2020; Convertino et al., 2018; Wang et al., 2012) associations, with few being statistically significant. Given the inconsistent findings in both *medium* and *low* confidence studies, the available evidence suggests PFOA is not associated with HDL in adults.

Nine *medium* confidence and 16 *low* confidence studies examined the association between PFOA and triglycerides or hypertriglyceridemia. All studies examined the cross-sectional association (Cong et al., 2021; Han et al., 2021; Liu 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., 2020b; Lin et al., 2020e; Lin et al., 2020a; Liu et al., 2020a; Chen et al., 2019; Christensen et al., 2019; Donat-Vargas et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Convertino et al., 2018; 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 PFOA and changes in triglycerides or incident hypertriglyceridemia (Liu et al., 2020a; Lin et al., 2019; Liu et al., 2018b). Higher PFOA was significantly associated with higher levels of triglycerides in the DPPOS (Lin et al., 2019) (see Appendix, (U.S. EPA, 2024a)). This study also reported that

PFOA was significantly associated with higher odds of hypertriglyceridemia at baseline and higher incidence of hypertriglyceridemia prospectively (Lin et al., 2019). Similarly, PFOA was associated with slightly higher levels of triglycerides in Liu et al. (2020a). The association was stronger and statistically significant for triglycerides in the apoC-III-containing combined fractions of IDL and LDL and apoC-III-negative HDL (Liu et al., 2020a). In contrast, the four *medium* studies using overlapping data from NHANES 2005–2014 reported positive (Christensen et al., 2019; Jain and Ducatman, 2019b) or inverse associations (Fan et al., 2020; Jain and Ducatman, 2019b; Liu et al., 2018d) between PFOA and triglycerides/hypertriglyceridemia. The direction of association appeared to differ by survey cycle, sex, and obesity status. No associations in these NHANES analyses were statistically significant. In an additional *medium* confidence study, PFOA was inversely associated with triglycerides, regardless of whether PFOA was measured concurrently or averaged between baseline and follow-up (Donat-Vargas et al., 2019). All participants in this study were free of diabetes for over 10 years, as opposed to the obese or prediabetic adults in Liu et al. (2020a) and Lin et al. (2019). It is unclear whether participants' different health status explained differences in the findings across *medium* studies.

In *low* confidence studies, a mix of positive (Liu et al., 2021; Ye et al., 2021; Canova et al., 2020; Khalil et al., 2020; Lin et al., 2020e in women; Lin et al., 2020a; Chen et al., 2019; He et al., 2018; Liu et al., 2018b association with concurrent triglycerides or changes in triglycerides in the first 6 months of the study; Sun et al., 2018; Yang et al., 2018), inverse (Li et al., 2020b; Lin et al., 2020e in men; Liu et al., 2018b association with changes in triglycerides in the 6–24 months of the study; Lin et al., 2013), and essentially null (Cong et al., 2021; Convertino et al., 2018; Wang et al., 2012) associations with triglycerides or hypertriglyceridemia were reported. Some associations were statistically significant. Overall, the available evidence suggests that PFOA was associated with elevated triglycerides in some adults. Whether PFOA increases triglycerides in all adults is unclear given inconsistency in reported associations.

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

3.4.3.1.2.6 Findings From Occupational Studies

Workers are usually exposed to higher levels of PFOA, in a more regular manner (sometimes daily), and potentially for a longer duration than adults in the general population. At the same time, 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 PFOA and TC or hypercholesterolemia in workers. Two of these studies examined the cross-sectional association between PFOA and TC in fluorochemical plant workers or firefighters exposed to aqueous film-forming foam (AFFF) (Rotander et al., 2015; Wang et al., 2012). One investigated the association between baseline PFOA and changes in TC over the course of a fluorochemical plant demolition project (Olsen et al., 2012). The cross-sectional studies reported positive (Wang et al., 2012) or inverse (Rotander et al., 2015) associations between PFOA and TC; neither association

was statistically significant. Olsen et al. (2012) reported that over the course of the demolition project, changes in PFOA were inversely associated with changes in TC; this association was not statistically significant (Olsen et al., 2012). Taken together, these studies suggest no association between PFOA and TC in workers.

Two studies examined PFOA and LDL in workers. One study examined PFOA and non-HDL, of which LDL is a major component. All studies were considered *low* confidence. The two studies on LDL reported positive (Wang et al., 2012) or inverse (Rotander et al., 2015) association between PFOA and concurrent LDL; neither association was statistically significant. The study examining non-HDL reported that changes in PFOA during the fluorochemical plant demolition project were inversely associated with changes in non-HDL, but the association was not statistically significant (Olsen et al., 2012). Overall, these studies suggest no association between PFOA and LDL in workers.

The studies that examined LDL or non-HDL also examined the association between PFOA and HDL (Rotander et al., 2015; Olsen et al., 2012; Wang et al., 2012). The two cross-sectional studies in this set of studies reported inverse association between PFOA and HDL, including a statistically significant finding in Wang (2012) (Rotander et al., 2015). Contrary to these findings, Olsen et al. (2012) reported that changes in PFOA over the demolition project were positively associated with changes in HDL (Olsen et al., 2012). This association was not statistically significant. When changes in TC to HDL ratio were examined as an outcome, however, a statistically significant, inverse association was observed. This suggests that increasing PFOA exposure was associated with decreases in TC/HDL over time, potentially partly due to a positive association between changes in PFOA and changes in HDL. Together, the occupational studies reported a consistently inverse association between PFOA and concurrent HDL, but this cross-sectional association was not coherent with longitudinal findings.

Two *low* confidence cross-sectional studies examined PFOA and triglycerides in workers and reported inverse associations between PFOA and triglycerides (Rotander et al., 2015; Wang et al., 2012). Neither association was statistically significant.

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

3.4.3.2 Animal Evidence Study Quality Evaluation and Synthesis

There are three studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and seven studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and cardiovascular effects in animal models. Study quality evaluations for these 10 studies are shown in Figure 3-43.

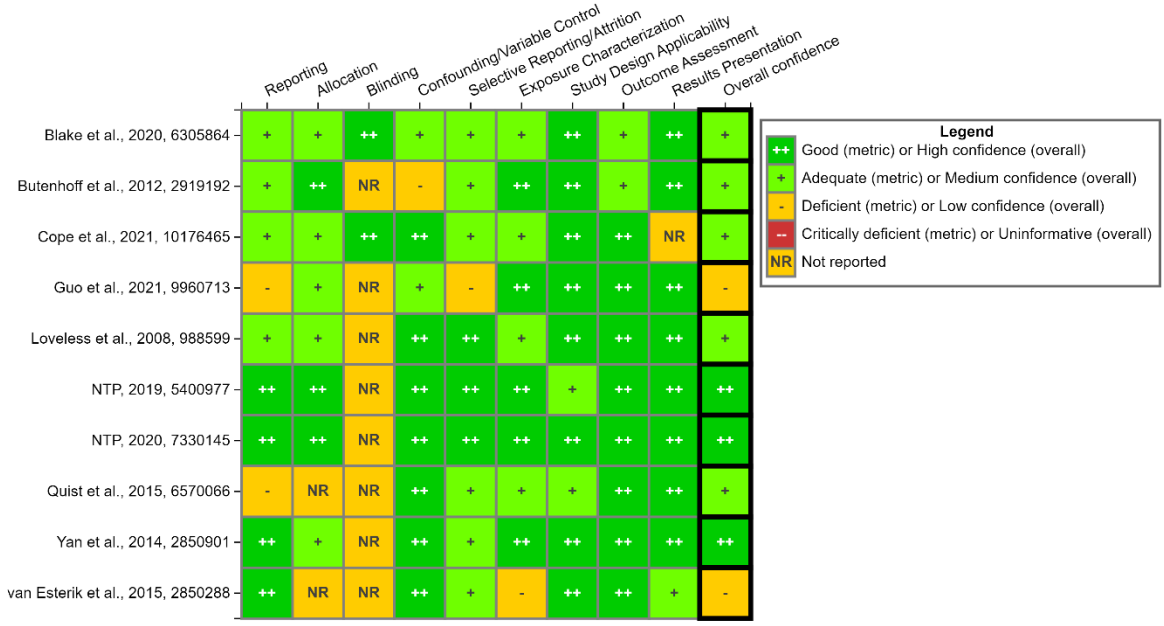


Figure 3-43. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

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

PFOA has been observed to cause perturbations in lipid homeostasis, which may have effects on the cardiovascular system. Alterations in serum lipid levels have been observed in mice and rats in subchronic, chronic, and developmental studies of oral exposure to PFOA (Figure 3-44). Overall, studies have generally reported consistent decreases in serum lipids including TC, triglycerides, LDL cholesterol, HDL cholesterol, and/or non-HDL cholesterol in rats (NTP, 2020, 2019; Elcombe et al., 2010; Loveless et al., 2008; Martin et al., 2007) and mice (Cope et al., 2021; Blake et al., 2020; Quist et al., 2015; Yan et al., 2014; Minata et al., 2010; Yahia et al., 2010; Dewitt et al., 2009; Loveless et al., 2008).

In a developmental study of female CD-1 P_0 mice exposed to PFOA (0, 1, and 5 mg/kg/day) by oral gavage from either GD 1.5–11.5 or GD 1.5–17.5, authors reported maximum decreases in serum triglyceride levels of 58% and 66%, respectively, at the highest dose of 5 mg/kg/day. No changes were observed for serum TC, HDL cholesterol, or LDL cholesterol (Blake et al., 2020). In a secondary developmental study of gestational PFOA exposure (0.1 and 1.0 mg/kg/day), female CD-1 P_0 mice were exposed via gavage from GD 1.5 to GD 17.5 (Cope et al., 2021). Male and female F_1 offspring were fed either a low-fat diet (LFD) or high-fat diet (HFD) at PND

22 and serum cholesterol markers were evaluated at PND 22 and at postnatal week (PNW) 18. At PND 22, there was a significant reduction in serum triglycerides in males and females and a significant reduction in LDL in males only but no effects in TC or HDL. At PNW 18, LFD female mice exhibited nonsignificant decreases in TC, HDL, LDL, and triglycerides. However, animals that were given a HFD no longer exhibited decreased levels of TC, HDL, or triglycerides and developed significantly higher levels of LDL (1.0 mg/kg/day) when compared with HFD control. Males fed the LFD exhibited nonsignificant increases in TC, HDL, LDL, and triglycerides; however, this trend was lost when animals were fed the HFD.

Male BALB/c mice exposed to PFOA by gavage for 28 days had significant decreases in serum TC and HDL levels at concentrations as low as 1.25 mg/kg/day (Yan et al., 2014). For serum triglyceride levels, significant increases were observed at lower exposure concentrations of PFOA (0.31 and 1.25 mg/kg/day) while significant decreases were seen following exposure to higher PFOA concentrations (5 and 10 mg/kg/day); no changes were observed in serum LDL cholesterol levels. In a study conducted by NTP, sex differences were observed in Sprague-Dawley rats exposed to PFOA by gavage for 28 days (NTP, 2019). Males had significantly decreased serum TC and triglyceride levels at exposure concentrations as low as 0.625 mg/kg/day. Female rats in the same study were exposed to 10-fold higher doses than their male counterparts due to sex differences in PFOA excretion (see Appendix, (U.S. EPA, 2024a)). Females had significant increases in both serum TC and triglyceride levels at the two highest doses (50 and 100 mg/kg/day). In the available chronic study (NTP, 2020), F_1 male and female Sprague-Dawley rats were exposed during gestation and lactation (perinatal exposure with postweaning exposure) or postweaning exposure only until animals were 19 weeks of age (e.g., 16-week interim time point; see further study design details in Section 3.4.4.2.1.2). Serum TC levels were significantly decreased only in males exposed during both the perinatal and postweaning phases (at postweaning doses of approximately 1 and 4.6 mg/kg/day); serum triglyceride levels were decreased in all exposure groups. Serum TC levels were significantly decreased only in the mid-dose F_1 females exposed during both perinatal and postweaning phases; TG levels were not altered in F_1 females.

Conclusions from these studies are met with limitations as the difference in serum lipid composition between humans and commonly used rodent models may impact the relevance to human exposures (Oppi et al., 2019; Getz and Reardon, 2012). It should be noted that human population-based PFOA exposure studies have consistently found that as PFOA exposure increases both serum cholesterol and serum triglycerides also increase. 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. The relatively high exposure and PFOA serum concentrations that produce these inverse effects are generally beyond the scope of human relevance, though there is some evidence in humans that similarly high serum PFOA serum concentrations result in decreased serum total cholesterol (e.g., Convertino et al. (2018)). This suggests that rodent models may be utilized accurately if the tested doses are within human health relevant exposure scenarios. 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 PFOA.

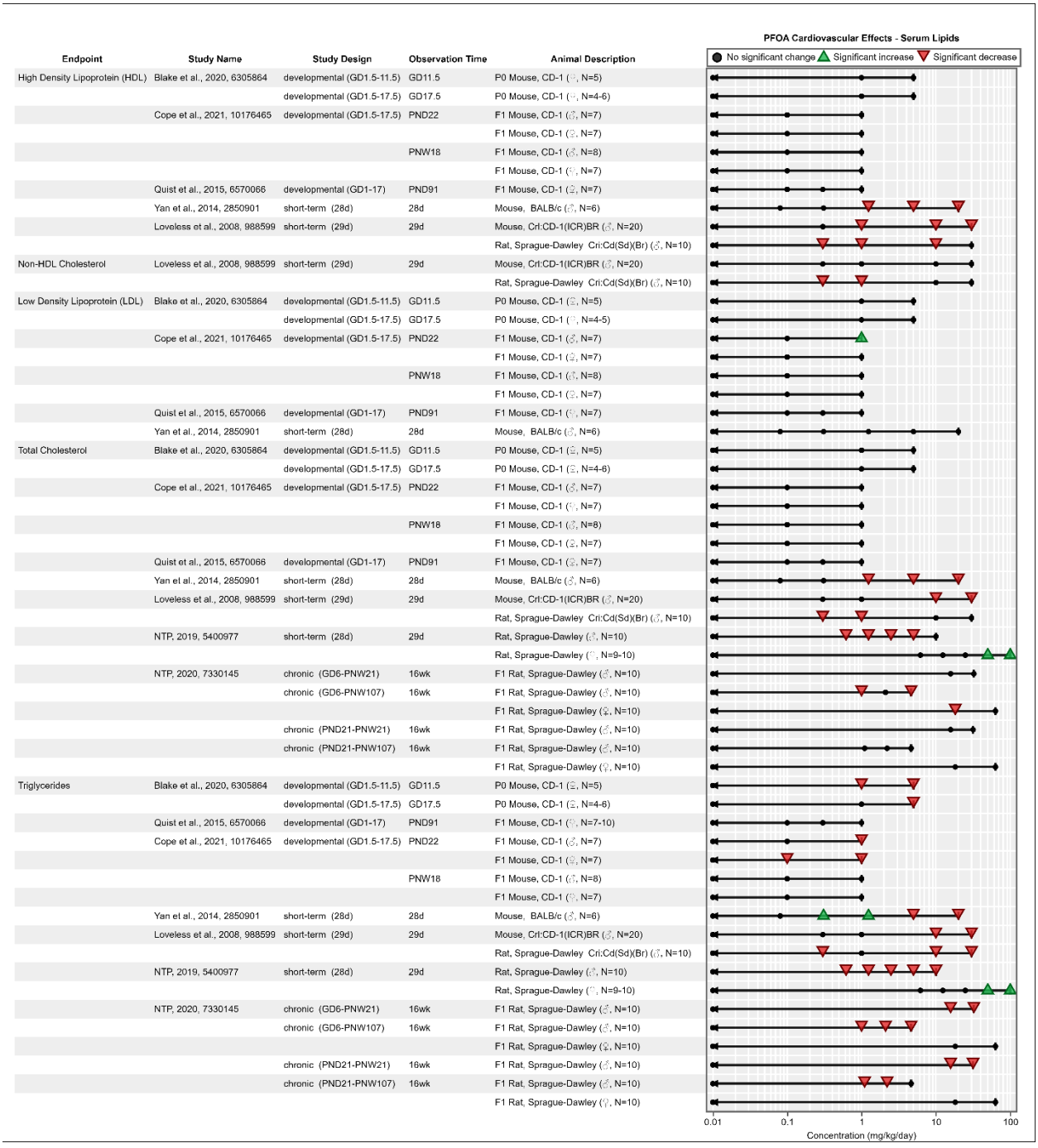


Figure 3-44. Serum Lipid Levels in Rodents Following Exposure to PFOA (logarithmic scale)

PFOA concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.

GD = gestation day; P_0 = parental generation; PNW = postnatal week; F_1 = first generation; PND = postnatal day; d = day; wk = week.

3.4.3.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse cardiovascular outcomes is discussed in Sections 3.1.1.1 and 3.4.1 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are eight studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA that lead to cardiovascular effects. A summary of these studies by mechanistic data category (see Appendix A, (U.S. EPA, 2024a)) and source is shown in Figure 3-45.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	0	1	0	1
Atherogenesis And Clot Formation	0	1	3	4
Big Data, Non-Targeted Analysis	1	0	0	1
Cell Growth, Differentiation, Proliferation, Or Viability	1	1	1	3
Cell Signaling Or Signal Transduction	0	0	2	2
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	1	0	0	1
Inflammation And Immune Response	0	0	1	1
Oxidative Stress	0	2	0	2
Grand Total	2	3	3	8

Figure 3-45. Summary of Mechanistic Studies of PFOA and Cardiovascular Effects

Interactive figure and additional study details available on HAWC.

3.4.3.3.1 Lipid Transport and Metabolism

Blood lipid levels are associated with risk factors for cardiovascular disease. Pouwer et al. (2019) investigated how PFOA influences plasma cholesterol and triglyceride metabolism using a transgenic mouse model of human-like lipoprotein metabolism (APOE*3-Leiden.CETP mice, which express the human CETP gene), human plasma samples, and in silico predictions. In the animal toxicological study, mice were fed a semisynthetic Western-type diet (0.25% cholesterol (wt/wt), 1% corn oil (wt/wt), and 14% bovine fat (wt/wt)) with varying levels of PFOA added (10, 300, or 30,000 ng/g/d). At the end of 4 or 6 weeks, mice were sacrificed and levels of triglycerides, TC, free fatty acids (FFA), ALT, glycerol, VLDL, HDL, and CETP were measured. The authors found that administration of PFOA at the 30,000 ng/g/d levels "reduced plasma TG and TC levels by affecting VLDL-TG production through decreased apoB synthesis and by increasing VLDL clearance." The authors also observed that PFOA at the highest dose decreased hepatic VLDL production rate, increased plasma VLDL clearance through enhanced LPL activity and affected gene expression of TG and cholesterol metabolism markers. Upon further analysis. PPARα was determined to be the major transcription factor affecting gene expression and fatty acid oxidation that regulates triglyceride and TC levels.

One study summarized in the 2016 PFOA HESD (U.S. EPA, 2016c) evaluated a subset of 290 individuals in the C8 Health Project for evidence that PFOA exposure can influence the transcript expression of genes involved in cholesterol metabolism, mobilization, or transport (Fletcher et al., 2013). Inverse associations were found between PFOA levels and expression of genes involved in cholesterol transport including Nuclear Receptor Subfamily 1 Group H Member 2 (NR1H2), Niemann-Pick disease type C (NPC1), and ATP Binding Cassette Subfamily G Member 1 (ABCG1). When males and females were analyzed separately, PFOA serum concentrations were negatively associated with expression of genes involved in cholesterol transport in both males and females, although the genes themselves differed between sexes (males: NPC1, ABCG1, PPARα; females: Nuclear Receptor Subfamily 1, Group H, Member 1 (NCEH1)). For additional information on the disruption of lipid metabolism, transport, and storage in the liver following PFOA exposure, please see Section 3.4.1.3.2.

3.4.3.3.2 Apoptosis and Cell Cycle Regulation

To elucidate the mechanisms involved in PFOA-induced vascular tissue apoptosis and CIMT, the levels of endothelial microparticles (CD62E, CD31+/CD42a−) and platelet microparticles (CD62P, CD31+/CD42a+) were measured in the serum of adolescents and young adults in another epidemiological study (Lin et al., 2016). The results showed that there was no association between PFOA serum levels and markers of apoptosis, endothelial activation, or platelet activation. This study also measured the relationship between oxidative stress and PFOA by measuring levels of 8-hydroxydeoxyguanosine (8-OHdG) in the urine. Similar to the markers of apoptosis, no association was found between PFOA and 8-OHdG. Another study by the same researchers also found that there was no association between PFOA and oxidative/nitrative stress markers 8-OHdG and 8-nitroguanine (8-NO_2Gua) in Taiwanese adults (Lin et al., 2020a).

One study evaluated the potential for PFOA to affect cell cycle regulation in the heart and other tissues (Cui et al., 2019). Male mice were orally dosed with 5 mg/kg/day PFOA for 28 days, and microRNA-34 (miR-34), a marker of tissue damage, was measured in the heart at the end of the exposure period. To further study the role of cardiovascular miR-34a under PFOA treatment, the authors also dosed miR-34a-knockout and wild-type mice for 28 days. In the wild-type mice, the expression of miR-34a in the heart was not significantly different in the treatment group compared with the control group. There were also no detectible levels miR-34b or miR-34c in the heart for either the treatment group or the control group.

3.4.3.3.3 Mechanisms of Atherogenesis and Clot Formation

Four groups of researchers published studies on the mechanism of atherogenesis and clot formation. The first two studies investigated how the structure of PFOA and other PFAS leads to activation of the plasma kallikrein-kinin system (KKS) using in vitro and ex vivo activation assays and in silico molecular docking analysis. KKS is a key component of plasma that plays a role in regulation of 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. The cascade activation of KKS includes the autoactivation of Hageman factor XII (FXII), cleavage of plasma prekallikrein (PPK), and activation of high-molecular-weight kininogen (HK) (Liu et al., 2018e). Results from the ex vivo mouse plasma study by Liu et al. (2017b) revealed that the addition of PFOA (5 mM) at the highest dose binds with FXII in a structure dependent manner and triggers the cascade to the rest

of the system. Liu et al. (2018e) observed no activation of the KKS cascade when mouse plasma was incubated with up to 500 μM PFOA.

Bassler et al. (2019) focused on several disease biomarkers, including plasminogen activator ihhibitor-1 (PAI-1), an indicator of clot formation that may lead to atherosclerosis. Human serum was collected from 200 patients as part of the larger C8 Health Project and analyzed for PFOA content. The authors found that there was no statistically significant difference in PAI-1 concentration in association with high exposure to PFOA concentrations.

The final study among the four groups of researchers, conducted by De Toni et al. (2020), investigated the effect of PFOA on platelet function, a key factor in atherosclerosis. Whole blood and peripheral blood samples were taken from healthy males that lived in low exposure areas and incubated with 400 ng/mL of PFOA. After isolating erythrocytes, leukocytes, and platelets and quantifying the amount of PFOA present, platelets were found to be the cell target of PFOA accumulation. The authors then used the platelets in an in vitro system and inoculated them with 400 ng/mL of PFOA and found that substantially more PFOA accumulated in the membrane of platelets versus the cytoplasm. Using molecular docking analysis, they were able to target the specific binding sites of PFOA to phosphatidylcholine, a major platelet phospholipid, suggesting that the accumulation of PFOA in the platelet may alter the activation process of platelets by impairing membrane stability.

3.4.3.4 Evidence Integration

There is *moderate* evidence for an association between PFOA exposure and cardiovascular effects in humans based on consistent positive associations with serum lipids, particularly LDL, and TC (Canova et al., 2020; Fan et al., 2020; Lin et al., 2020e; Donat-Vargas et al., 2019; Dong et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Liu et al., 2018d; Winquist and Steenland, 2014; Eriksen et al., 2013; Fitz-Simon et al., 2013; Nelson et al., 2010; Steenland et al., 2009). Additional evidence of positive associations with blood pressure and hypertension in adult populations supported this classification. The lack of evidence of consistent or precise effects for CVD or atherosclerotic changes raise uncertainty related to cardiovascular health effects following PFOA exposure. 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 PFOA exposure and these outcomes.

On the basis of this systematic review of 43 epidemiologic studies, the available evidence revealed positive associations between PFOA exposure and TC, LDL, and triglycerides effects in some human populations. For TC, the association was consistently positive in adults from the general population, positive but less consistently so in children and pregnant women, and generally null in workers. For LDL, the association was generally positive among adults, positive but less consistently so in children, and generally null in workers. Data were not available for PFOA and LDL in pregnant women. For triglycerides, positive, often nonsignificant associations were observed in some adults and children, but not pregnant women and workers. Except for workers, these results are consistent with findings from the 2016 PFOA HESD. Differences in findings from occupational studies between the 2016 PFOA HESD and this review may be attributable to limitations of occupational studies in this review. Similar to the 2016 PFOA HESD, the available evidence in this review does not support an inverse association between PFOA and HDL in any populations. The positive associations with TC are also supported by the

recent meta-analysis restricted to 14 general population studies in adults (U.S. EPA, 2022b). Similarly, a recent meta-analysis including data from 11 studies reported consistent associations between serum PFOA 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 epidemiological studies identified since the 2016 assessments do not provide additional clarity on the association between PFOA and CVD. Most of the CVD evidence identified in this review focused on blood pressure in the general adult population (13 studies). The findings from a single *high* confidence study and five *medium* confidence studies conducted in the general adult population did not provide consistent evidence for an association between PFOA and blood pressure. The evidence for an association between PFOA and increased risk of hypertension overall and in gender-stratified analysis was inconsistent. Evidence in children and adolescents also is less consistent. Five studies in children and adolescents, and one study in pregnant women suggest no associations with elevated blood pressure in these populations. Evidence for other CVD-related outcomes across all study populations was more limited, and similarly inconsistent. Consequently, the evidence for these CVD outcomes is broadly consistent with the conclusions of the C8 Science Panel and in the 2016 PFOA assessment, which found no probable link between PFOA exposure and multiple other conditions, including high blood pressure and CAD. It is challenging to compare findings on CVD-related mortality in the current assessment to the prior assessment due to differences in how this outcome was defined. Findings from the prior assessment were mixed, with one study reporting an increased risk of cerebrovascular disease mortality observed in the highest PFOA exposure category among occupationally exposed subjects. However, no association was reported with IHD mortality. The current evidence from a single study indicated PFOA was not associated with an increased risk of mortality due to cardiovascular causes, including hypertensive disease, IHD, stroke, and circulatory diseases. Future analyses of cause-specific CVD mortality could help elucidate whether there is a consistent association between PFOA and cerebrovascular disease mortality. No studies or endpoints were considered for the derivation of PODs since findings for an association between PFOA and CVD outcomes are mixed.

The animal evidence for an association between PFOA exposure and cardiovascular toxicity is *moderate* based on effects on serum lipids observed in animal models in six *high* or *medium* confidence studies. The most consistent results are for TC 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 heart weight and histopathology in the heart and aorta.

The underlying mechanisms for the observed cardiovascular effects related to PFOA exposure are likely related to changes in lipid metabolism, as described in detail in Section 3.4.1.3. Specifically, alterations in lipid metabolism lead to alterations in serum levels of triglycerides and cholesterol, as evidenced by in vivo in animal models. The events that precede and result in the alterations in serum levels have been proposed as the following, based on experimental evidence: (1) PFOA accumulation in liver activates nuclear receptors, including PPARα; (2) expression of genes involved in lipid homeostasis and metabolism is altered by nuclear receptor activation; (3) gene products (translated proteins) modify the lipid content of liver to favor

triglyceride accumulation and potentially cholesterol accumulation; (4) altered lipid content in the liver leads to accumulation of lipid droplets, which can lead to the development of steatosis and liver dysfunction. It should be noted that the results for PFOA-induced changes to serum lipid levels contrast between rodents (generally decreased) and humans (generally increased). Evidence is ultimately limited regarding a clear mechanism of alterations to serum lipid homeostasis caused by PFOA exposure. In humans, as discussed in the 2016 PFOA HESD (U.S. EPA, 2016c) data from the C8 Health Project indicated that PFOA exposure can influence expression of genes involved in cholesterol metabolism, mobilization, or transport. Specifically, an inverse association was found between PFOA levels and expression of genes involved in cholesterol transport, with sex-specificity for some of the individual gene expression changes. The authors of the study suggested that exposure to PFOA may promote a hypercholesterolaemic environment. Results were inconsistent regarding effects of PFOA on indicators or mechanisms related to atherosclerosis, including a lack of effect on an indicator of clot formation in human serum samples, and dose-dependent effects on the plasma kallikrein-kinin system in mouse plasma. A single study found that PFOA accumulates in platelets in human blood samples exposed in vitro, which may alter the activation process of platelets, although it was not directly evaluated. PFOA did not induce apoptosis or oxidative stress in vascular tissue in humans, as evidenced in two studies that evaluated serum levels of endothelial microparticles and platelet microparticles, and urinary 8-hydroxydeoxyguanosine (8-OHdG) in relation to PFOA levels.

3.4.3.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the *evidence indicates* that PFOA exposure is likely to cause adverse cardiovascular effects, specifically serum lipid effects, in humans under relevant exposure circumstances (Table 3-12). The hazard judgment is driven primarily by consistent evidence of serum lipid responses from epidemiological studies at median PFOA exposure levels representative of the NHANES population (median = 3.7 ng/mL). The evidence in animals showed coherent results for perturbations in lipid homeostasis in rodent models in developmental, subchronic, and chronic studies following exposure to doses as low as 0.3 mg/kg/day PFOA. 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 3-12. Evidence Profile Table for PFOA 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 27 *Medium* confidence studies 22 *Low* confidence studies 19 *Mixed*[a] confidence 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 (29), there is evidence of positive associations with TC (13/15) in *medium* confidence studies. Positive associations were also observed for LDL (6/8) in *medium* confidence studies, and mostly null, but some positive associations with TG (4/11) in *medium* confidence studies. Evidence from studies of children (19) was mixed, and observed associations often failed to reach significance, but findings were mostly positive for TC (10/19). In studies of pregnant women (6), evidence indicated positive associations with TC (3/4) and HDL (2/4) but no other serum lipid	• *High* and *medium* confidence studies • *Consistent* findings of positive associations with serum lipid measures in adults from the general population • *Coherence* of findings across serum lipids serum lipid effects	• *Low* confidence studies • *Inconsistent* findings in children, likely due to variations in measured exposure windows • *Inconsistent* findings by sex or health status	⊕⊕⊙ *Moderate* Evidence for cardiovascular effects is based on numerous *medium* confidence studies reporting positive associations with serum lipids, such as TC, LDL, and TG in adults from the general population. Results from occupational studies were generally consistent with studies of general population adults, particularly for increases in TC. Results from some studies of children and pregnant women also observed positive effects for TC and TG, however, interpretations of changes in serum lipids for children are less clear. *High* and *medium* confidence studies of adults reported positive associations with blood pressure and risk of	*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 rodent models in developmental, subchronic, and chronic studies following exposure to PFOA. 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**	
	measures. In occupational studies (10), positive associations or increased risks were observed for TC and high cholesterol (8/10), LDL (3/5), and TG (4/8). Findings on HDL in occupational studies were mixed.			hypertension, though other *medium* and *low* confidence studies reported nonsignificant associations. Observed effects were inconsistent for CVD and imprecise for atherosclerotic changes across all study populations.	
Blood pressure and hypertension 2 *High* confidence studies 18 *Medium* confidence studies 7 *Low* confidence studies	Studies examining changes in blood pressure, including DBP and SBP, and risk for hypertension in general population adults (15), showed consistent positive associations for SBP (5/6), DBP (6/6), combined BP (2/2), and hypertension (9/10) in *high* and *medium* confidence studies. In studies of children (9), mixed results were observed for SBP (7), DBP (5), and general BP (3). The only study examining hypertension in children reported a positive, dose-dependent association. In occupational studies, one study reported a positive association for hypertension (1/3). In the	• *High* and *medium* confidence studies • *Consistent* findings of effects for blood pressure measures, including hypertension, among adults • *Consistent* findings of effects observed in studies of children for blood pressure measures and hypertension	• *Low* confidence studies • *Imprecision* of findings • *Inconsistent findings* in children, likely due to variation in measured exposure window*s*		

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 study of pregnant women (1), a positive association was reported with hypertension. Hypertension analyses provided evidence of modification by sex, with males having higher risk in some studies.				
Cardiovascular disease 1 *High* confidence study 6 *Medium* confidence studies 6 *Low* confidence studies	CVD measures included CHD, stroke, angina, heart attack, MVD, IHD, PAD, and arrhythmia. Studies of general population adults (9) reported mixed results. The most commonly investigated endpoints were CHD (5), general CVD (5), and stroke (3); in all cases, positive and inverse associations were observed. A significant positive association for risk of heart attack was observed in a *medium* confidence study (1/1). Observations for other outcomes were limited to nonsignificant, imprecise findings by singular studies. In occupational studies (4), consistent inverse associations were observed for IHD (3/3),	• *High* and *medium* confidence studies	• *Low* confidence studies • *Inconsistent* findings for CVD-related outcomes • *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**	
	but results remained mixed for stroke (1/2).				
Atherosclerotic changes 1 *High* confidence study 3 *Medium* confidence studies 3 *Low* confidence studies	In studies of children (2), one study reported significantly increased associations in brachial artery distensibility (1/1). No significant associations were observed for CIMT among Taiwanese children (1/1) or pulse wave velocity among American children (1/1). Studies of adults (4) reported mixed results for measures of atherosclerotic changes. Most studies did not report associations that reached significance, however, one study reported decreased left ventricular relative wall thickness (1/3).	• *High* and *medium* confidence studies	• *Low confidence studies* • *Imprecision* of findings across children and adult study populations		
Evidence from In Vivo Animal Toxicological Studies (Section 3.4.3.2)					
Serum lipids 3 *High* confidence studies 4 *Medium* confidence studies	Significant decreases in serum TC were observed in 4/7 studies that examined this endpoint, regardless of species, sex, or study design. In three developmental studies, no changes were observed in mice. Similar decreases	• *High* and *medium* confidence studies • *Consistency of* findings across species, sex, or study design • *Dose-response* relationship	• *Incoherence* of findings in other cardiovascular outcomes • *Biological significance* of the magnitude of effect is unclear	⊕⊕⊙ *Moderate* Evidence based on six *high* or *medium* confidence studies observed that PFOA affects serum lipids in	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	were observed in serum TG (6/7). In a developmental study, decreased serum TG were observed in mice at PND 22 but not during adulthood. In a short-term exposure study, female rats were given 10-fold higher doses of PFOA than males due to sex differences in excretion, and it was found that serum TC and TG were decreased in males but increased in females. Fewer studies examined HDL and LDL, with decreases found in HDL (2/5). Three studies found no changes in LDL, but one developmental study in mice observed increased LDL in males at PND 22 but no changes during adulthood.	observed within multiple studies		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 indicate a disruption in lipid metabolism. No effects or minimal alterations were noted for heart weight and histopathology in the heart and aorta. However, many of the studies identified may not be adequate in exposure duration to assess potential toxicity to the cardiovascular system.	
Histopathology 2 *High* confidence studies 1 *Medium* confidence study	No changes in heart histopathology were reported in two studies. One chronic study reported decreased incidence of chronic myocarditis in female rats in the mid-dose group	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	only. No changes in aorta histopathology were noted in two studies.				
Organ weight 2 *High* confidence studies, 1 *Medium* confidence study	No changes in absolute or relative heart weights were found in one short-term study and one chronic study in rats. One chronic study in rats reported decreased absolute heart weights in males and females, but those reductions were found to be related to reduced body weights.	• *High* and *medium* confidence studies	• *Limited number* of studies examining outcome • *Confounding* variables such as decreases in body weights may limit ability to interpret these responses		

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

Key findings and interpretation:
• Alterations in lipid metabolism results in alterations in serum levels of TG and TC via:
  o PFOA accumulation in liver activates nuclear receptors, including PPARα.
  o Nuclear receptor activation alters the expression of genes involved in lipid homeostasis and metabolism.
PPARα is a major transcription factor affecting expression of genes that regulate fatty acid oxidation and triglyceride and total cholesterol levels.
Limitations:
• Only a single study demonstrating PFOA accumulation in platelets in vitro.
• Results are inconsistent and conflicting regarding effects on indicators or mechanisms related to atherosclerosis, primarily related to clot formation.
	Findings support plausibility that cardiovascular effects, specifically changes to serum TG and TC levels, can occur through changes in lipid metabolism related to PFOA exposure.

Notes: CHD = coronary heart disease; CIMT = carotid intima-media thickness; CVD = cardiovascular disease; DBP = diastolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MVD = microvascular disease; PAD = peripheral arterial disease; PPARα = peroxisome proliferator-activated receptor alpha; SBP = systolic blood pressure; TC = total cholesterol; TG = triglyceride.

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

3.4.4 Developmental

EPA identified 100 epidemiological and 19 animal toxicological studies that investigated the association between PFOA and developmental effects. Of the epidemiological studies, 30 were classified as *high* confidence, 39 as *medium* confidence, 19 as *low* confidence, 5 as *mixed* (2 *high/medium*, 1 *medium/low*, 2 *low/uninformative*) confidence, and 7 were considered *uninformative* (Section 3.4.4.1). Of the animal toxicological studies, 2 were classified as *high* confidence, 12 as *medium confidence,* and 4 as *low* confidence, and 1 was considered *mixed* (*medium/low*) (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 PFOA exposure and potential in utero and perinatal effects or developmental delays, as well as effects attributable to developmental exposure. The latter includes all studies where exposure is limited to gestation and/or early life up to 2 years of age. Developmental endpoints can include gestational age, measures of fetal growth (e.g., birth weight), birth defects, and fetal loss (i.e., spontaneous abortion/miscarriage and stillbirths), as well as infant/child development.

The 2016 PFOA HESD (U.S. EPA, 2016c) summarized epidemiological studies that examined developmental effects in relation to PFOA exposure. There are 22 studies from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and developmental effects. Study quality evaluations for these 22 studies are shown in Figure 3-46. Studies included ones conducted both in the general population as well as in communities known to have experienced high PFOA exposure (e.g., the C8 population in West Virginia and Ohio). Results from studies summarized in the 2016 PFOA HESD are described in Table 3-13 and below.

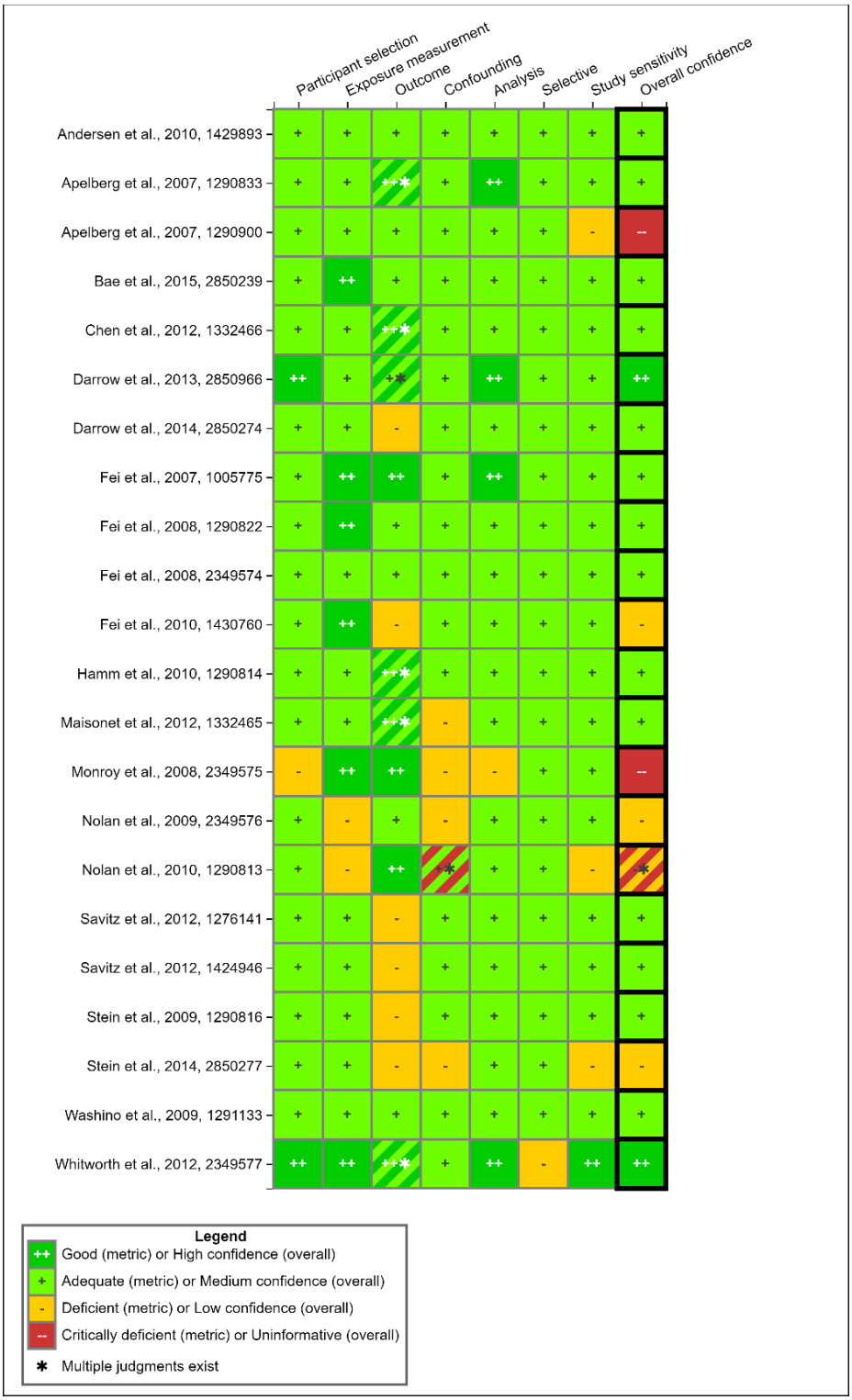


Figure 3-46. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Developmental Effects Published before 2016 (References from 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

As noted in the 2016 PFOA HESD, several available studies measured fetal growth outcomes. Apelberg et al. (2007b) found that birth weight was inversely associated with umbilical cord PFOA concentration (β per log unit increase: −104 g; 95% CI: −213, –5) in a study of 293 infants born in Maryland in 2004–2005 (mean PFOA concentration of 0.0016 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 PFOA concentration (median concentration of 0.0037 μg/mL) was inversely associated with birth weight (β per log unit increase: −34.2 g; 95% CI: −54.8, –13). A study of 252 pregnant women in Alberta, Canada found no statistically significant association between PFOA concentration measured in maternal blood during the second trimester (mean concentration of 0.0021 μg/mL) and birth weight (Hamm et al., 2010). In a Japanese prospective cohort of 428 infants in the Hokkaido Study on Environment and Children's Health (2002–2005), Washino et al. (2009) observed a large nonsignificant association between PFOA concentration in maternal blood during pregnancy (mean PFOA concentration of 0.0014 μg/mL) and birth weight (β per each $\log_{10}$ increase: –75.1 g; 95% CI: –191.8 to 41.6). Chen et al. (2012) examined 429 mother-infant pairs from the Taiwan Birth Panel Study and found no statistically significant association between umbilical cord blood PFOA concentration (geometric mean (GM) of 0.0018 μg/mL) and birth weight (β per each ln-unit increase: –19.2 g; 95% CI: –63.5, 25.1).

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. Fei et al. (2007) found a small, nonsignificant inverse association between maternal PFOA concentration (blood samples taken in the first and second trimester) and birth weight (β per unit increase: −8.7; 95% CI: −19.5, 2.1). Fei et al. (2008b) found an inverse association between maternal PFOA levels and birth length and abdominal circumference in the DNBC. Change in birth length per unit increase was 0.069 cm (95% CI: 0.024, 0.113) and change in abdominal circumference per unit increase was 0.059 cm (95% CI: 0.012, 0.106). Andersen et al. (2010) examined the association between maternal PFOA concentrations and measures of standardized birth weight, birth length, and infant body mass index (BMI) and body weight at 5 and 12 months of age in DNBC participants. Andersen et al. (2010) also reported an inverse association with birth weight, but the study population overlapped with participants reported in Fei et al. (2007). Regarding post-natal growth, they observed a positive association between adiposity and maternal PFOA concentration based on BMI measured at 5 and 12 months in boys, but not girls.

Some studies described in the 2016 PFOA HESD evaluated developmental outcomes in the C8 Health Project study population, which comprises a community known to have been subjected to high PFAS exposure (Darrow et al., 2014; Darrow et al., 2013; Savitz et al., 2012a; Savitz et al., 2012b; Stein et al., 2009). 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. As noted in the 2016 PFOA HESD, none of the studies reported statistically significant or large magnitude associations between PFOA and either birth weight or the risk of low birth weight. Darrow et al. (2013) reported a non-statistically significant increased risk (ORs ranging 1.3 to 1.49) for participants in the upper three quintiles of PFOA exposure (PFOA concentrations ≥11.1 ng/mL) compared with the lowest (PFOA concentration >8.6 ng/mL), but results from other C8 studies reported null associations for preterm birth. In the *low* confidence study (Stein et al., 2014) on the C8 Health Project

community population, modeled maternal serum PFOA was associated with brain birth defects (albeit with only 13 cases), but no associations were observed for other birth defects. Additionally, two studies (Nolan et al., 2010, 2009) evaluated birth weight, gestational age of infants, and frequencies of congenital anomalies in this community based on whether participants were supplied with contaminated public drinking water (PFOA concentrations were not measured in participants). The studies found no associations between these developmental effects and water supply status. These two studies were rated *low* confidence for most endpoints and *uninformative for* congenital anomalies in Nolan et al. (2010).

Table 3-13. Associations Between Elevated Exposure to PFOA and Developmental Outcomes in Children from Studies Identified in the 2016 PFOA 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[d] *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
Fei, 2007, 1005775[c] *Medium*	Cohort	↓	↑	–	NA	↑↑	NA	NA	NA
Hamm, 2010, 1290814 *Medium*	Cohort	↑	NA	↓	↓	↑	NA	NA	NA
Maisonet, 2012, 1332465 *Medium*	Cohort	↓↓	NA	NA	↓	NA	NA	NA	–
Nolan, 2009, 2349576 *Low*	Cross-sectional	–	NA	NA	–	NA	NA	NA	NA
Nolan, 2010, 1290813 *Mixed*[e]	Cross-sectional	NA	NA	NA	–	NA	–	NA	NA
Savitz, 2012, 1276141 *Medium*	Cohort	NA	–	NA	NA	–	–	–	NA
Savitz, 2012, 1424946 *Medium*	Cohort	↓	–	↓	NA	↑	NA	–	NA
Stein, 2009, 1290816	Cohort	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]
Medium									
Stein, 2014, 2850277 *Low*	Cohort	NA	NA	NA	NA	NA	–	NA	NA
Washino, 2009, 1291133[f] *Medium*	Cohort	↓	NA	NA	NA	NA	NA	NA	NA
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 = post-natal 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] Fei (2007) reports results from a population overlapping with Meng et al. (2018), which was considered the most updated data.

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

[e] Nolan (2010) was rated *uninformative* for congenital abnormalities and *low* confidence for all other outcomes.

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

3.4.4.1.2 Study Evaluation Considerations

There were multiple developmental outcome-specific considerations that informed domain-specific ratings and overall study confidence. For the Confounding domain, downgrading of studies occurred when key confounders of the fetal growth and PFAS relationship, such as parity, were not considered. Some hemodynamic factors related to physiological changes during pregnancy were also considered in this domain as potential confounders (e.g., GFR and blood volume changes over the course of pregnancy) because these factors may be related to both PFOA levels and the developmental effects examined here. More confidence was placed in the epidemiologic studies that adjusted for GFR in their regression models or if they limited this potential source of confounding by sampling PFAS levels earlier in pregnancy. An additional source of uncertainty was the potential for confounding by other PFAS (and other co-occurring contaminants). Although scientific consensus on how best to address PFAS co-exposures remains elusive, this was considered in the study quality evaluations and as part of the overall weight of evidence determination. Further discussion of considerations for potential confounding by co-occurring PFAS can be found in Section 5.1.

For the Exposure domain, all the available studies analyzed PFAS in serum or plasma using standard methods. Given the estimated long half-life of PFOA in humans noted in Section 3.3.1.4.5, samples collected during all three trimesters, before birth or shortly after birth were considered adequately representative of the most critical in utero exposures for fetal growth and gestational duration measures. The postnatal anthropometric studies were evaluated with consideration of fetal programming mechanisms (i.e., Barker hypothesis) where in utero perturbations, such as poor nutrition, can lead to developmental effects such as fetal growth restriction and ultimately adult-onset metabolic-related disorders and related complications (see more on this topic in (De Boo and Harding, 2006) and (Perng et al., 2016). There is some evidence that birth weight (BWT) deficits can be followed by increased weight gain that may occur especially among those with rapid growth catch-up periods during childhood (Perng 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, BWT measures have been shown to be quite accurate and precise, while other fetal and early childhood anthropometric measures may result in more uncertainty. Mismeasurement and incomplete case ascertainment can affect the accuracy of effect estimates by impacting both precision and validity. For example, the spontaneous abortion studies were downgraded for incomplete case ascertainment in the Outcome domain given that some pregnancy losses go unrecognized early in pregnancy (e.g., before implantation). This incomplete ascertainment, referred to as left truncation, can result in decreased study sensitivity and loss of precision. Often, this type of error can result in bias toward the null if ascertainment of fetal loss is not associated with PFOA exposures (i.e., non-differential). In some situations, differential loss is possible and bias away from the null can manifest as an apparent protective effect. Fetal and

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

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

3.4.4.1.3 Study Inclusion for Updated Literature Search

There are 79 epidemiological studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and developmental effects. Although every study is included in the endpoint-specific study quality evaluation heat maps for comprehensiveness, six developmental epidemiological studies identified in the literature search were excluded from this synthesis due to study population overlap with other included studies (i.e., were considered duplicative). The Li et al. (2017c) Guangzhou Birth Cohort Study overlaps with a more recent study by Chu et al. (2020). Four other 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 as Kashino et al. (2020). For those studies with the same endpoints analyzed across different

subsets from the same cohort, such as mean BWT, the analysis with the largest sample size was used in forest plots and tables (e.g., (Kashino et al., 2020) for the Hokkaido birth cohort study). 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 study by Bae et al. (Bae et al., 2015) was the only study to examine sex ratio and was not further considered here. In general, to best gauge consistency and magnitude of reported associations, EPA largely focused on the most accurate and most prevalent measures within each fetal growth endpoint. Three additional studies with overlapping cohorts were all included in the synthesis, as they provided some unique data for different endpoints. For example, the Woods et al. (2017) publication on the Health Outcomes and Measures of the Environment (HOME) cohort overlaps with Shoaff et al. (2018) but the authors provided additional mean BWT data. The mean BWT results for singleton and twin births from Bell et al. (2018) are included in forest plots here, while the postnatal growth trajectory data in the same UPSTATE KIDS cohort by Yeung et al. (2019) are also included as they target different developmental endpoints. 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 seven studies above, 72 developmental epidemiological studies were available for the synthesis. One study by Bae et al. (2015) was the only study to examine sex ratio and was not further considered here. 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 deficiencies in some risk of bias domains (e.g., confounding) or multiple domain deficiencies and are not further examined here. Thus, 66 studies were included across various developmental endpoints for further examination and synthesis. Forty-six of the 66 studies examined PFOA in relation to fetal growth restriction measured by the following fetal growth restriction endpoints: SGA, low BWT, head circumference, as well as mean and standardized BWT and birth length measures. Twenty studies examined different measures of gestation duration, five examined fetal loss, four examined birth defects, and 13 examined post-natal growth.

High and *medium* confidence studies were the focus of the evidence synthesis for endpoints with numerous studies, though *low* confidence studies were still considered for consistency in the direction of association (see Appendix, (U.S. EPA, 2024a)). For endpoints with fewer studies, the evidence synthesis below included details on any *low* confidence studies available. Studies considered *uninformative* were not considered further in the evidence synthesis.

3.4.4.1.4 Growth Restriction: Fetal Growth

3.4.4.1.4.1 Birth Weight

Of the 43 studies examining different BWT measures in relation to PFOA exposures, 37 examined mean birth weight 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., 2018; Meng et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Bach et al., 2016). Twenty-six of the 37 mean BWT were prospective birth cohort studies, and the remaining 11 were cross-sectional analyses defined here as if biomarker samples were collected at birth or post-partum (Yao et al., 2021; Gao et al., 2019; Wang et al., 2019; Xu et al., 2019a; Bell et al., 2018; Gyllenhammar et al., 2018; Shi et al., 2017; Callan et al., 2016; de Cock et al., 2016; Kwon et al., 2016; Wu et al., 2012).

Eight of the 37 studies with data on the overall population 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., 2018). 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 of the prospective birth cohort studies examined pre-conception maternal serum samples (Robledo et al., 2015). Twenty-four studies had maternal exposure 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 (Buck Louis et al., 2018; Lauritzen et al., 2017), 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; Wang et al., 2016; Wu et al., 2012), 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 PFOA in umbilical cord blood and one which measured PFOA in maternal blood samples collected in trimesters 1 and 2. For comparability with other studies of mean BWT, only one biomarker measure was used (e.g., preferably maternal samples when collected in conjunction with umbilical cord samples or maternal only when more than the parent provided samples). In addition, other related publications (e.g., Gyllenhammar et al. (2017)) or additional information or data provided by study authors were used.

Sixteen of the 37 studies reporting mean BWT changes in relation to PFOA in the overall population were rated *high* in overall study confidence (Luo et al., 2021; Chu et al., 2020; Eick et al., 2020; Wikström et al., 2020; Bell et al., 2018; Buck Louis 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; Wang et al., 2016), while 13 were rated *medium* (Chang et al., 2022; Chen et al., 2021; Yao et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018; 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 eight 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; Wu et al., 2012) as shown in Figure 3-47, Figure 3-48, and Figure 3-49.

Of the 29 *high* or *medium* confidence studies highlighted in this synthesis, two had deficient study sensitivity (Bell et al., 2018; de Cock et al., 2016). Nine studies (Chen et al., 2021; Yao et al., 2021; Wikström et al., 2020; Lauritzen et al., 2017; Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016; Wang et al., 2016; Robledo et al., 2015) were considered to have good study sensitivity, and 18 studies (Chang et al., 2022; Luo et al., 2021; Chu et al., 2020; Eick et al., 2020; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Buck Louis et al., 2018; Gyllenhammar et al., 2018; Meng et al., 2018; Sagiv et al., 2018; Ashley-Martin et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Bach et al., 2016; Govarts et al., 2016; Kwon et al., 2016) were considered adequate. The median exposure values across all studies ranged from 0.86 ng/mL (Callan et al., 2016) to 42.8 ng/mL (Yao et al., 2021).

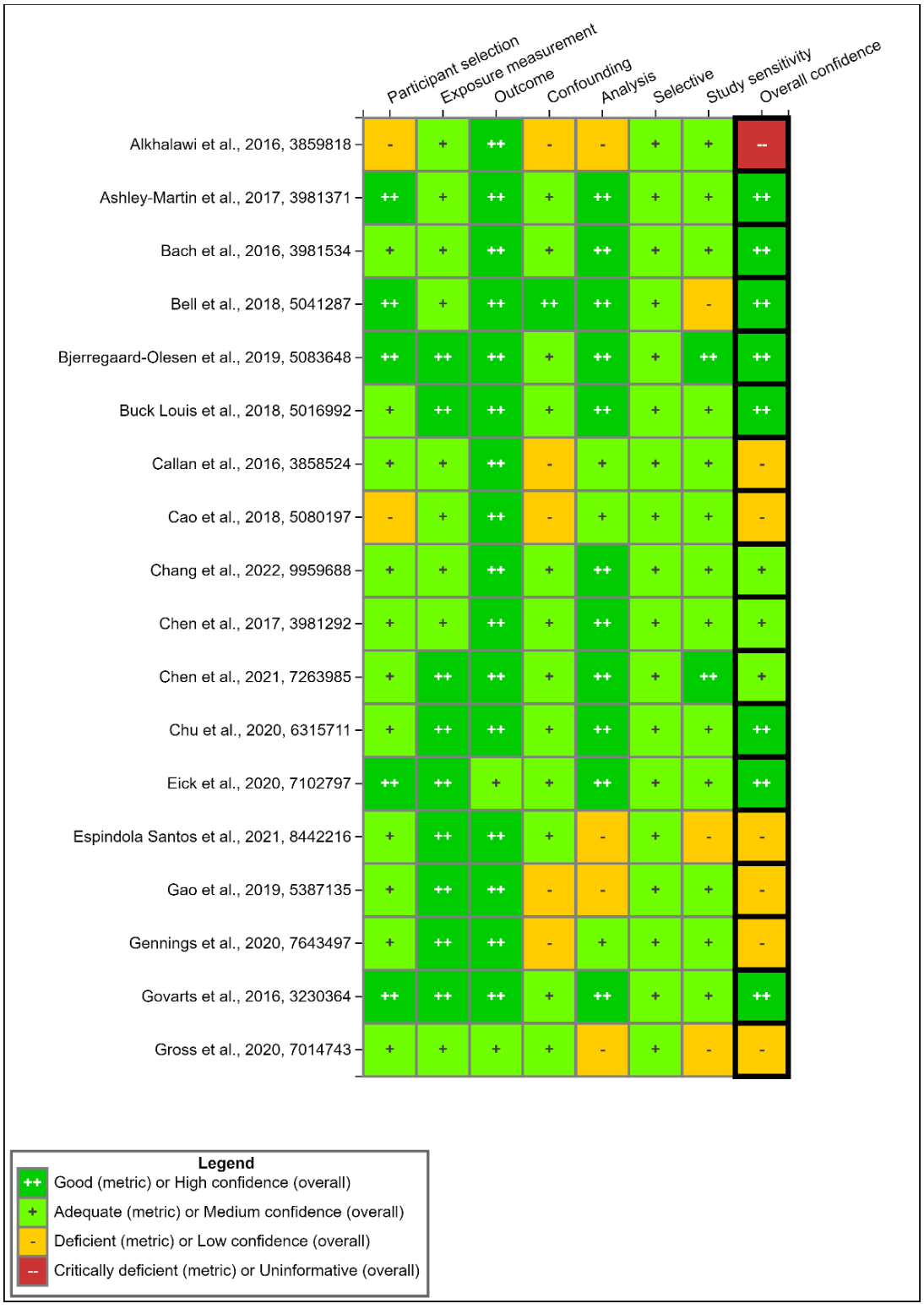


Figure 3-47. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Weight Effects

Interactive figure and additional study details available on HAWC.

Figure 3-48. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Birth Weight Effects (Continued)

Interactive figure and additional study details available on HAWC.

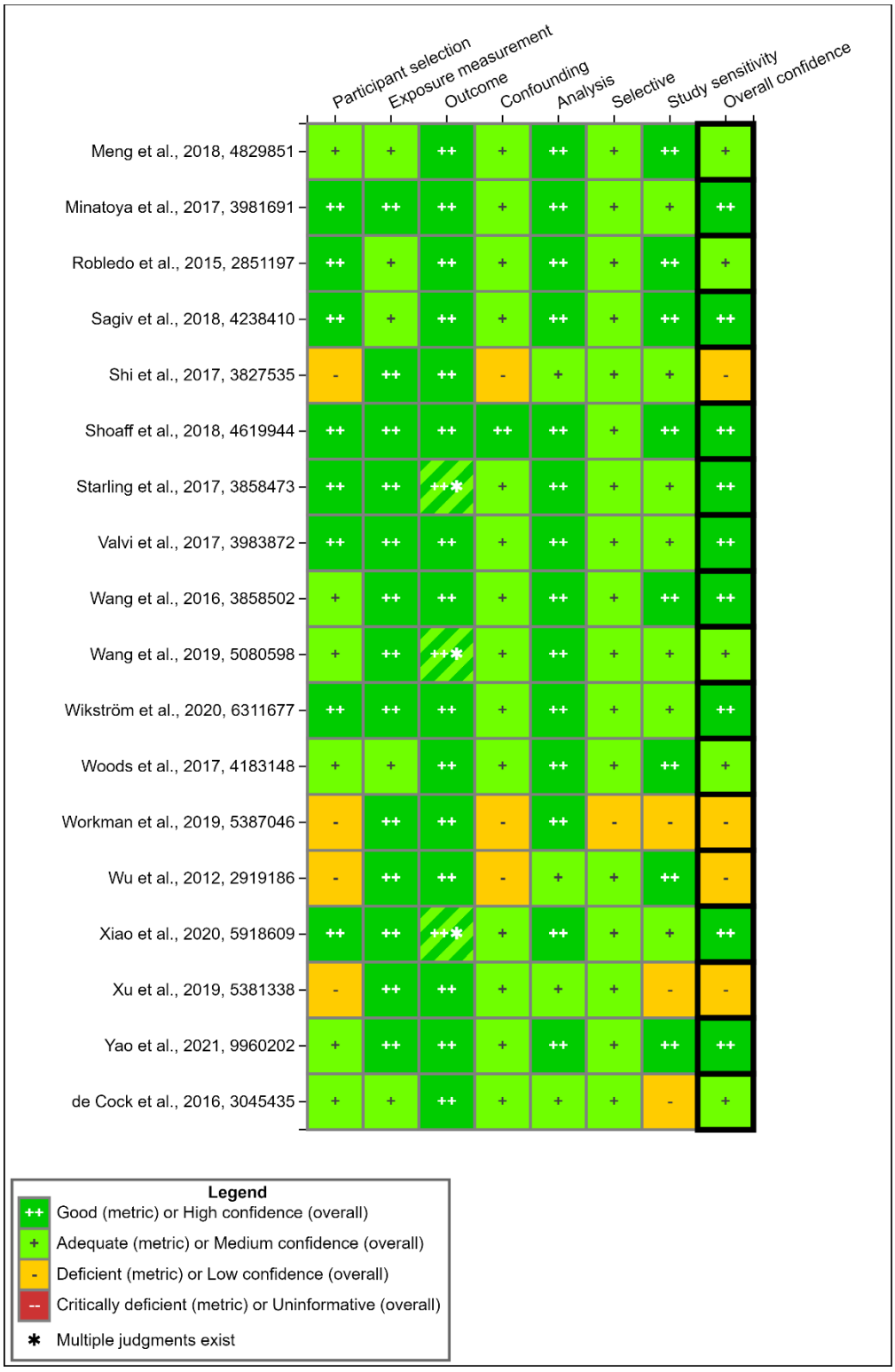


Figure 3-49. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA and Birth Weight Effects (Continued)

Interactive figure and additional study details available on HAWC.

3.4.4.1.4.1.1 Mean Birth Weight Study Results: Overall Population Studies

Thirty-two of the 37 included studies with mean BWT data that examined 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; Buck Louis et al., 2018; Cao et al., 2018; Gyllenhammar et al., 2018; 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; Wang et al., 2016; Robledo et al., 2015; Wu et al., 2012), while five reported sex-specific data only (Marks et al., 2019; Ashley-Martin et al., 2017; Lind et al., 2017a; Wang et al., 2016; Robledo et al., 2015). Twenty-one of the 32 PFOA studies reported some mean BWT deficits in the overall population, albeit these were not always statistically significant (see Appendix, (U.S. EPA, 2024a)). Five of these mean BWT studies in the overall population reported null associations (Bell et al., 2018; Buck Louis et al., 2018; Valvi et al., 2017; Woods et al., 2017; Bach et al., 2016), while six reported increased mean BWT deficits with increasing PFOA exposures (Chen et al., 2021; Eick et al., 2020; Gao et al., 2019; Xu et al., 2019a; Shi et al., 2017; de Cock et al., 2016). Seventeen of the 25 *medium* and *high* confidence studies reported some BWT deficits in relation to PFOA exposures. Among the 10 studies presenting results based on categorical data, two studies (Meng et al., 2018; Starling et al., 2017) showed inverse monotonic exposure-response relationships (Figure 3-50, Figure 3-51, Figure 3-52, and Figure 3-53).

Among the 21 studies showing some inverse associations in the overall population, there was a wide distribution of deficits ranging from –14 to –267 grams across both categorical and continuous exposure estimates with results based on a per unit (continuous measure) when studies presented both. Among those with continuous PFOA results in the overall population, 14 of 20 studies reported deficits from –27 to –82 grams with increasing PFOA exposures. There were no clear patterns were observed by confidence level, but there was a preponderance of inverse associations based on studies with later biomarker sampling timing (i.e., trimester two onward) including 15 of the overall 21 studies and 6 of the 9 *high* confidence studies. The two largest associations (one *medium* and one *low* confidence study) expressed per each PFOA change were detected in studies with later pregnancy samples, while three of the four smallest associations were based on earlier biomarker samples. Thus, some of these reported results may be related to pregnancy hemodynamic influences on the PFOA biomarkers during pregnancy. For example, 11 of the 12 largest mean BWT deficits (–48 grams or larger per unit change) in the overall population were detected among studies with either later pregnancy samples (i.e., maternal samples during trimesters 2, 3, or post-partum or umbilical cord samples). However, five (Chang et al., 2022; Wikström et al., 2020; Hjermitslev et al., 2019; Meng et al., 2018; Sagiv et al., 2018) of nine *medium* and *high* confidence studies still reported some evidence of reductions in mean BWT based on early pregnancy biomarker samples.

Sampling Period	Reference, Confidence Rating	Study design	Exposure Matrix	Sub-population	Exposure levels	Comparison	EE
Early pregnancy	Bach et al. (2016, 3981534), High	Cohort	maternal serum	Term births (GA >= 37 weeks)	median=2.0 ng/mL (25th-75th percentile: 1.5-2.6 ng/mL)	Regression coefficient for Q2 (1.54-2.02 ng/mL) vs. Q1 (<1.54 ng/mL)	-36
						Regression coefficient for Q3 (2.03-2.64 ng/mL) vs. Q1 (<1.54 ng/mL)	-7
						Regression coefficient for Q4 (2.65-15.10 ng/mL) vs. Q1 (<1.54 ng/mL)	4
						Regression coefficient per IQR (1.1 ng/mL) increase	13
	Buck Louis et al. (2018, 5016992), High	Cohort	maternal blood	--	Median (25th-75th percentiles): 1.985 ng/mL (1.297-3.001 ng/mL)	Regression coefficient (change in birth weight per SD increase in log-PFOA)	-5.9
Later pregnancy	Bell et al. (2018, 5041287), High	Cross-sectional	blood	Singleton	median=1.10 ng/mL (25th-75th percentile: 0.69-1.63 ng/mL)	Regression coefficient (per log(PFOA+1) unit increase)	-11.6
	Chu et al. (2020, 6315711), High	Cohort	maternal serum	--	median=1.538 ng/mL (25th percentile=0.957 ng/mL, 75th percentile=2.635 ng/mL)	Regression coefficient (per 1 ln change in PFOA)	-73.6
	Eick et al. (2020, 7102797), High	Cohort	serum	full-term births	median= 0.76 ng/mL (25th-75th percentile= 0.46 - 1.12 ng/mL)	Regression Coefficient [for T2 (1.40-1.96 ng/ml) vs. T1 (<1.40 ng/ml)]	62.9
						Regression Coefficient [for T3 (>1.96 ng/ml) vs. T1 (<1.40 ng/ml)]	86.1
	Govarts et al. (2016, 3230364), High	Cohort	cord blood	--	geometric mean = 1.52 ug/L (25th-75th percentile = 1.10-2.10 ug/L)	Regression coefficient (per IQR change in PFOA z-score)	-34.5

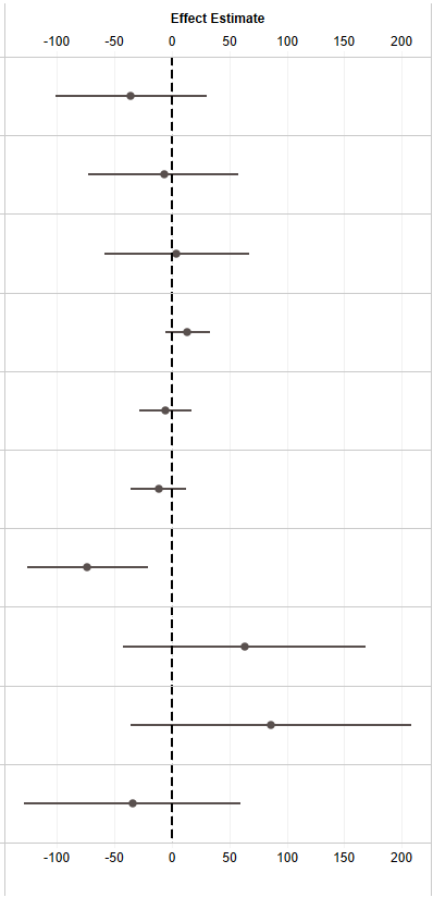


Figure 3-50. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA

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): 2.35 ng/mL (1.25 ng/mL)	Regression coefficient (change in birth weight per doubling of PFOA)	-9.3
						Regression coefficient for birth weight (Q2 vs Q1)	-29.6
						Regression coefficient for birth weight (Q3 vs Q1)	-33
						Regression coefficient for birth weight (Q4 vs Q1)	-32.8
	Sagiv et al. (2018, 4238410), High	Cohort	maternal blood	--	median=25.7 ng/mL (IQR: 16.0 ng/mL)	Regression coefficient per IQR increase	-18.5
						Regression coefficient (for Q2 [4.2 - 5.8 ng/mL] vs Q1 [0.3 - 4.1 ng/mL])	-8.3
						Regression coefficient (for Q3 vs Q1)	-60.3
						Regression coefficient (for Q4 vs Q1)	-36.7
Later pregnancy	Lauritzen et al. (2017, 3981410) High	Cohort	maternal serum	--	Norway: median=1.62 ng/mL (range: 0.31-7.97 ng/mL); Sweden: median=2.33 ng/mL (range: 0.60-6.70 ng/mL)	Regression coefficient per unit increase in lnPFOA	-81.7
	Luo et al. (2021, 9959610), High	Cohort	maternal blood, cord blood	--	median (25th-75th percentile): 3.51 ng/mL (2.23-4.80)	Regression coefficient (per ln-ng/mL increase PFOA)	-62.4
	Starling et al. (2017, 3858473), High	Cohort	maternal serum	--	median=1.1 ng/mL (25th percentile=0.7, 75th percentile=1.6)	Regression coefficient (per 1 ln increase in PFOA)	-51.4
						Regression coefficient for tertile 2 (0.9-1.4 ng/mL) vs tertile 1 (0.1-0.8 ng/mL)	-15.9
						Regression coefficient for tertile 3 (1.4-17.0 ng/mL) vs. tertile 1 (0.1-0.8 ng/mL)	-92.4

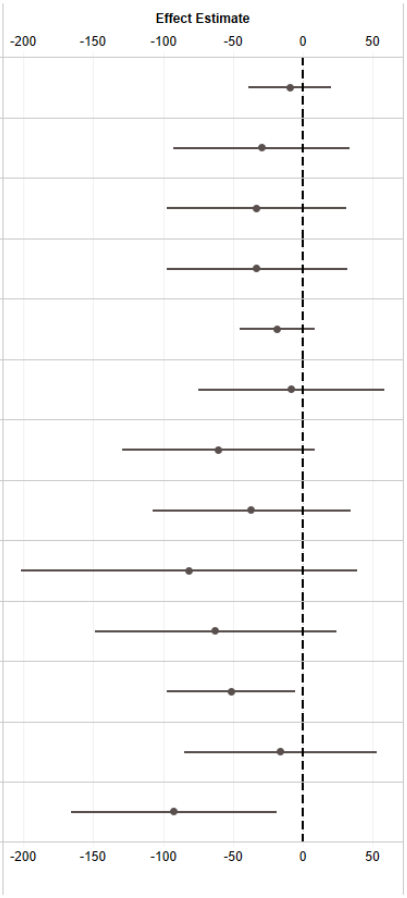


Figure 3-51. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

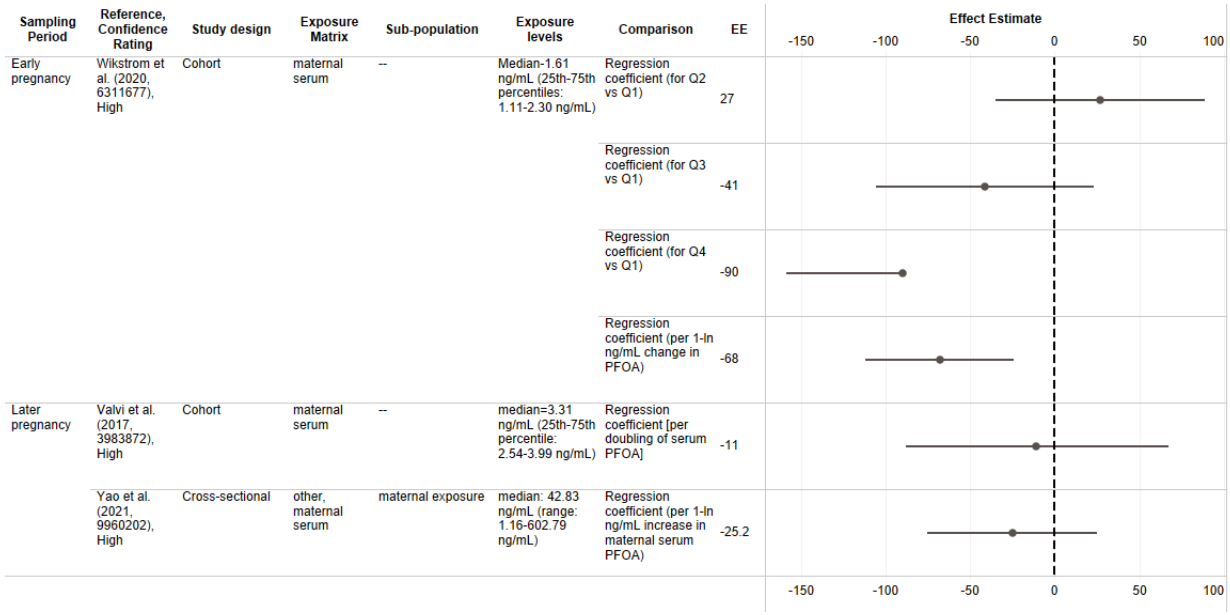


Figure 3-52. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.
Wikström et al. (2020) has a manuscript error in the regression coefficient for Q4 vs. Q1.

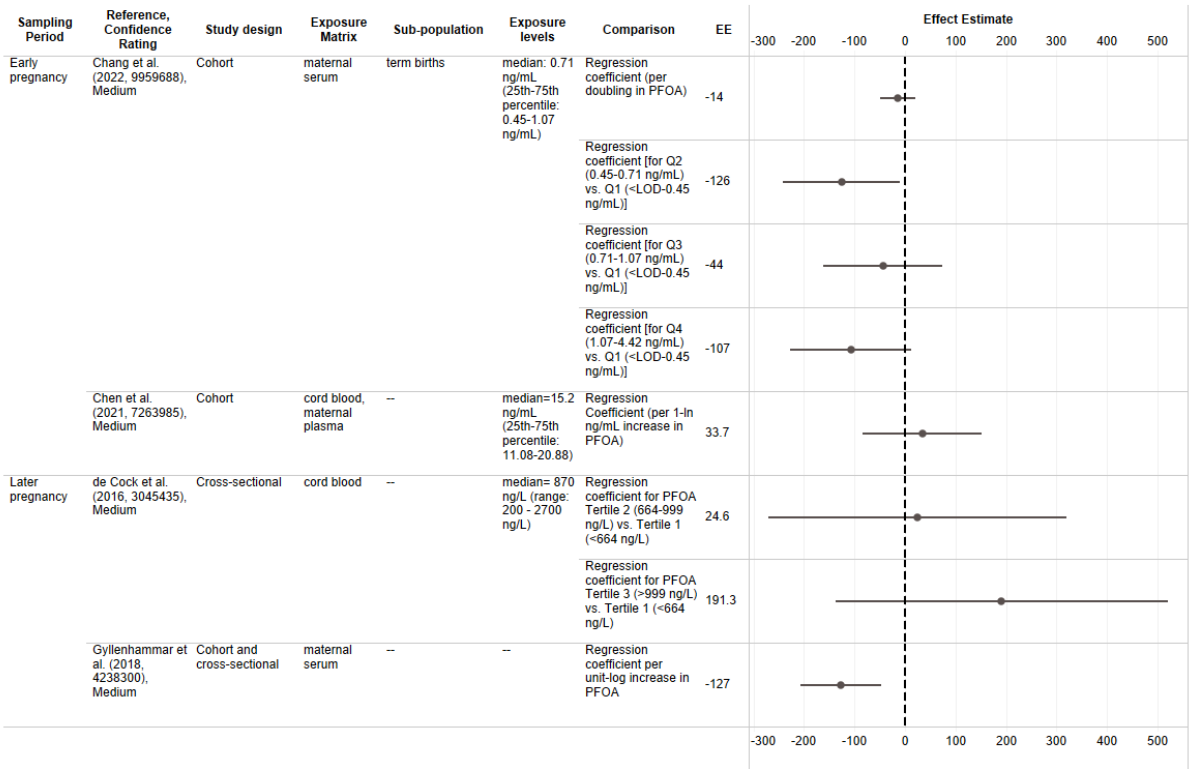


Figure 3-53. Overall Mean Birth Weight from Epidemiology Studies Following Exposure to PFOA (Continued)

Interactive figure and additional study details available on HAWC.

3.4.4.1.4.1.2 Mean BWT-Overall Population Summary

Overall, 21 of the 32 PFOA studies reported some mean BWT deficits in the overall population with limited evidence of exposure-response relationships. Seventeen of the 21 studies were *medium* or *high* confidence (out of 25 in total), but the majority of studies that showed inverse associations were based on later biomarker sampling timing (i.e., trimester two onward). While some of the changes were relatively large in magnitude (most were from –27 to –82 grams per each unit PFOA change), there was also a pattern of stronger associations detected amongst studies with later pregnancy biomarker samples. These patterns may be indicative of pregnancy hemodynamic influences on the PFOA biomarkers during pregnancy.

3.4.4.1.4.1.3 Mean Birth Weight Study Results: Sex-Specific Studies

Mean BWT findings were reported for 18 and 19 studies in female and male neonates, respectively. Eleven of 18 epidemiological studies examining sex-specific results in female neonates showed some BWT deficits including 10 of 16 *medium* and *high* confidence studies. Twelve of 19 *medium* and *high* confidence epidemiological studies examining sex-specific results in male neonates showed some BWT deficits. The remaining 7 studies (Hjermitslev et al., 2019; Wang et al., 2019; Lind et al., 2017a; Shi et al., 2017; Bach et al., 2016; de Cock et al., 2016; Robledo et al., 2015) in male neonates were either null or showed larger birth weights with increasing PFOA exposures. The *low* confidence study by Marks et al. (2019) of boys only reported large deficits in the upper two PFOA tertiles (−53 and −46 grams, respectively) with no exposure-response relationship. None of the other five studies with categorical data in either girls or boys showed evidence of monotonic exposure-response relationships.

Nine of the 18 studies examining mean BWT associations in both boys and girls detected some deficits in both sexes with one of these reporting comparable BWT deficits (Lenters et al., 2016). Five of the 9 studies showed larger deficits in girls (Wikström et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Cao et al., 2018; Ashley-Martin et al., 2017) and 3 showed larger deficits among boys (Chu et al., 2020; Meng et al., 2018; Lauritzen et al., 2017). One study showed comparable results irrespective of sex (Lenters et al., 2016). Three additional studies each reported mean BWT deficits either only in boys (Kashino et al., 2020; Manzano-Salgado et al., 2017a; Valvi et al., 2017) or girls (Hjermitslev et al., 2019; Wang et al., 2016; Robledo et al., 2015).

Overall, no consistent patterns in magnitude of deficits were observed with the sex-specific studies by sample timing and other study characteristics; however, the three largest deficits in male studies were later pregnancy sampled studies. Although other studies based on different exposure measures were more variable, some consistency in the magnitude of deficits (range: −80 to −90 g) was observed among four studies in girls (Wikström et al., 2020; Wang et al., 2019; Ashley-Martin et al., 2017; Wang et al., 2016) including three *high* confidence studies based on analyses of continuous PFOA measurements (i.e., per each ln or log10 PFOA exposures increase). The magnitude of deficits in boys across 7 studies (Kashino et al., 2020; Wikström et al., 2020; Wang et al., 2019; Meng et al., 2018; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017a; Lenters et al., 2016) was fairly consistent per each continuous unit PFOA change (range: −21 to −49 g), although 3 studies (Chu et al., 2020; Lauritzen et al., 2017; Valvi et al., 2017) reported larger deficits in excess of –71 grams.

3.4.4.1.4.1.4 Standardized Birth Weight Measures

Fifteen studies examined standardized BWT measures including 14 studies reporting changes in standardized BWT scores on a continuous scale per each PFOA comparison. Eight of the 15 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), 4 were *medium* (Wang et al., 2019; Gyllenhammar et al., 2018; Meng et al., 2018; Chen et al., 2017b) and 3 were *low* confidence (Espindola-Santos et al., 2021; Gross et al., 2020; Workman et al., 2019).

Eight out of 15 studies with standardized BWT scores in the overall population showed some inverse associations and 5 of these were *high* confidence. The *high* confidence study by Gardener et al. (2021) reported that participants in PFOA quartiles 2 (OR = 0.84; 95% CI: 0.40–1.80) and 3 (OR = 0.91; 95% CI: 0.41–2.02) had a lower odds of being in the lowest standardized birth weight category (vs. the top 3 birth weight z-score quartiles). They also reported that there were no statistically significant interactions for their BWT z-score measures by sex.

Among the 14 studies examining continuous standardized BWT measures in the overall population, 8 showed some inverse associations of at least –0.1. The ranges of deficits were –0.1 (Wang et al., 2019; Sagiv et al., 2018; Ashley-Martin et al., 2017), –0.2 (Wikström et al., 2020; Shoaff et al., 2018; Chen et al., 2017b), and –0.3 (Gross et al., 2020; Xiao et al., 2019). More associations were detected among the *high* confidence studies (5/8), compared with 2 of the 4 *medium*, and 1 of the 3 *low* confidence studies. None of the 5 studies (Eick et al., 2020; Wikström et al., 2020; Sagiv et al., 2018; Shoaff et al., 2018; Bach et al., 2016) showed any evidence of exposure-response relationships. Overall, four out of six studies in boys (Gross et al., 2020; Wikström et al., 2020; Xiao et al., 2019; Chen et al., 2017b) and 3 of 5 in girls (Gross et al., 2020; Wikström et al., 2020; Xiao et al., 2019) showed lower BWT z-scores with increasing PFOA exposures. For example, the *low* confidence study by Gross et al. (2020) reported BWT z-score deficits in both sexes (males β: –0.17; SE = 0.29; p-value = 0.57; females β: –0.38; SE = 0.26; p-value = 0.16) for PFOA levels greater than the mean level. Gardener et al. (2021) only reported that there were no statistically significant interactions for standardized BWT measures by sex in their analysis.

3.4.4.1.4.1.5 Standardized BWT summary

Eight out of 15 studies with standardized BWT scores in the overall population showed some inverse associations with PFOA exposures. Seven of these 8 studies were either *medium* or *high* confidence studies (of 17 in total), and most of these had moderate or large exposure contrasts. Although some studies may have been underpowered to detect associations small in magnitude relative to PFOA exposure, there was consistent lower BWT z-scores reported across all confidence levels. There was no apparent pattern related to magnitude of deficits across study confidence, but more associations were evident across *high* confidence levels in general. Many studies (5 of 8) showing inverse associations were based on later(Gross et al., 2020; Wang et al., 2019; Xiao et al., 2019; Shoaff et al., 2018; Chen et al., 2017b) 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. There was no evidence of exposure-response relationships in the 5 studies reporting categorical data. There were also few evident patterns and minimal differences seen across sexes. Overall, 9 out of 15

overall studies in the overall population showed some suggestion of inverse associations with the same studies showing associations in 4 out of 5 studies of male neonates and 3 of 5 studies in females.

3.4.4.1.4.2 Small for Gestational Age/Low Birth Weight

Eleven informative and two *uninformative* non-overlapping epidemiological studies examined associations between PFOA exposure and different dichotomous fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints), low birth weight (LBW), or both (i.e., (Manzano-Salgado et al., 2017a)) (Figure 3-54). Five studies were rated *high* confidence (Chu et al., 2020; Wikström et al., 2020; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wang et al., 2016), three were rated *medium* confidence (Govarts et al., 2018 Hjermitslev, 2020, 5880849; Meng et al., 2018), three were low confidence studies (Chang et al., 2022; Souza et al., 2020; Xu et al., 2019a) and two were *uninformative* (Gundacker et al., 2021; Arbuckle et al., 2013). Of the informative studies, four studies had good study sensitivity (Meng et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wang et al., 2016), four were considered adequate (Chang et al., 2022; Chu et al., 2020; Wikström et al., 2020; Hjermitslev et al., 2019) and three were deficient (Souza et al., 2020; Xu et al., 2019a; Govarts et al., 2018).

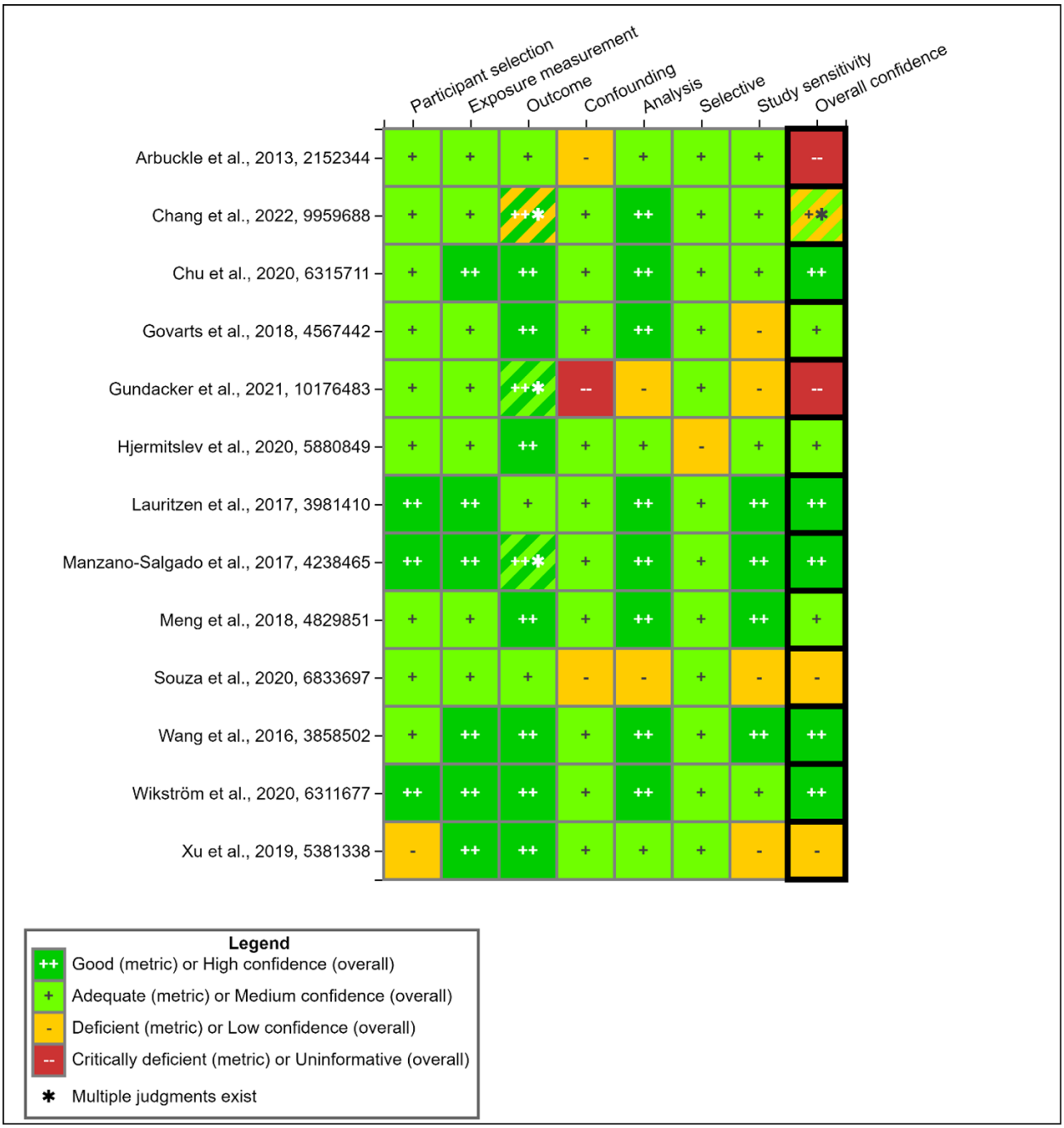


Figure 3-54. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Small for Gestational Age and Low Birth Weight Effects[a]

Interactive figure and additional study details available on HAWC.
[a] Manzano-Salgado et al. (2017a): *High* confidence for SGA; *medium* confidence for LBW.

Six of eight SGA studies (Chang et al., 2022; Souza et al., 2020; Wikström et al., 2020; Govarts et al., 2018; Lauritzen et al., 2017; Wang et al., 2016) showed some increased risk, while two studies were entirely null (Xu et al., 2019a; Manzano-Salgado et al., 2017a) (Figure 3-55, Figure 3-56, Figure 3-57). Although they were not always statistically significant, the relative risks reported in the five studies examining the overall population based on either categorical or

continuous exposures (per each unit increase) were fairly consistent in magnitude (odds ratio (OR) range: 1.21 to 2.81). The *medium* confidence study by Govarts et al. (2018) reported an increased risk (OR = 1.64; 95% CI: 0.97, 2.76) per each PFOA IQR increase. The *high* confidence study by Lauritzen et al. (2017) showed a slight increased risk in the overall population (OR = 1.21; 95% CI: 0.69, 2.11 per each ln-unit PFOA increase), but this was driven by associations only in participants from Sweden (OR = 5.25; 95% CI: 1.68, 16.4) including large risks detected for both girls and boys. One (Souza et al., 2020) of the three studies examining exposure quartiles detected an exposure-response relationship in the overall population (OR range: 1.26–2.81). The *medium* confidence study by Chang et al. (2022) reported nonmonotonic but consistent statistically significant ORs across the upper three quartiles (range: 2.22–2.44) in their study of African American pregnant women. The *high* confidence study by Wikström et al. (2020) reported comparable ORs for the 4th quartile (OR = 1.44; 95% CI: 0.86, 2.40) as well as per each per ln-unit increase (OR = 1.43; 95% CI: 1.03, 1.99). Among females only, they reported a twofold increased risk per each ln-unit increase risk (OR = 1.96; 95% CI: 1.18, 3.28) and nonmonotonic increased risks in the upper two quartiles (OR range: 1.64–2.33). The *high* confidence study by Wang et al. (2016) only reported sex-specific results but also showed an increased risk (OR = 1.48; 95% CI: 0.63, 3.48 per each ln-unit increase) for SGA among girls only. SGA findings from *low* confidence studies are not included in figures.

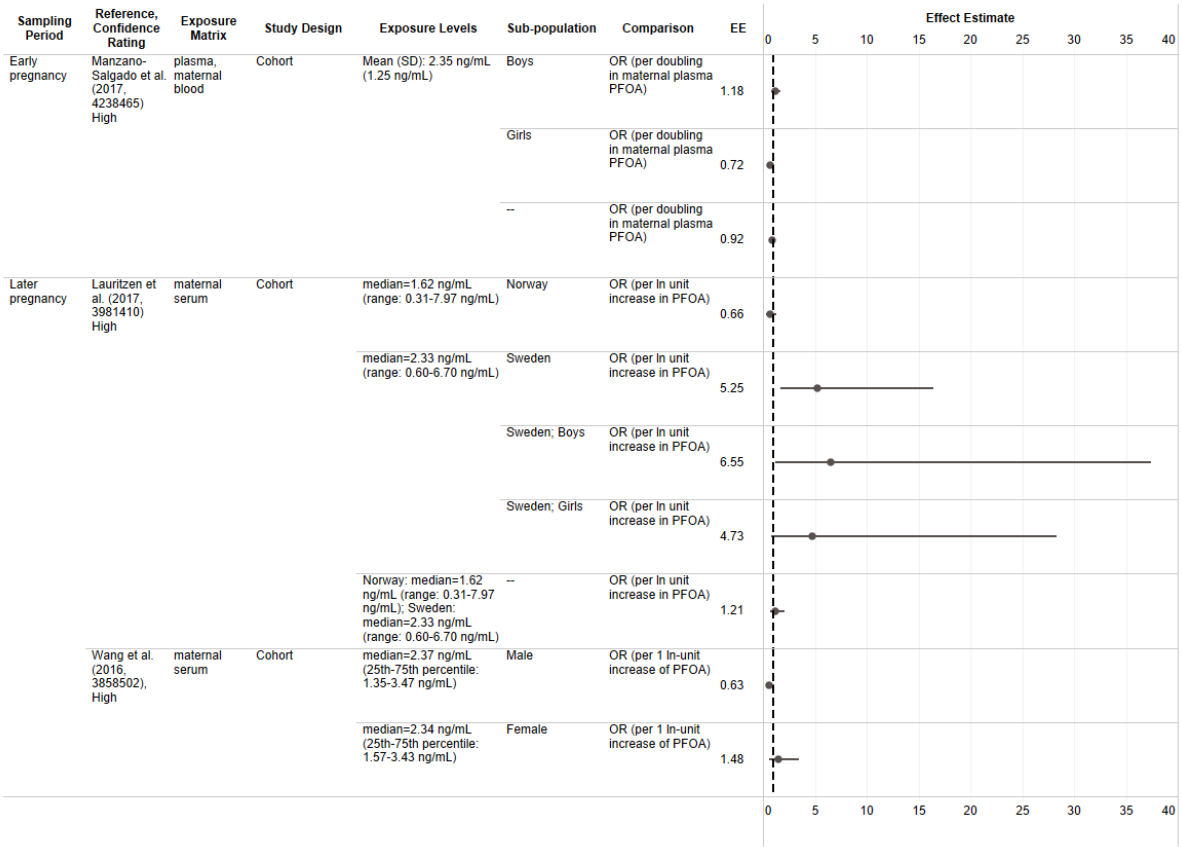


Figure 3-55. Odds of Small for Gestational Age in Children from High Confidence Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.

Small-for-gestational-age defined as birthweight below the 10th percentile for the reference population.

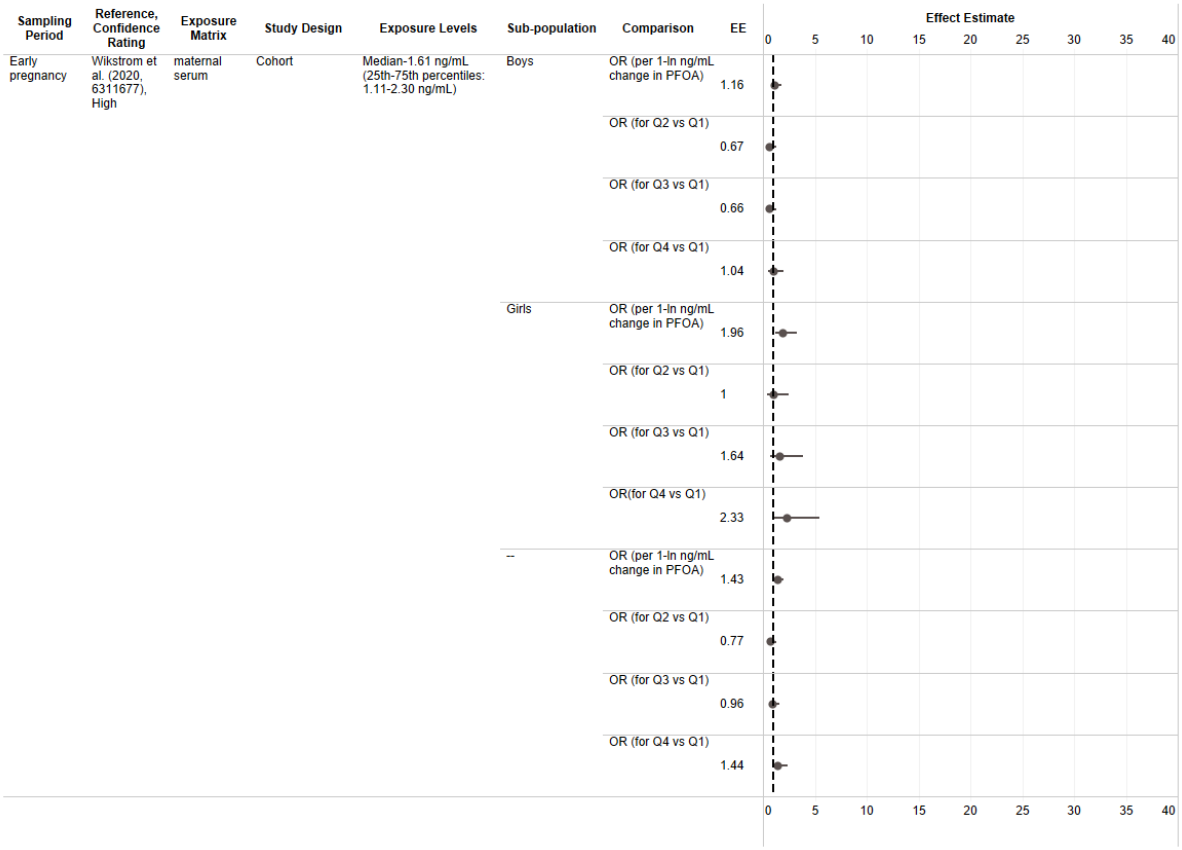


Figure 3-56. Odds of Small for Gestational Age in Children from High Confidence Epidemiology Studies Following Exposure to PFOA (Continued)

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

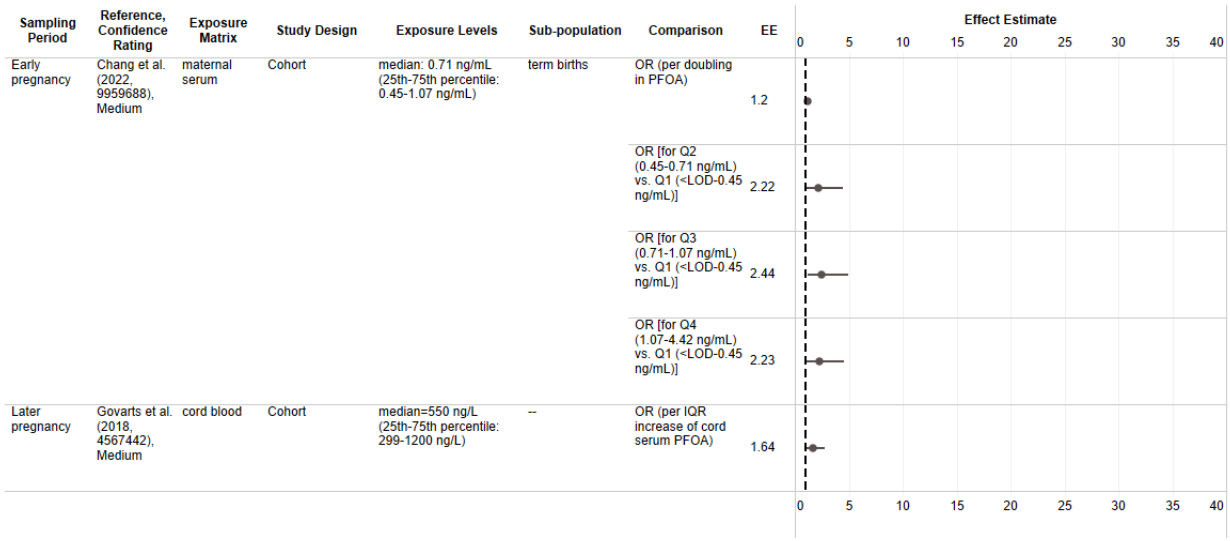


Figure 3-57. Odds of Small for Gestational Age in Children from Medium Confidence Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
Odds of Small-for-gestational-age in Children from *Medium* Confidence Epidemiology Studies Following Exposure to PFOA

Four studies examined LBW in relation to PFOA including two each that were rated *high* (Chu et al., 2020; Manzano-Salgado et al., 2017a) or *medium* confidence (Hjermitslev et al., 2019; Meng et al., 2018) confidence. Two of four LBW studies (Meng et al., 2018; Manzano-Salgado et al., 2017a) showed some associations within the overall population, and/or in boys or girls (Figure 3-58). The *medium* confidence study by Meng et al. (2018) reported nonsignificant increased ORs (range: 1.2–1.5) across all quartiles but saw no evidence of an exposure-response relationship. The *high* confidence Manzano-Salgado (Manzano-Salgado et al., 2017a) study showed some suggestion of an increased risk (OR = 1.67; 95% CI: 0.72, 3.86) for term LBW in boys only.

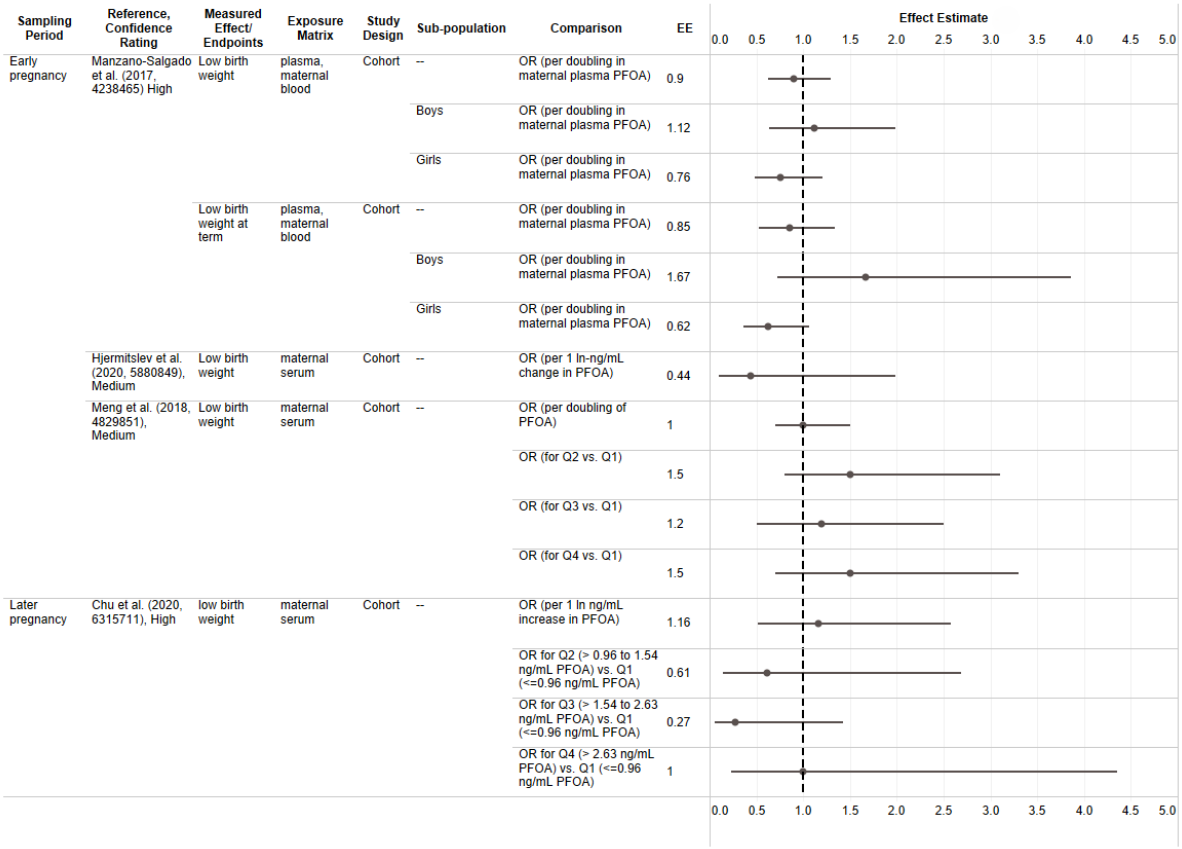


Figure 3-58. Odds of Low Birthweight in Children from Epidemiology Studies Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
Low birthweight defined as birthweight <2,500 g.

Overall, eight of the 11 informative studies reporting main effects for either SGA or LBW or both showed some increased risks with increasing PFOA exposures. The magnitude of the associations was typically from 1.2 to 2.8 with limited evidence of exposure-response relationships among the studies with categorical data. Although the number of studies was fairly small, few discernible patterns across study characteristics or confidence ratings were evident across the SGA or LBW findings. For example, four (Chang et al., 2022; Wikström et al., 2020; Meng et al., 2018; Manzano-Salgado et al., 2017a) of the eight studies showing increased odds of either SGA or LBW were based on early sampling biomarkers, suggesting the results were not overly influenced by pregnancy hemodynamics. Collectively, the majority (8 of 11) of epidemiological studies were supportive of an increased risk of either SGA or LBW with increasing PFOA exposures.

3.4.4.1.4.3 Birth Length

As shown in Figure 3-59 and Figure 3-60, 34 birth length studies were considered as part of the study evaluation. 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. Among the 26 non-overlapping informative studies examined birth length in relation to PFOA, including five studies with standardized birth length measures (Espindola-Santos et al., 2021; Xiao et al., 2019; Gyllenhammar et al., 2018; Shoaff et al., 2018; Chen et al., 2017b), and one study evaluated standardized and mean birth length changes (Workman et al., 2019). Eighteen studies examined mean birth length differences in the overall study population. 13 studies examined sex-specific data with three studies (Marks et al., 2019; Wang et al., 2016; Robledo et al., 2015) reporting only sex-specific results.

Nine of the 26 studies were *high* confidence (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Bell et al., 2018; Buck Louis et al., 2018; Shoaff et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Wang et al., 2016), eight were *medium* (Chen et al., 2021; Luo et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018; Chen et al., 2017b; Robledo et al., 2015) and nine were *low* confidence (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; Wu et al., 2012). Eight PFOA studies had good study sensitivity (Chen et al., 2021; Bjerregaard-Olesen et al., 2019; Shoaff et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wang et al., 2016; Robledo et al., 2015; Wu et al., 2012), 14 had adequate (Luo et al., 2021; Kashino et al., 2020; Gao et al., 2019; Hjermitslev et al., 2019; Marks et al., 2019; Wang et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Cao et al., 2018; Gyllenhammar et al., 2018; Chen et al., 2017b; Shi et al., 2017; Valvi et al., 2017; Callan et al., 2016) sensitivity and four (Espindola-Santos et al., 2021; Workman et al., 2019; Xu et al., 2019a; Bell et al., 2018) considered deficient.

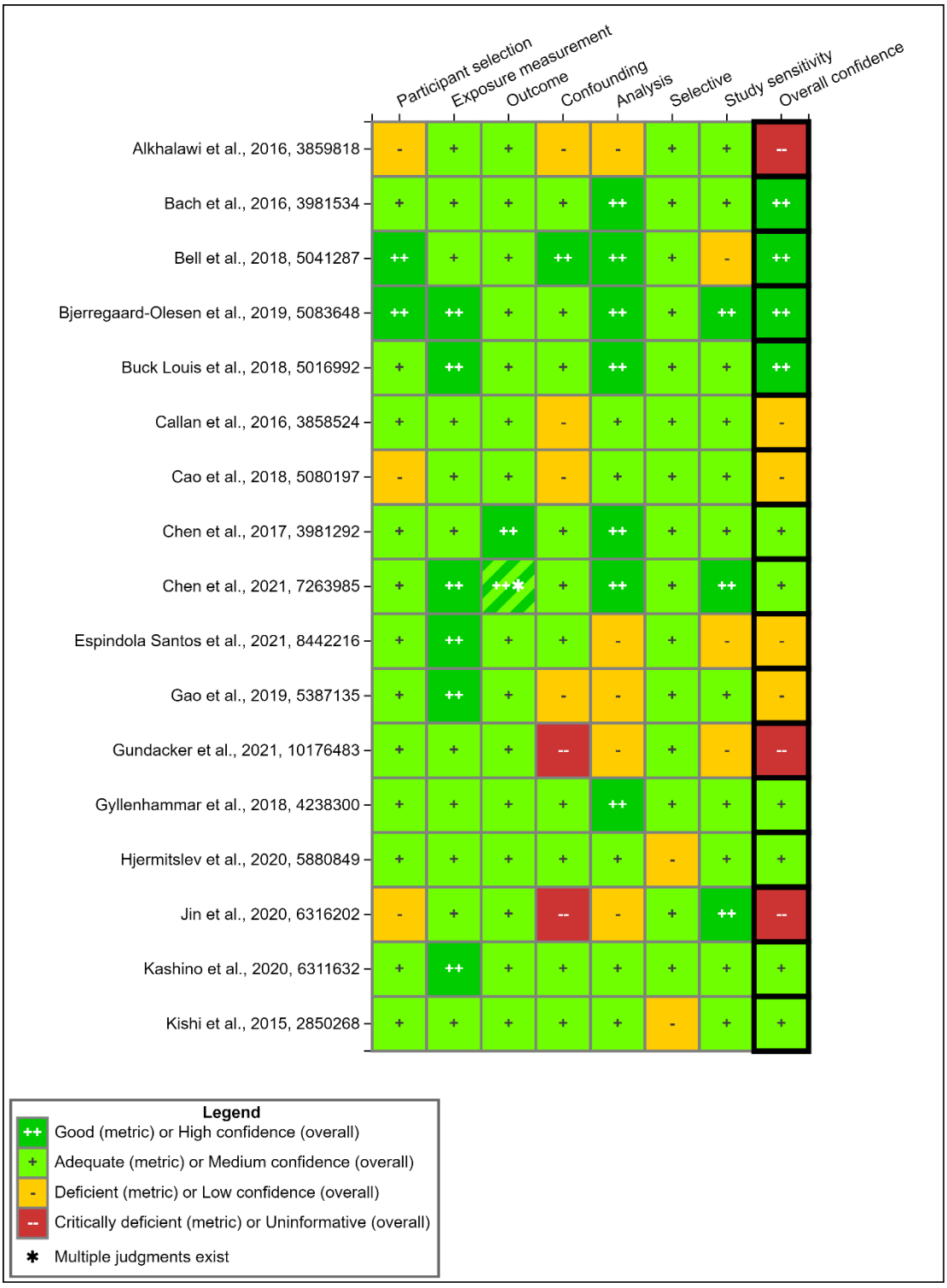


Figure 3-59. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Length Effects

Interactive figure and additional study details available on HAWC.

Figure 3-60. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Length Effects (Continued)

Interactive figure and additional study details available on HAWC.

Amongst the 26 birth length studies (examining mean differences or changes in standardized scores), nine of them reported some inverse associations including three of the six studies that reported standardized birth length data. There was limited evidence of exposure-response relationships in the three studies that examined categorical data. The *high* confidence study by Xiao et al. (2019) reported a reduced birth length z-score (β per log2 increase in PFOA: −0.14; 95% CI: −0.40, 0.13) in the overall population that appeared to be driven by male neonates (β: −0.27; 95% CI: −0.65, 0.10). The *low* confidence Workman et al. (Workman et al., 2019) study reported a nonsignificant deficit similar in magnitude (β: –0.26; 95% CI: −1.13, 0.61). The other study *high* confidence study by Shoaff et al. (2018) of standardized birth length measures showed a deficit only for tertile 3 (β: −0.32; 95% CI: −0.72, 0.07) compared with tertile 1. In contrast, the *low* confidence study by Espindola-Santos et al. (2021) reported a larger birth length z-score (β per $\log_{10}$ PFOA increase: 0.26; 95% CI: -0.21, 0.73).

Among the 21 studies examining mean birth length differences, eight different studies showed inverse associations. This included six different studies (out of 18) based on the overall population as well two out of three studies (Wang et al., 2016; Robledo et al., 2015) reporting only sex-specific results. The *high* confidence study by Wang et al. (2016) only showed deficits among females for only PFOA quartiles 1 (β: −0.39 cm; 95% CI: −1.80, 1.02) and 3 (β: −0.60 cm; 95% CI: −1.98, 0.77). The *medium* confidence study by Chen et al. (Chen et al., 2021) reported similar birth length deficits in the overall population (β per ln-unit PFOA increase: –0.27 cm; 95% CI: –0.61, 0.07), males (β: –0.21; 95% CI: –0.73, 0.32) and females (β: –0.21; 95% CI: –0.74, 0.33). In the *medium* confidence study by Robledo et al. (2015), smaller deficits in birth length were detected for both male and female neonates per each 1 standard deviation (SD) PFOA increase. The *high* confidence study by Lauritzen et al. (2017) showed a deficit in the overall population (β: −0.49 cm; 95% CI: −0.99, 0.02), but detected the strongest association when restricted to the Swedish population (β: −1.2 cm; 95% CI: −2.1, −0.3) and especially Swedish boys (β: −1.6 cm; 95% CI: −2.9, −0.4). Overall, four sex-specific studies showed deficits for both boys and girls with two studies showing larger deficits among boys. One study showed larger deficits amongst girls and the fourth study showed results equal in magnitude.

In the overall population studies showing inverse associations, the reported magnitude of deficits was quite variable (range: −0.16 to −1.91 cm). For example, the low confidence study by Wu et al. (2012) showed the largest deficit (β per log10 increase: −1.91 cm; 95% CI: −3.31, −0.52). The *low* confidence study by Cao et al. (2018) showed consistent results across their overall population (β: −0.45 cm; 95% CI: −0.79, −0.10 per each ln-unit PFOA increase), male (β: −0.36 cm; 95% CI: −0.80, 0.09), and female neonates (β: −0.58 cm; 95% CI: −1.12, −0.04) with evidence of exposure-response relationships in all three of these groups. Overall, 6 of 12 studies in girls and 4 of 13 studies in boys showed some birth length deficits. One of the three studies in either or both boys and girls showed some additional evidence of exposure-response relationships. The same study by Cao et al., (Cao et al., 2018) was the only study in the overall population to show evidence of exposure-response.

Overall, 9 different studies out of 26 studies examining birth length reported deficits in relation to PFOA exposures, including 6 *medium* or *high* confidence studies. There was no apparent relationship between studies showing inverse associations and study confidence ratings. However, seven of these studies sampled PFOA biomarkers later in pregnancy (Workman et al., 2019; Xiao et al., 2019; Cao et al., 2018; Shoaff et al., 2018; Lauritzen et al., 2017; Wang et al.,

2016; Wu et al., 2012) and may be more prone to potential bias from pregnancy hemodynamic changes. Among the mean birth length studies, most showed consistent deficits ranging from –0.21 to –0.49 cm per different PFOA comparisons. An unusually large result (β per log10 PFOA increase = –1.91 cm; 95% CI: –3.21, –0.52) was reported in an earlier study (Wu et al., 2012) that reported the largest exposure range. There was a preponderance of inverse associations among females (6 of 12 studies) compared with males (4 of 13); however, amongst the four studies that reported associations in both sexes, more studies reported larger deficits in male neonates.

3.4.4.1.4.4 Head Circumference at Birth

As shown in Figure 3-61, 21 informative studies examined head circumference at birth in relation to PFOA exposures. Six of the 21 studies 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), while seven studies were *medium* (Chen et al., 2021; Kashino et al., 2020; Hjermitslev et al., 2019; Wang et al., 2019; Gyllenhammar et al., 2018; Lind et al., 2017a; Robledo et al., 2015) and eight were *high* confidence (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Bell et al., 2018; Buck Louis et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Valvi et al., 2017; Wang et al., 2016). Four studies were deficient in study sensitivity (Espindola-Santos et al., 2021; Workman et al., 2019; Xu et al., 2019a; Bell et al., 2018), while five were good (Chen et al., 2021; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wang et al., 2016; Robledo et al., 2015) and 12 had adequate study sensitivity (Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Marks et al., 2019; Wang et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Cao et al., 2018; Gyllenhammar et al., 2018; Lind et al., 2017a; Valvi et al., 2017; Callan et al., 2016).

Figure 3-61. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Head Circumference Effects

Interactive figure and additional study details available on HAWC.

Eighteen of the 21 included studies reported PFOA in relation to mean head circumference differences including 17 studies that provided results based on the overall population. Including

the Xiao et al. (2019) z-score data, 13 of these 21 studies reported sex-specific head circumference data with four other studies (Marks et al., 2019; Lind et al., 2017a; Wang et al., 2016; Robledo et al., 2015) providing sex-specific data only.

Among the 21 studies, 10 reported some inverse associations between PFOA exposures and different head circumference measures in the overall population, in either or both male and female neonates, across different racial strata, or different countries in the same study population. For example, the *high* confidence study by Lauritzen et al. (2017) reported a similar deficit only in their Swedish population (β per ln-unit PFOA increase: −0.4 cm; 95% CI: −1.0, 0.1) ; this was largely due to an association seen in male neonates (β: –0.6 cm; 95% CI: –1.3, 0.1). The *high* confidence study by Buck Louis et al. (2018), reported nonsignificant head circumference differences (β: −0.14 cm; 95% CI: −0.29, 0.02) among Black neonates but no main effect association in the overall population. Six out of 17 studies based on the overall population reported some inverse associations between PFOA exposures and either mean head circumference measures or standardized z-scores. The *high* confidence study by Xiao et al. (2019) reported a reduced head circumference z-score (β: −0.17; 95% CI: −0.48, 0.15) in the overall population per each log2 increase in PFOA that appeared to be driven by female neonates (β: −0.30; 95% CI: −0.74, 0.13) (data not shown on figures). Although it was not statistically significant, the *low* confidence study by Espindola-Santos et al. (2021) reported a larger head circumference z-score (β per log10 PFOA increase: 0.62; 95% CI: –0.06, 1.29). The *medium* confidence study by Gyllenhammar et al. (2018) was null based on their standardized head circumference measure.

Among the 14 studies that examined mean head circumference at birth in the overall population, four of them reported inverse associations. Nine studies were largely null, and one study showed larger mean head circumference in the overall population with increasing PFOA exposures. Of the 11 different studies examining sex-specific results associations were observed 5 of 10 in female neonates (Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Wang et al., 2019; Cao et al., 2018; Robledo et al., 2015) and three (Wang et al., 2019; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a) of 11 studies in male neonates. The *medium* confidence study by Wang et al. (2019) reported an association in the overall population (β: −0.37 cm; 95% CI: −0.70, -0.40) with larger deficits noted in female (β: −0.57 cm; 95% CI: −1.07, −0.08) than in male neonates (β: −0.35 cm; 95% CI: −0.79, −0.10). The *medium* confidence study by Hjermitslev et al. (2019) showed a significant reduction in head circumference for the term births in the overall population (β per ng/mL PFOA increase: −0.30 cm; 95% CI: −0.56, –0.04) which seemed to be driven by results in females (β: −0.25 cm; 95% CI: −0.65, 0.14). The *high* confidence study by Manzano-Salgado et al. (2017a) reported a nonsignificant decrease only in quartile 4 (β: −0.16 cm; 95% CI: −0.38, 0.06) compared with quartile 1 from the overall population and a deficit among male neonates only (β per log2 PFOA increase: −0.13 cm; 95% CI: −0.27, 0.0). In the *medium* confidence study by Robledo et al. (2015), opposite results were seen for male (0.18 cm; 95% CI: –0.25, 0.60) and female neonates (β per 1 SD PFOA increase: −0.18 cm; 95% CI: −0.59, 0.23). In their *low* confidence study, Cao et al. (2018) reported an overall null association, while divergent and large changes were seen for male (β per ln-unit PFOA increase: 0.72 cm; 95% CI: –0.51, 1.94) and female neonates (β: −1.46 cm; 95% CI:

−2.96, 0.05). The *low* confidence study by Callan et al. (2016) reported a −0.40 cm (95% CI: −0.96, 0.16) difference per each ln-unit PFOA change.

Among the 21 epidemiological studies examining PFOA and mean differences and standardized measures of head circumference, 10 different studies reported some evidence of inverse associations in the overall population or across sexes or race. This included 4 of 15 studies in the overall population and 5 of 12 sex-specific studies in either or both sexes. No definitive patterns across sex were observed as deficits were found in four or fewer studies in both male and female neonates. Apart from the Wang et al. (2019) study, no other sex-specific studies reported reduced head circumference in both sexes. Few patterns were seen based on study characteristics or overall confidence levels although nearly all of the *high* and *low* confidence studies were null. Among the nine different studies reporting associations across various populations examined there was no definitive pattern of results by biomarker sample timing as five studies relied on early sampling periods (Bjerregaard-Olesen et al., 2019; Hjermitslev et al., 2019; Buck Louis et al., 2018; Manzano-Salgado et al., 2017a; Robledo et al., 2015). This suggests that pregnancy hemodynamics is not fully explaining the inverse asociations detected here.

3.4.4.1.4.5 Fetal Growth Restriction Summary

The majority of studies examining fetal growth restriction showed some evidence of associations with PFOA 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 but still reported many inverse associations. For example, 10 (out of 21) different epidemiological studies of PFOA examining head circumference reported some evidence of inverse associations in either the overall population or across the sexes, which included 8 of 15 *medium* or *high* confidence studies. Nine different studies out of 26 studies reported some birth length deficits in relation to PFOA exposures with limited evidence of exposure-response relationships. This included 6 of 17 *medium* or *high* confidence studies of birth length. Across the fetal growth measures, there was not consistent evidence of sexual dimorphic differences across the fetal growth measures; however, as noted above, many of the individual study results lacked precision and statistical power to detect sex-specific differences that vary considerably in magnitude. There was minimal evidence of exposure-response relationships reported among those examining categorical exposure data, but 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 PFOA exposure metrics considered. For example, nearly two-thirds of studies showed BWT deficits based on differences in means or standardized measures. 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. Associations were also seen for the majority of studies examining SGA and low birth weight measures. The magnitude of some fetal growth measures were at times considered large especially when considering the per unit PFOA increases across the exposure distributions. The range of deficits detected in the overall population across all categorical and continuous exposure estimates ranged from−14 to −267 grams. Among those with continuous PFOA results in the overall population. For example, 14 of the 21 studies reported deficits from −27 to −82 grams in

the overall population based on each unit increase in PFOA exposures. Interestingly, 11 of the 12 largest mean BWT deficits (–48 grams or larger per unit change) in the overall population were detected among studies with later biomarker sampling. However, five (Chang et al., 2022; Wikström et al., 2020; Hjermitslev et al., 2019; Meng et al., 2018; Sagiv et al., 2018) of nine *medium* and *high* confidence studies still reported some evidence of reductions in mean BWT based on early pregnancy biomarker samples.

The current database (since the 2016 PFOA HESD) is fairly strong given the wealth of studies included here with most of them considered *high* or *medium* confidence (e.g., 17 out of 25 mean BWT studies with data in the overall population) and most of them had adequate or good study sensitivity. As noted earlier, one source of uncertainty is that previous 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 later biomarker sampling. For many of these endpoints, such as birth weight measures, there was a preponderance of associations amongst studies with later biomarker samples (i.e., either exclusive trimester 2/3 maternal sample or later, such as umbilical cord or post-partum maternal samples). This would seem to comport with the PFOA meta-analysis by Steenland et al. (2018a) that suggested that results for mean BWT may be impacted by some bias due to pregnancy hemodynamics. Therefore, despite some consistency in evidence across these fetal growth endpoints, some important uncertainties remain mainly around the degree that some of the results examined here may be influenced by sample timing. This source of uncertainty and potential explanation of different results across studies may indicate some bias due to the impact of pregnancy hemodynamics.

3.4.4.1.5 Postnatal Growth

Thirteen studies examined PFOA exposure in relation to postnatal growth measures. The synthesis here is focused on postnatal growth measures including 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 rapid growth during infancy (Tanner et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b), as well as mean and standardized weight (all 13 studies except Gross et al. (2020), Tanner et al. (2020), and Jensen et al. (2020) depicted in Figure 3-62), and height (Yeung et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018; Lee et al., 2018; Shoaff et al., 2018; Chen et al., 2017b; Wang et al., 2016; de Cock et al., 2014) measures.

Six postnatal growth studies were *high* confidence (Jensen et al., 2020; Tanner et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Wang et al., 2016), four were *medium* confidence (Gyllenhammar et al., 2018; 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., 2018). Five postnatal growth studies had good study sensitivity (Tanner et al., 2020; Lee et al., 2018; Shoaff et al., 2018; Manzano-Salgado et al., 2017b; Wang et al., 2016), six were adequate (Jensen et al., 2020; Starling et al., 2019; Yeung et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018; Chen et al., 2017b) and two were considered deficient (Gross et al., 2020; de Cock et al., 2014). The synthesis here is focused on postnatal body mass index (BMI)/adiposity measures, head circumference and mean and standardized weight and height measures. Rapid growth during infancy is also included as it was examined in five studies (Tanner et al.; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et

al., 2017b). The *medium* confidence study by de Cock et al. (2014) did not report effect estimates for postnatal infant height (p-value = 0.045), weight (p-value = 0.35), and BMI (p-value = 0.81) up to 11 months of age. But their lack of reporting of effect estimates precluded consideration of magnitude and direction of any associations and are not further considered below in the summaries.

The *medium* confidence study by Manzano-Salgado et al. (2017b) had null associations for their overall population and female neonates measured at 6 months but reported an increased weight gain z-score for males (0.13; 95% CI: 0.01, 0.26) per each log2 PFOA increases. The *medium* confidence study by Chen et al. (2017b) did not report associations between each per ln-unit PFOA exposure increase and height z-score measures up to 24 months of age. The sex-specific data were not always consistent across time. For example, nonsignificant increases small in magnitude for boys (0.11; 95% CI: −0.04, 0.27) and decreases in greater height per each ln-unit PFOA increase in the 12- to 24-month window. The *low* confidence study by Lee et al. (2018) reported statistically significant associations detected for mean height differences at age 2 years (−0.91 cm; 95% CI: −1.36, −0.47 for each PFOA ln-unit increase), as well as height change from birth to 2 years (−0.86 cm; 95% CI: −1.52, −0.20). Large differences were seen for mean weight differences at age 2 years (−210 g; 95% CI: −430, 0.20) but not for weight change from birth to 2 years. An exposure-response relationship was detected when examined across PFOA categories with the highest exposure associated with smaller statistically significant height increases at age 2 compared with lower exposures.

In the *medium* confidence study by Gyllenhammar et al. (2018), no associations were detected for infant height deficits among participants followed from 3 months to 60 months of age per each IQR PFOA change. They also did not report statistically significant standardized BWT deficits per each IQR PFOA change, but they did show slight weight deficits (approximately −0.2) at 3 months that gradually decreased over time (to approximately −0.1) at 60 months of age. Compared to the PFOA tertile 1 referent, the *low* confidence study by Cao et al. (2018) reported slight increases (1.37 cm; 95% CI: −0.5, 3.28) in postnatal length (i.e., height) amongst infants (median age of 19.7 months), while large postnatal weight deficits were reported for tertile 2 (−429.2 g; 95% CI: −858.4, −0.12) and tertile 3 (−114.9 g; 95% CI: −562.0, 332.1). These height increases were predominately due to female infants, while the weight deficits were driven by males. Few differences were observed in the overall population for postnatal head circumference with slight nonsignificant deficits seen amongst females only.

In their *high* confidence study, Wang et al. (2016) reported statistically significant childhood weight (−0.14; 95% CI: −0.39, 0.11) and height (−0.15; 95% CI: −0.38, 0.08) z-scores for female neonates when averaged over the first 11 years and per 1-ln-unit PFOA increase. Results were null for male neonates for childhood average weight (0.03; 95% CI: −0.11, 0.18) and height (0.01; 95% CI: −0.24, 0.25) z-scores. However, when they examined the first 2 years only, statistically significant deficits in both height and weight z-scores were only seen for male neonates. These weight deficits dissipated in males later during childhood, while the height deficits detected at age 2 years continued through age 11. In contrast, the height deficits in female children that were detected at birth were no longer evident in older kids until later ages (i.e., 11 years). The weight deficits in female children detected at birth did not persist.

In their *high* confidence study, Yeung et al. (2019) reported statistically significant negative growth trajectories for weight-for-length z-scores in relation to each log SD increase in PFOA

exposures among singletons followed for three years. In contrast, the authors showed positive infant length (i.e., height) growth trajectory across two different measures. Some sex-specific results were detected with larger associations seen in singleton females for weight-for-length z-score (−0.13; 95% CI: −0.19, –0.06). An infant weight deficit of –12.6 g (95% CI: –49.5, 24.3 per each 1 log SD PFOA increase) was also observed and appeared to be driven by results in females (–30.2 g; 95% CI: –84.1, 23.6). 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 for weight-for-age (−0.46; 95% CI: −0.78, −0.14) z-score, and weight-for-length z-score (−0.34; 95% CI: −0.59, −0.08) in PFOA tertile 3 compared with tertile 1 with exposure-response relationships detected for infant weight-for-length z-score. Deficits comparable in magnitude that were not statistically significant were observed in tertile 3 for height measured as length for age z-score (−0.32; 95% CI: −0.72, 0.07). No associations were found in the overall population from the *high* confidence study by Starling et al. (2019) for postnatal measures at 5 months of age, but an exposure-response relationship of increased adiposity was seen among male neonates with increasing PFOA tertiles (2.81; 95% CI: 0.79, 4.84 for tertile 3). Similarly, no associations were found in the overall population for weight-for-age or weight-for-length z-scores and PFOA exposures, but both measures were increased among male neonates.

Overall, seven of nine studies with quantitative estimates (including six *high* and *medium* confidence studies) showed some associations between PFOA exposures and different measures of infant weight. Two of four studies with categorical data showed some evidence of inverse monotonic exposure-response relationships. Three (two *high* and one *low* confidence) of seven studies with quantitative estimates examining different infant height measures showed some evidence of inverse associations with PFOA. Study quality ratings, including study sensitivity and overall confidence, did not appear to be explanatory factors for heterogeneous results across studies.

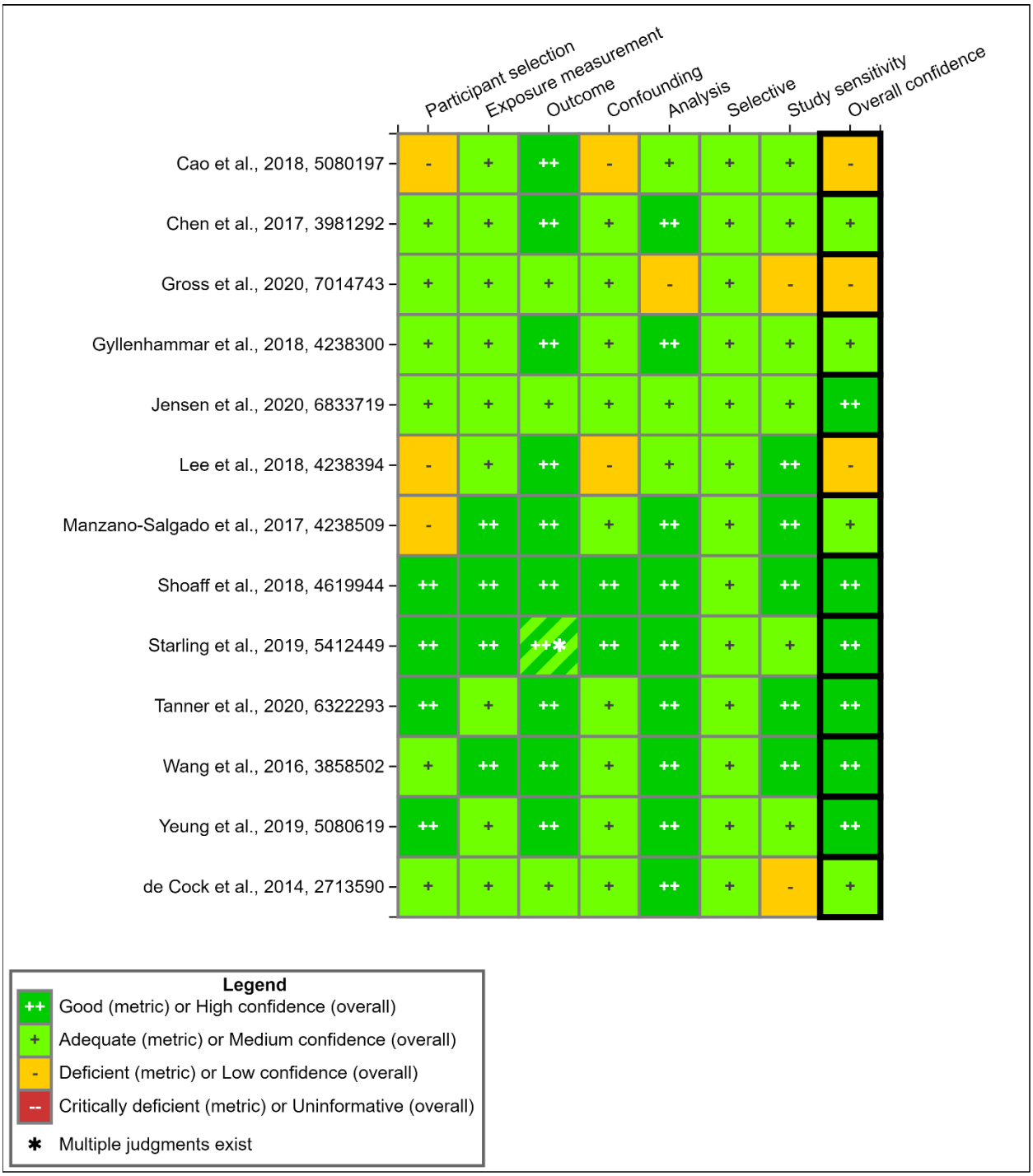


Figure 3-62. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Postnatal Growth

Interactive figure and additional study details available on HAWC.

3.4.4.1.5.1 Adiposity/BMI

The *medium* confidence study by Chen et al. (2017b) reported lower BMI z-scores (−0.16; 95% CI: −0.37, 0.05) per each ln-unit PFOA increase in the birth to 6–months window. 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 for infant BMI z-score (−0.36; 95% CI: −0.60, −0.12) in PFOA tertile 3 compared with tertile 1 with exposure-response relationships detected for infant BMI z-score. The *high* confidence study by Yeung et al. (2019) reported statistically significant negative growth trajectories for BMI, BMI z-score in relation to each log SD increase in PFOA exposures among singletons followed for three years. Some sex-specific results were detected with larger associations seen in singleton females for BMI (−0.18 kg/m^2; 95% CI: –0.27, –0.09) and BMI z-scores (−0.13; 95% CI: −0.19, −0.07). An exposure-response relationship was evident with decreasing BMI z-scores across PFOA quartiles in the overall population and for female neonates. An exposure-response relationship of increased adiposity was seen among male neonates with increasing PFOA tertiles (2.81; 95% CI: 0.79, 4.84 for tertile 3) in the *high* confidence study by Starling et al. (2019). The *high* confidence study by Jensen et al. (2020) reported null associations between adiposity and per each 1-unit increase in PFOA measured at 3 and 18 months. The *low* confidence study by Gross et al. (2020) reported a null association (OR = 0.91; 95% CI: 0.36 to 2.29) of being overweight at 18 months for PFOA levels greater than the mean level. They showed discordant sex-specific results with higher odds of being overweight at 18 months in males (OR = 2.62; p-value = 0.22) and lower odds among females (OR = 0.41; p-value = 0.27).

Overall, there was very limited evidence of adverse associations between PFOA exposures and either increased BMI or adiposity measures. Only one out of seven studies in the overall population showed evidence of increased adiposity or BMI changes in infancy in relation to PFOA. One of these studies did report increased odds of being overweight at 18 months for higher PFOA levels in males only. Only one of two studies showed an inverse monotonic relationship between either BMI or adiposity with increasing PFOA exposures.

3.4.4.1.5.2 Rapid Weight Gain

Five studies (Tanner et al., 2020; Starling et al., 2019; Yeung et al., 2019; Shoaff et al., 2018; Manzano-Salgado et al., 2017b) examined rapid infant growth, with all five considered *high* confidence. Limited evidence of associations was reported with these studies, 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 of rapid weight gain with increasing PFOA. For example, Starling et al. (2019) reported small increased ORs (range: 1.25 to 1.43) for rapid growth in the overall population based on either weight-for-age-based z-scores or weight-for-length-based z-scores. The most detailed evaluation by Tanner et al. (2020) also showed some adverse associations including higher prenatal PFOA concentrations related to a longer duration of time needed to complete 90% of the infant growth spurt (Δtertile 1: 0.06; 95% CI: 0.01, 0.11). Higher prenatal PFOA concentrations were also significantly related to delayed infant peak growth velocity (δ1: 0.58; 95% CI: 0.17, 0.99) and a higher post-spurt weight plateau (α1: 0.81; 95% CI: 0.21, 1.41).

3.4.4.1.5.3 Postnatal Growth Summary

Seven of the nine studies reporting quantitative results for different infant weight measures showed some evidence of adverse associations with PFOA exposures, with two of these studies showing adverse results predominately in females and one in males only. Two other studies showed increased weight among males only and lack of reporting of effect estimates in one study precluded further consideration of adversity. Two (Starling et al., 2019; Manzano-Salgado et al.,

2017b) of three studies did not report adverse associations in either the overall population or females, but did detect increased infant weight measures among males. Three of the seven studies reporting quantitative results showed some evidence of inverse associations between PFOA exposures and infant height. Only one out of seven studies in the overall population showed evidence of increased adiposity or BMI changes in infancy in relation to PFOA. One study showed increased adiposity amongst males only, while four studies each were null or reported some inverse associations (i.e., lower adiposity/BMI with increasing PFOA). Two of the studies showed exposure-response relationships for PFOA and decreased BMI scores, while a third showed the opposite exposure-response for increased adiposity. Although the data across different endpoints was not entirely consistent, the majority of infant weight studies indicated that PFOA may be associated with post-natal growth measures up to 2 years of age.

3.4.4.1.6 Gestational Duration

Twenty-two different studies examined gestational duration measures (i.e., PTB or gestational age measures) in relation to PFOA 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., 2020c). Two of these studies were *uninformative* and not considered further below (Gundacker et al., 2021; Lee et al., 2013).

3.4.4.1.6.1 Gestational Age

Eighteen different informative studies examined the relationship between PFOA and gestational age (in weeks) (Figure 3-63). Seventeen of these examined associations in the overall population and one study reported sex-specific findings only (Lind et al., 2017a). Ten of these 18 studies were *high* confidence (Chu et al., 2020; Eick et al., 2020; Huo et al., 2020a; Bell et al., 2018; Buck Louis 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., 2018; Meng et al., 2018) and four were *low* confidence (Gao et al., 2019; Workman et al., 2019; Xu et al., 2019a; Wu et al., 2012). Six of the studies had good study sensitivity (Huo et al., 2020a; Meng et al., 2018; Sagiv et al., 2018; Lauritzen et al., 2017; Manzano-Salgado et al., 2017a; Wu et al., 2012), nine were adequate (Yang et al., 2022In Press; Chu et al., 2020; Eick et al., 2020; Gao et al., 2019; Hjermitslev et al., 2019; Buck Louis et al., 2018; Gyllenhammar et al., 2018; Lind et al., 2017a; Bach et al., 2016) and three (Workman et al., 2019; Xu et al., 2019a; Bell et al., 2018) were deficient.

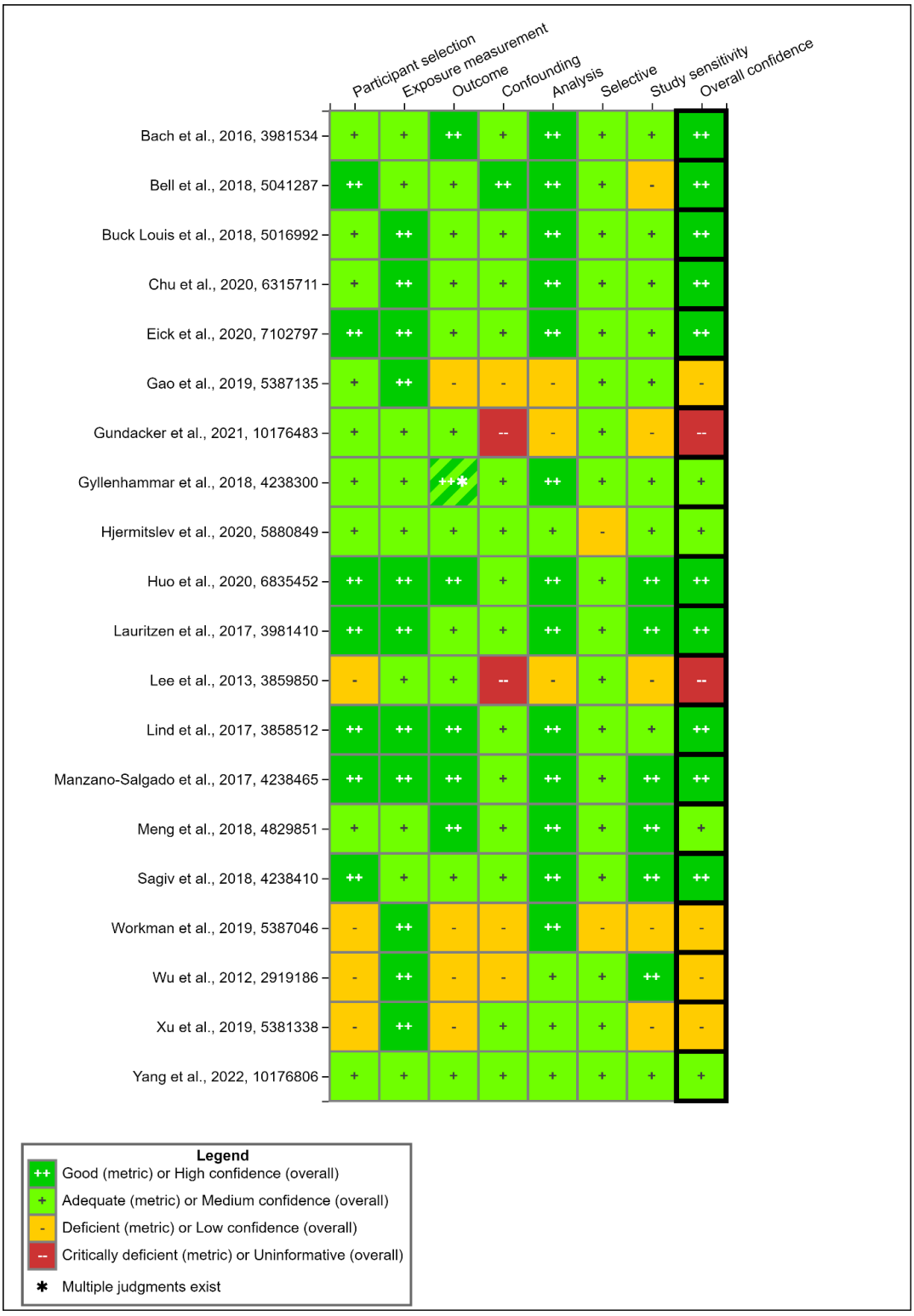


Figure 3-63. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Gestational Age

Interactive figure and additional study details available on HAWC.

Five (3 *low* confidence and 1 each *medium* and *high* confidence) of the 18 studies showed some evidence of increased gestational age (Gao et al., 2019; Hjermitslev et al., 2019; Workman et al., 2019; Xu et al., 2019a; Bach et al., 2016) in relation to PFOA while six others were largely null (Huo et al., 2020a; Bell et al., 2018; Buck Louis et al., 2018; Gyllenhammar et al., 2018; Sagiv et al., 2018; Manzano-Salgado et al., 2017a). The remaining seven studies showed some evidence of adverse impacts on gestational age either in the overall population or either. The *high* confidence study by Lind et al. (2017a) examined only sex-specific data and reported larger deficits in female (−0.21 cm; 95% CI: −0.61, 0.19 per each ln-unit PFOA increase) than male neonates (−0.10 cm; 95% CI: −0.41, 0.21). Among the other six studies with results based on the overall population, three were *high* confidence, two were *medium*, and one was *low* confidence. The *low* confidence study by Wu et al. (2012) study reported an extremely large difference (−2.3 weeks; 95% CI: −4.0, −0.6) in gestational age per each log10 unit PFOA change. The *medium* confidence study by Yang et al. (Yang et al., 2022In Press) reported a larger (–1.04 weeks; 95% CI: –3.72, 1.63 per each PFOA IQR increase) difference in gestational age among preterm births than among term births (–0.38 weeks; 95% CI: –1.33, 0.57 per each PFOA IQR increase). The *medium* confidence study by Meng et al. (2018) reported statistically significant gestational age deficits (range: −0.17 to −0.24 weeks) across all quartiles but no evidence of an exposure-response relationship. The *high* confidence study by Lauritzen et al. (2017) reported a slight decrease in the overall population (−0.2 weeks; 95% CI: −0.34, 0.14). They also showed larger deficits in their Swedish population (−0.3 weeks; 95% CI: −0.9, 0.3) which was predominately driven by results among male neonates (−0.4 weeks; 95% CI: −1.2, 0.5). The *high* confidence study by Chu et al. (2020) showed larger deficits in the overall population (−0.21 weeks; 95% CI: −0.44, 0.02) which was driven by female neonates (−0.83 weeks; 95% CI: −0.53, −0.23). The *high* confidence study by Eick et al. (Eick et al., 2020) reported decreased gestational age only among tertile 2 only in the overall population (–0.29 weeks; 95% CI: –0.74, 0.17), males (–0.24 weeks; 95% CI: –0.91, 0.43) and females (–0.31 weeks; 95% CI: –0.95, 0.34) relative to tertile 1.

Overall, seven of the 18 studies showed some evidence of adverse impacts on gestational age. Six of the seven studies were either *medium* or *high* confidence studies. Few patterns emerged based on study confidence or other study characteristics. For example, three of the null studies were rated as having good sensitivity, along with two studies with adequate and one with deficient ratings. There was a preponderance of associations related to sample timing possibly related to pregnancy hemodynamic influences on the PFOA biomarkers, as five of the seven studies reporting inverse associations were sampled later in pregnancy (i.e., exclusively trimester two or later).

3.4.4.1.6.2 Preterm Birth

As shown in Figure 3-64, eleven studies examined the relationship between PFOA and PTB; all of the studies were either *medium* (Yang et al., 2022In Press; Liu et al., 2020c; 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., 2020b; Sagiv et al., 2018; Manzano-Salgado et al., 2017a; Bach et al., 2016). Nine of the 11 studies were prospective birth cohort studies, and the two studies by Liu et al. (2020c) and Yang et al. (Yang et al., 2022In Press) were case-control studies nested with prospective birth cohorts. Four studies had maternal exposure measures that were sampled either 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. (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., 2020b; Liu et al., 2020c; Hjermitslev et al., 2019). The *medium* confidence study by Meng et al. (2018) pooled exposure data from two study populations, one which measured PFOA in umbilical cord blood and one which measured PFOA in maternal blood samples collected in trimesters 1 and 2. The *medium* confidence study by Yang et al. (2022In Press) collected umbilical cord blood samples. Four studies (Huo et al., 2020b; Meng et al., 2018; Sagiv et al., 2018; Manzano-Salgado et al., 2017a) were considered to have *good* sensitivity and one was *deficient* (Liu et al., 2020c). The other six studies were rated *adequate* in this domain. The median exposure values across all studies ranged from 0.76 ng/mL (Eick et al., 2020) to 11.85 ng/mL (Huo et al., 2020b).

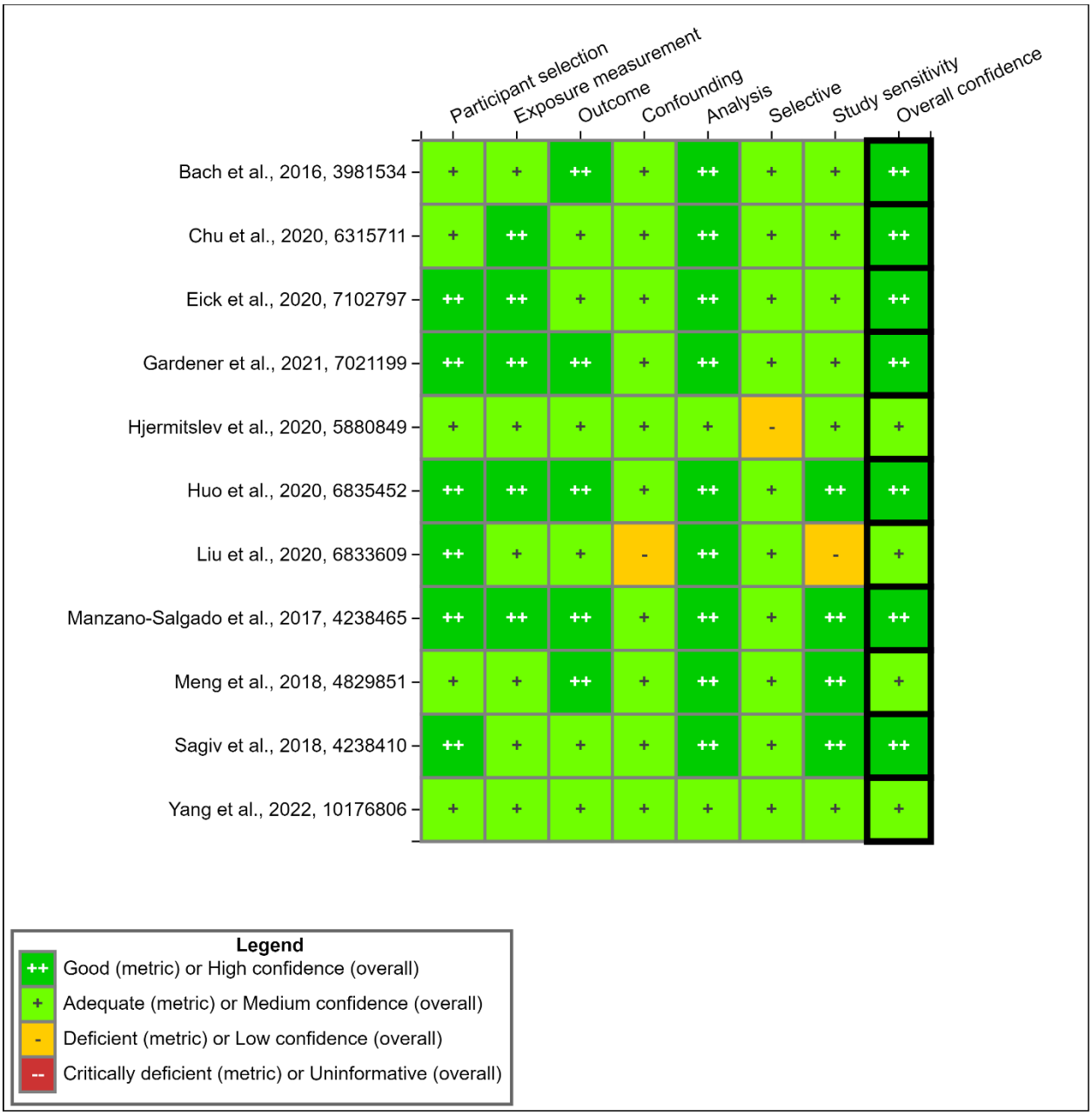


Figure 3-64. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Preterm Birth Effects

Interactive figure and additional study details available on HAWC.

Six of the 11 studies reported an increased risk of PTB with elevated exposure to PFOA. Null or inverse associations were reported by Bach et al. (2016), Hjermitslev et al. (2019), Liu et al. (2020c), Manzano-Salgado et al. (2017a) and Yang et al. (2022In Press). The *medium* confidence study by Meng et al. (2018) reported consistently elevated nonmonotonic ORs for PTB in the upper three PFOA quartiles (OR range: 1.7–3.2), but little evidence was observed per each doubling of PFOA exposures (OR = 1.1; 95% CI: 0.8, 1.5). Although they were not statistically significant, the *high* confidence study by Chu et al. (2020) reported increased ORs of similar

magnitude per each ln ng/mL increase (OR = 1.49; 95% CI: 0.94, 2.36) and when quartile 3 (OR = 1.60; 95% CI: 0.60, 4.23) and quartile 4 (OR = 1.84; 95% CI: 0.72, 4.71) exposures were compared with the referent. ORs similar in magnitude were detected in the *high* confidence study by Eick et al. (2020) study albeit in a more monotonic fashion across all quantiles (tertile 2: OR = 1.48; 95% CI: 0.66, 3.31); 95% CI: tertile 3: OR = 1.63; 95% CI: 0.74, 3.59). Associations between PFOA and overall PTB near or just below the null value were consistently detected for either categorical or continuous exposures in the *high* confidence Huo et al. (2020b) study. Few patterns emerged across PTB subtypes in that study, although there was an increase in clinically indicated PTBs (OR = 1.71; 95% CI: 0.80, 3.67 per each ln-unit PFOA increase) which seemed to be largely driven by results in female neonates (OR = 2.64; 95% CI: 0.83, 8.39). The *high* confidence study by Sagiv et al. (2018) reported increased nonsignificant risks (OR range: 1.1–1.2) for PTB across all PFOA quartiles. Relative to the referent, the *high* confidence study by Gardener (Gardener et al., 2021) showed higher odds of PTB in PFOA quartiles 2 and 3 (range: 3.1–3.2) than that found in quartile 4 (OR = 1.38; 95% CI: 0.32–5.97). Outside of the aforementioned Eick et al. (2020) study, none of the other seven studies with categorical data showed evidence of exposure-response relationships.

Overall, 6 of the 11 studies showed increased risk of PTB with PFOA exposures with limited evidence of exposure-response relationships. Although small numbers limited the confidence in many of the sub-strata comparisons, there were few apparent patterns by study evaluation ratings or other characteristics that explained the heterogeneous results across studies. However, there were more associations amongst studies with later sample timing data collection, as three of the five studies with later PFOA biomarker sampling showed some increased odds of preterm birth compared with two of six studies with earlier sampling.

3.4.4.1.6.3 Gestational Duration Summary

Overall, there was mixed evidence of exposure to PFOA and both inverse associations with gestational age and increased risk of preterm birth. Most of the associations for either gestational duration measures were reported in *medium* or *high* confidence studies. Few other patterns were evident that explained any between study heterogeneity.

3.4.4.1.7 Fetal Loss

Five (two *high*, two *medium* and one *low* confidence) studies examined PFOA exposure and fetal loss with limited evidence as only one study showing increased risks of miscarriage. Two studies had good study sensitivity (Wang et al., 2021; Wikström et al., 2021), while three had adequate sensitivity (Liew et al., 2020; Buck Louis et al., 2016; Jensen et al., 2015) (Figure 3-65).

The *high* confidence study by Wikström et al. (2021) showed a statistically significant association between PFOA and miscarriages (OR = 1.48; 95% CI: 1.09, 2.01 per doubling of PFOA exposures. The authors also reported a monotonic exposure-response relationship across PFOA quartiles (ORs/95% CIs: Q2: 1.69; 0.8, 3.56; Q3: 2.02; 0.95, 4.29; Q4: 2.66; 1.26, 5.65). The *medium* confidence study by Liew et al. (2020) detected a 40% increased risk of miscarriage (OR = 1.4; 95% CI: 1.0, 1.9) per each PFOA doubling with increased risks detected for quartiles three (OR = 1.4; 95% CI: 0.8, 2.6) and four (OR = 2.2; 95% CI: 1.2, 3.9) only. No associations were detected in the *high* confidence study by Wang et al. (Wang et al., 2021) for preclinical spontaneous abortion (OR = 0.99; 95% CI: 0.94, 1.05) or in the *medium* confidence study by Buck Louis et al. (2016) (hazard ratio (HR) =0.93; 95% CI: 0.75, 1.16 per each SD PFOA

increase). In the *low* confidence study by Jensen et al. (Jensen et al., 2015), a decreased risk of miscarriages was reported (OR = 0.64; 95% CI: 0.36, 1.18 per each ln-unit PFOA increase).

Overall, there was positive evidence for fetal loss with increased relative risk estimates in two out of five studies. In those two studies, the magnitude of associations detected ranged from 1.4 to 2.7 with an exposure-response relationship detected in one study. No patterns in the results were detected by study confidence ratings including sensitivity.

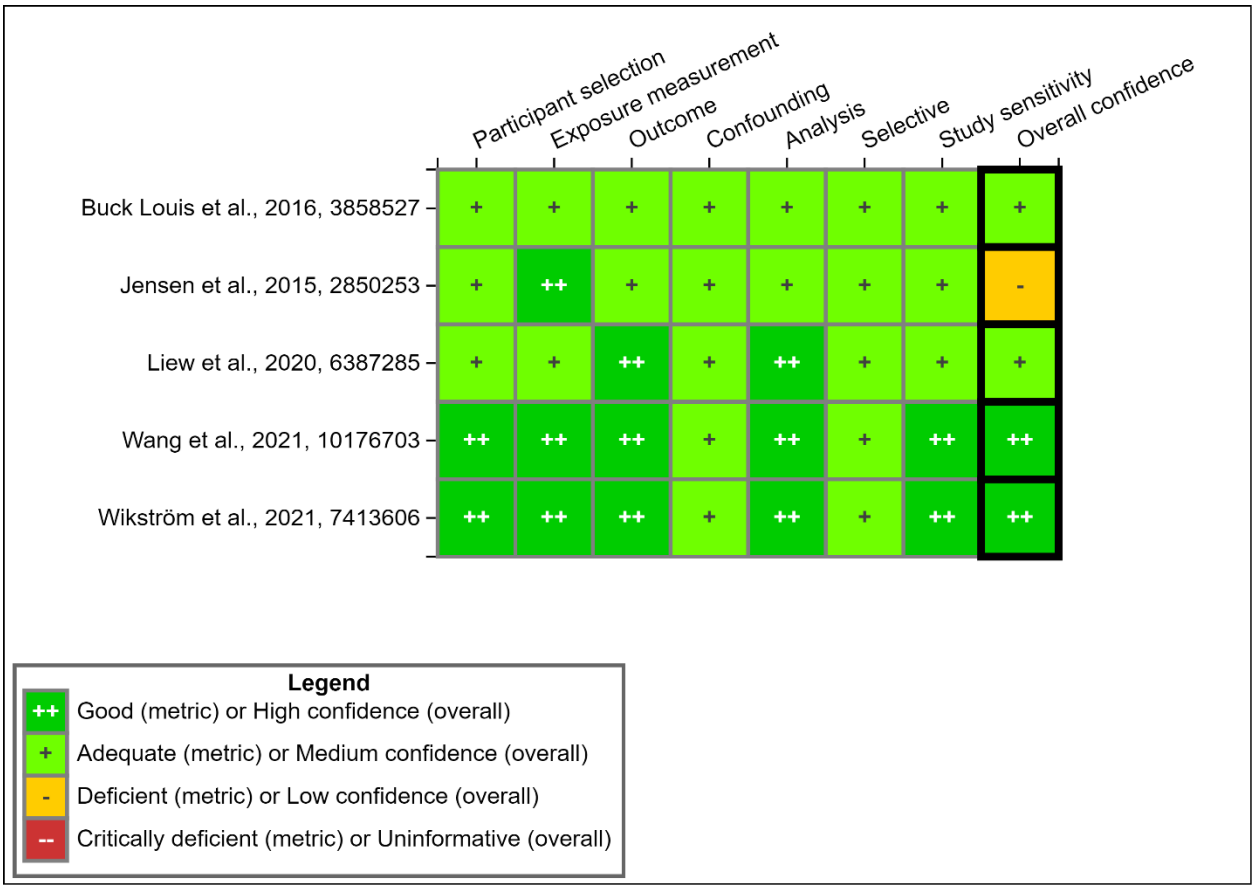


Figure 3-65. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Fetal Loss

Interactive figure and additional study details available on HAWC.

3.4.4.1.8 Birth Defects

Four birth defect studies examined PFOA exposure with three of these four having adequate study sensitivity (one was deficient) as shown in Figure 3-66. This included a *medium* confidence study by Vesterholm Jensen et al. (2014) that reported no increased risk for cryptorchidism (OR = 0.83; 95% CI: 0.44, 1.58 per each ln-unit PFOA increase). A *medium* confidence study by Ou et al. (2021) reported decreased risks for septal defects (OR = 0.54; 95% CI: 0.18, 1.62), conotruncal defects (OR = 0.28; 95% CI: 0.07, 1.10), and total congenital heart defects (OR = 0.64; 95% CI: 0.34, 1.21) among participants with maternal serum levels over >75th PFOA percentile (relative to those <75th percentile). A *low* confidence study (Cao et al., 2018) of a nonspecific all birth defect grouping reported limited evidence of an association

(OR = 1.24; 95% CI: 0.57, 2.61), but interpretation of an all-birth defect grouping is challenging given that etiological heterogeneity may occur across individual defects. Compared to the referent group of no Little Hocking Water Association supplied water, no associations (both ORs were 1.1) were reported in a *low* confidence study from Washington County, Ohio among infants born to women partially or exclusively supplied in part by the Little Hocking Water Association (Nolan et al., 2010). The study was considered *uninformative* for examination of individual defects given the lack of consideration of confounding and other limitations in those analyses.

Overall, there was negligible evidence of associations between PFOA and birth defects based on the four available epidemiological studies including two *medium* confidence studies which reported decreased odds of birth defects relative to exposures. 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.

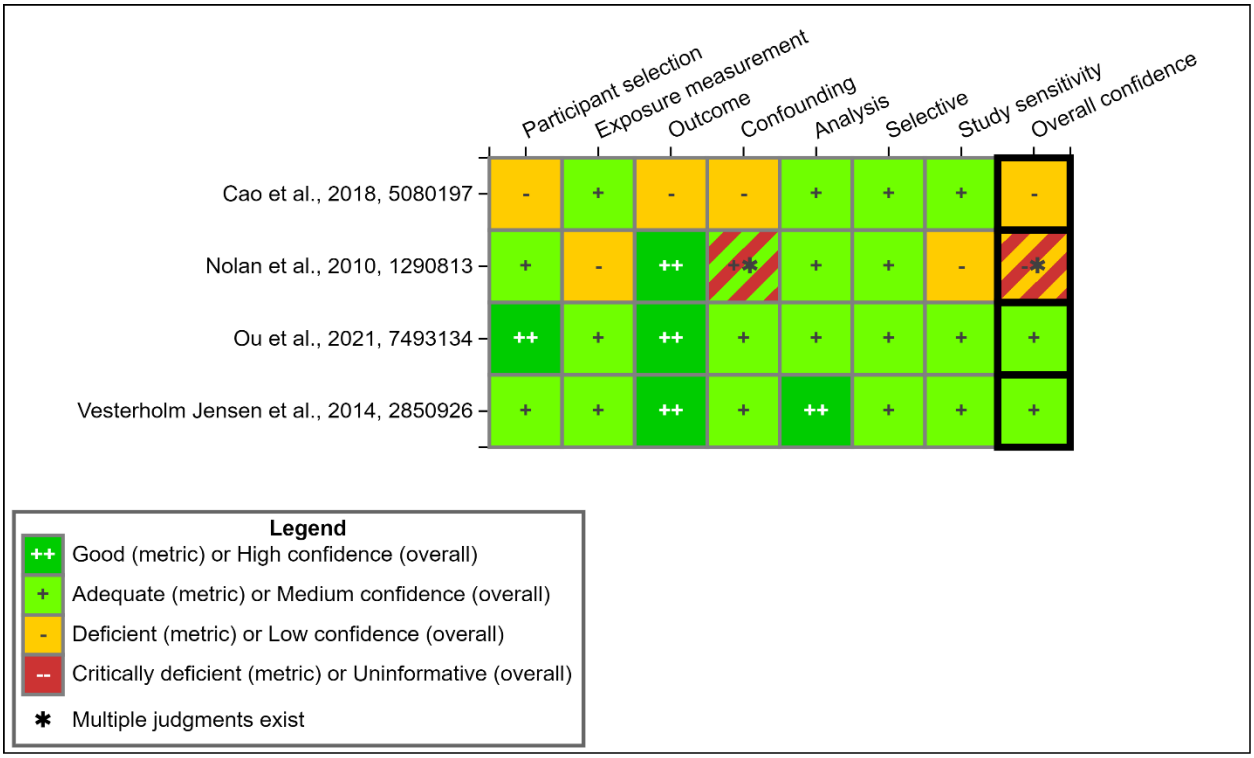


Figure 3-66. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Birth Defects

Interactive figure and additional study details available on HAWC.

3.4.4.2 Animal Evidence Study Quality Evaluation and Synthesis

There are 6 studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and 13 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and developmental effects in animal models. Study quality evaluations for these 19 studies are shown in Figure 3-67.

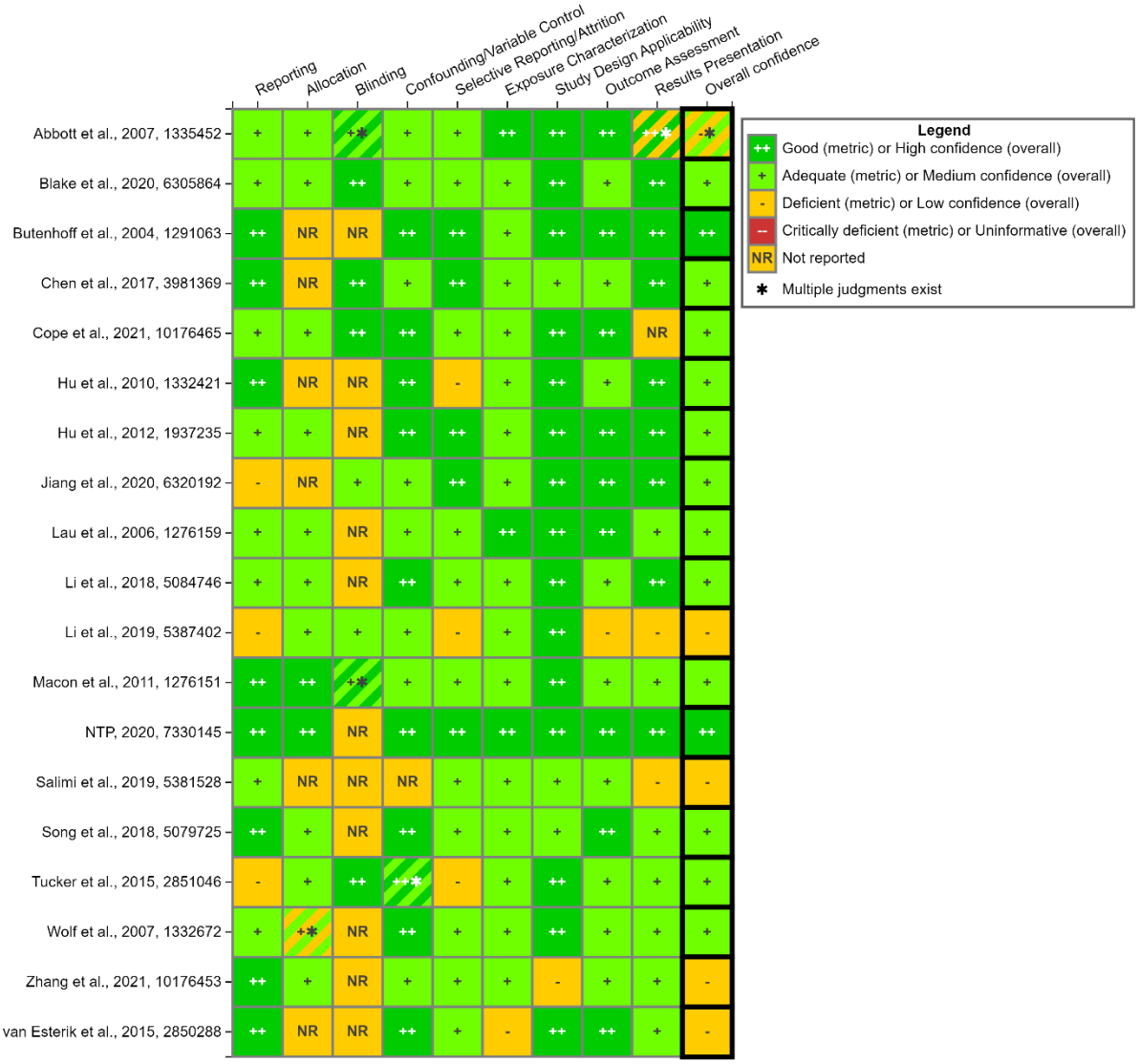


Figure 3-67. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Developmental Effects

Interactive figure and additional study details available on HAWC.

Evidence suggests that PFOA exposure can adversely affect development. Oral studies in mice and rats report effects in offspring including decreased survival, decreased body weights, structural abnormalities (e.g., reduced skeletal ossification), delayed eye opening, and altered mammary gland development. Doses that elicited responses were generally lower in mice than in rats. Additionally, three studies of gestational PFOA exposure to mice reported effects on placental weight and histopathological changes in placental tissue, suggesting that the placenta may be a target of PFOA. In some cases, adverse developmental effects of PFOA exposure that relate to other health outcomes may be discussed in the corresponding health outcome section (e.g., neurodevelopmental effects are discussed in the Appendix (U.S. EPA, 2024a)).

3.4.4.2.1 Maternal Effects

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

3.4.4.2.1.1 Studies in Mice

Li et al. (2018a) reported marked, dose-related decreases in maternal body weight gain at ≥10 mg/kg/day in pregnant Kunming mice exposed from gestation day 1 to 17 (GD 1 to GD 17; no statistical tests performed). Dose-related decreases in body weight gain were also seen in pregnant CD-1 mice exposed to 10, 20, or 40 mg/kg/day (significant at 20 and 40 mg/kg/day) by Lau et al. (2006); significantly delayed time to parturition was also seen at 3, 10, and 20 mg/kg/day in this study (all litters at 40 mg/kg/day were resorbed). Yahia et al. (2010) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day from GD 0 to GD 17 (sacrificed on GD 18) or GD 0 to GD 18 (allowed to give birth), and at 10 mg/kg/day, observed significant decreases in body weight gain from GD 12 onward in dams allowed to give birth as well as significantly decreased terminal body weight in dams sacrificed on GD 18. In the same study, a significant decrease in food intake during early gestation was also reported for the dams allowed to give birth, but data were not shown. Delayed parturition was also observed at 10 mg/kg/day (data not shown). Pregnant CD-1 mice exposed to 25 mg/kg/day from GD 11 to GD 16 exhibited significantly decreased body weight from GD 13 to GD 16 (Suh et al., 2011). Hu et al. (2010) exposed pregnant C57BL/6N mouse dams to 0.5 or 1.0 mg/kg/day PFOA and found no significant differences relative to controls on GD 19. No significant effects on maternal body weight were noted in C57BL/6N mouse dams exposed to 0.02, 0.2, or 2 mg/kg/day PFOA from time of mating through PND 21 (Hu et al., 2012). In contrast to the above-described findings, two studies in pregnant CD-1 mice reported significantly increased maternal body weight gain after exposure to 5 mg/kg/day (Blake et al., 2020) or 3 or 5 mg/kg/day PFOA (Wolf et al., 2007) from GD 1 to GD 17. Abbott et al. (2007) found no effects of 0.1, 0.3, 0.6, or 1 mg/kg/day PFOA on maternal weight changes in 129S1/SvlmJ wild-type mice (exposure to 5, 10, and 20 mg/kg/day PFOA led to increased maternal death) (Figure 3-68).

3.4.4.2.1.2 Studies in Rats

A two-generation oral gavage reproductive toxicity study in Sprague-Dawley rats reported no effect on parental generation (P_0) maternal body weight or food consumption but found significantly decreased body weight in first-generation (F_1) parental females at 30 mg/kg/day during pre-cohabitation, gestation (GD 0–GD 14), and lactation day 5 to 15 (LD 5–LD 15). Decreased absolute food consumption was reported, but data were not shown; relative feed consumption was unaffected (Butenhoff et al., 2004a). In pregnant Sprague-Dawley rats dosed with 30 mg/kg/day from GD 4 to LD 21, body weight gain was decreased during gestation and body weight was 4% lower than controls during lactation (statistical significance not indicated) (Hinderliter et al., 2005).

In a two-year chronic toxicity/carcinogenicity assay conducted by the NTP (2020), female Sprague-Dawley (Hsd:Sprague-Dawley® SD®) rat dams were exposed to 0, 150, or 300 parts per million (ppm) PFOA in feed during the perinatal period. In study 1, F_1 male rats were

administered 0, 150, or 300 ppm PFOA and F_1 female rats were administered 0, 300, or 1,000 ppm PFOA in feed during the postweaning period. For study 2, lower postweaning exposure levels (0, 20, 40, or 80 ppm) were utilized for males due to unexpected toxicity in male offspring using the original exposure regime. Exposure for all F_1 generations in both studies occurred for 107 weeks or until the 16-week interim necropsy. The perinatal and postweaning exposure regimes for females and males for both studies are presented in Table 3-14. Dose groups for this study are referred to as "[perinatal exposure level]/[postweaning exposure level]" (e.g., 300/100).

Table 3-14. Study Design for Perinatal and Postweaning Exposure Levels for F_1 Male and Female Rats for the NTP (2020) Study

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

Notes: F_1 = first generation; X = exposure level used.

In pregnant Sprague-Dawley rats exposed to 150 or 300 ppm via diet (equivalent to approximately 11 and 22 mg/kg/day during gestation and 22 and 45 mg/kg/day from LD 1 to LD 14), no consistent effects were observed on body weight or body weight gain during gestation or lactation (Figure 3-68). Food consumption was marginally but significantly decreased (up to 4%) at one or both dose levels at various intervals. In a repeat of this study that tested a single dose level of 300 ppm (approximately 21.8 mg/kg/day during gestation and 48.3 mg/kg/day from LD 1 to LD 14), no effects were observed on maternal body weight or body weight gain during gestation; from LD 1 to LD 14, there was a marginal but significant decrease (2%–3%) in maternal body weight and body weight gain and a significant decrease (5%) in food consumption (NTP, 2020).

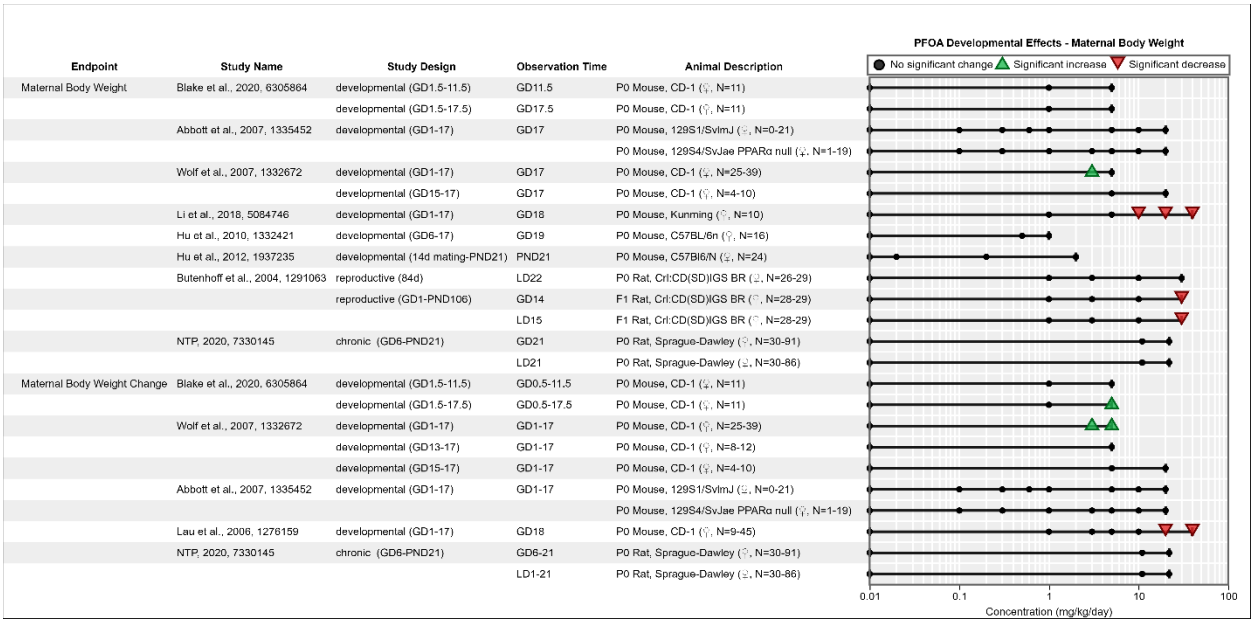


Figure 3-68. Maternal Body Weight in Rodents Following Exposure to PFOA (logarithmic scale)

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

3.4.4.2.2 Placenta Effects

Two oral gavage studies in CD-1 mice reported significant decreases in embryo to placenta weight ratio at 5 mg/kg/day PFOA (Blake et al., 2020) or doses ≥2 mg/kg/day (Suh et al., 2011), as well as treatment-related histopathological lesions at 5 mg/kg/day (Blake et al., 2020) or doses ≥10 mg/kg/day (Suh et al., 2011). A third study in Kunming mice reported decreased placenta to body weight ratio at PFOA doses ≥5 mg/kg/day and histopathological changes in placental tissue at doses ≥2.5 mg/kg/day (Jiang et al., 2020) (Figure 3-69).

Blake et al. (2020) administered 0, 1, or 5 mg/kg/day to pregnant CD-1 mice from GD 1.5 through sacrifice on GD 11.5 or GD 17.5, Suh et al. (2011) administered 0, 2, 10, or 25 mg/kg/day to CD-1 mice from GD 11 through sacrifice on GD 16, and Jiang et al. (2020) administered 0, 2.5, 5, or 10 mg/kg/day to Kunming mice from GD 1 through sacrifice on GD 13. The embryo to placental weight ratio was significantly decreased at 5 mg/kg/day in Blake et al. (2020) and at doses ≥2 mg/kg/day in Suh et al. (2011). Blake et al. (2020) observed significantly increased placental weight at 5 mg/kg/day at GD 17.5 and no changes in the numbers of viable fetuses or resorptions, whereas Suh et al. (2011) observed significantly decreased placental weight and increased numbers of resorptions and dead fetuses at ≥2 mg/kg/day. Jiang et al. (2020) observed significantly decreased relative placental weight at ≥5 mg/kg/day (decreases were also seen at lower dose levels, but they did not reach statistical significance). Histopathological changes in placental tissue were also observed at PFOA doses ≥2.5 mg/kg/day (increased area of spongiotrophoblast, decreased blood sinusoidal area in labyrinth), ≥5 mg/kg/day (increased interstitial edema of spongiotrophoblast), or 10 mg/kg/day (decreased labyrinth area, increased ratio of spongiotrophoblast to labyrinth area). Jiang et al.

(2020) found no effect on fetus to maternal body weight ratio. Viable fetus weight was significantly decreased in Blake et al. (2020) at 5 mg/kg/day and in Suh et al. (2011) at ≥10 mg/kg/day and corresponded with treatment-related lesions in the placenta. The incidence of GD 17.5 placentas within normal limits was significantly lower in mice exposed to 5 mg/kg/day (Blake et al., 2020), and the lesions observed in placentas from that group included labyrinth atrophy (3/40 placentas), labyrinth congestion (23/40), and early fibrin clot (1/40). In dams treated with 1 mg/kg/day, labyrinth necrosis was observed in 1/32 placentas and placental nodules were observed in 2/32 placentas. Histopathologic examination by Suh et al. (2011) showed normal placental tissue in 0 and 2 mg/kg/day groups and dose-dependent necrotic changes in placentas from the 10 and 25 mg/kg/day groups (incidences of specific lesions and statistical significance not reported).

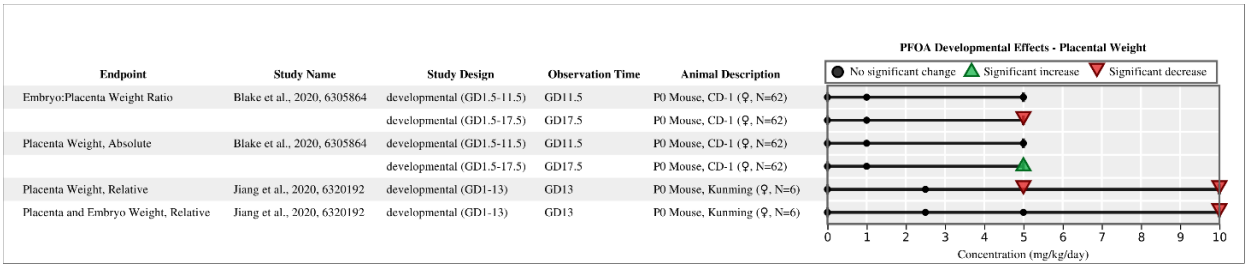


Figure 3-69. Placental Weights in Mice Following Exposure to PFOA

Interactive figure and additional study details available on HAWC.
GD = gestation day; P_0 = parental generation.

3.4.4.2.3 Offspring Mortality

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

3.4.4.2.3.1 Mice, Prenatal Evaluations

In two studies of gestational PFOA exposure in pregnant Kunming mice, Li et al. (2018a) reported significantly decreased GD 18 fetal survival at 10 and 20 mg/kg/day and total fetal resorption at 40 mg/kg/day (fetal survival was also decreased at 5 mg/kg/day, but the effect did not reach statistical significance), and Chen et al. (2017c) reported a significant increase in the number of resorbed fetuses at GD 13, but not GD 7, after exposure to 10 mg/kg/day PFOA beginning on GD 1 (there were no effects on the number of implantation sites). Suh et al. (2011) exposed pregnant CD-1 mice to 0, 2, 10, or 25 mg/kg/day from GD 11 to GD 16 (dams were sacrificed on GD16) and observed significant increases in the number of resorptions and dead fetuses at all dose levels; post-implantation loss was 3.87%, 8.83%, 30.98%, and 55.41% at 0, 2, 10, and 25 mg/kg/day, respectively. In pregnant CD-1 mice exposed from GD 1 to GD 17, Lau et

al. (2006) reported significant increases in the number of full-litter resorptions at PFOA doses ≥5 mg/kg/day, with complete loss of all pregnancies at the high dose of 40 mg/kg/day (no effect was observed on the number of implantation sites in litters that were fully resorbed). At 20 mg/kg/day, a significant increase in the percentage of prenatal loss per live litter was observed. White et al. (2011) reported significantly fewer implants in F_1-generation CD-1 mouse dams that had been exposed to 5 mg/kg/day PFOA (Figure 3-70).

3.4.4.2.3.2 Mice, Postnatal Evaluations

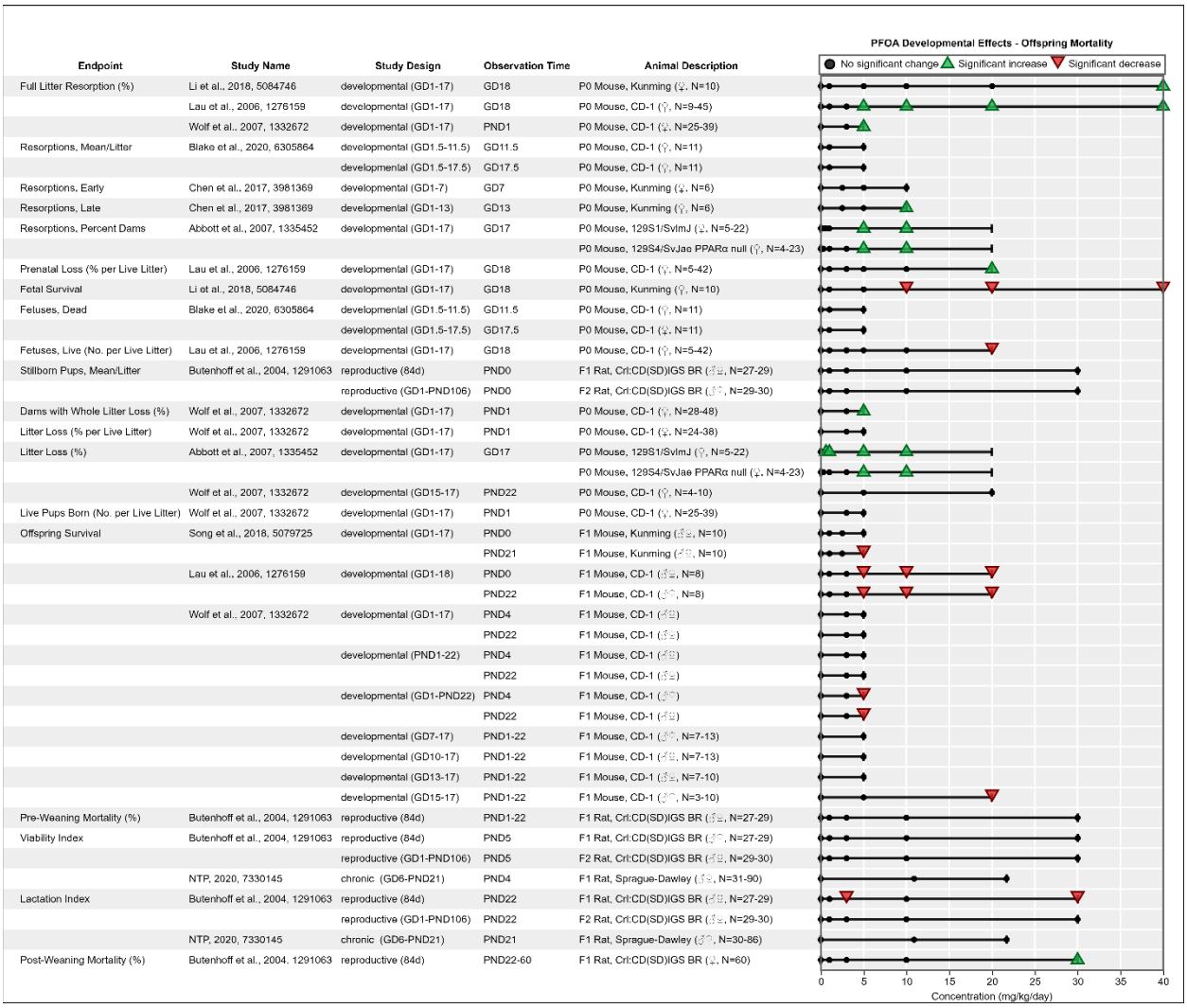
Wolf et al. (2007) reported a significant increase in total litter loss following oral PFOA exposure of pregnant CD-1 mice to 5 mg/kg/day (no effect on the number of implantation sites). In offspring exposed to 5 mg/kg/day PFOA in utero and throughout lactation, significantly decreased pup survival was observed from postnatal day (PND) 4 to 22; this effect was not seen in cross-fostered offspring exposed during gestation only or during lactation only. In a separate study, these authors exposed pregnant CD-1 mice to 5 mg/kg/day PFOA for different lengths of time (GD 7–GD 17, GD 10–GD 17, GD 13–GD 17, or GD 15–GD 17) and to 20 mg/kg/day from GD 15–17. Control mice received deionized water from GD 7 to GD 17. Although gestational PFOA exposure from GD 1 to GD 6 was not required to elicit adverse developmental responses in pups, the severity of postnatal responses, including decreased pup weight during lactation and delayed eye opening, increased with earlier and longer exposure durations (i.e., GD 7–GD 17 exposure resulted in more severe decreases in pup body weight when compared with pups exposed from GD 15 to GD 17). The authors could not attribute the observed adverse effects to a sensitive window of development as the pups exposed for longer durations had higher serum PFOA levels than pups exposed for shorter durations. Notably, significantly decreased offspring survival was observed in pups exposed to 20 mg/kg/day with the shortest exposure duration from GD 15 to GD 17.

Lau et al. (2006) reported significant increases in the incidence of stillbirths and pup mortality at 5, 10, and 20 mg/kg/day PFOA in CD-1 mice exposed from GD 1 to GD 18 and allowed to deliver naturally. Complete loss of all pregnancies was observed at the high dose of 40 mg/kg/day, though there were no effects on the number of implantation sites. At 10 and 20 mg/kg/day, most of the pups died on PND 1. After exposure of pregnant Kunming mice to 1, 2.5, or 5 mg/kg/day from GD 1 to GD 17, Song et al. (2018) reported a significant decrease in the number of surviving pups per litter on PND 7, 14, and 21 at 5 mg/kg/day (a dose-related trend was observed, but statistical significance was achieved only at the high dose). Yahia et al. (2010) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day PFOA from GD 0 to GD 18, and the dams were allowed to give birth naturally. Approximately 58% of pups born to high-dose dams were stillborn, and the remaining pups died within 6 hours of birth. Mean PND 4 survival rate was 98%, 100%, 84.4%, and 0% at 0, 1, 5, and 10 mg/kg/day, respectively (with significant decreases at 5 and 10 mg/kg/day). In the same study, some of the pregnant mice were exposed to the same dose levels from GD 0 to GD 17 and sacrificed on GD 18, and the number of live GD 18 fetuses from these dams was not significantly affected at any dose level. White et al. (2011) conducted a multigenerational study and dosed pregnant CD-1 mice with 0, 1, or 5 mg/kg/day from GD 1 to GD 17. Exposure to 5 mg/kg/day significantly increased prenatal loss, significantly decreased the number of live pups born, and significantly reduced postnatal survival. In adult female F_1 animals, no effects were observed on the prenatal loss or postnatal pup survival of the second generation (F_2) offspring.

Abbott et al. (2007) exposed pregnant 129S1/SvImJ wild-type and PPARα-null mice from GD 1 to GD 17 to dose levels ranging from 0.1 to 20 mg/kg/day and allowed the mice to deliver naturally. There were no treatment-related effects on the number of implantation sites, but wild-type dams exposed to ≥0.6 mg/kg/day PFOA and PPARα-null dams exposed to ≥5 mg/kg/day PFOA had significantly increased litter loss compared with their respective controls. At doses ≥5 mg/kg/day in wild-type dams and 20 mg/kg/day in PPARα-null dams, 100% litter loss occurred. The percentage of dams with full litter resorptions significantly increased in the 5, 10, and 20 mg/kg/day groups, with 100% full litter resorption in the 20 mg/kg/day group. When excluding dams with full litter resorptions, wild-type dams exposed to 1 mg/kg/day had a significant increase in litter loss. Pup survival from birth to weaning was significantly decreased in wild-type litters exposed to PFOA doses ≥0.6 mg/kg/day. No effect was seen in PPARα-null litters. Survival was significantly decreased for wild-type and heterozygous pups born to wild-type dams dosed with 1 mg/kg/day and for heterozygous pups born to PPARα-null dams dosed with 3 mg/kg/day. In the wild-type mice, the number of live and dead pups per litter were not affected by PFOA. Similarly, the number of pups per litter in CD-1 mice exposed to 0.1 or 1 mg/kg/day PFOA from GD 1.5 to GD 17.5 did not significantly differ from control groups (Cope et al., 2021) (Figure 3-70).

3.4.4.2.3.3 Rats, Postnatal Evaluations

The NTP two-year carcinogenicity studies in Sprague-Dawley rats found no effects on offspring survival (NTP, 2020), but Butenhoff et al. (2004a) reported an increase in the total number of dead F_1 rat pups during lactation (26/388 deaths at 30 mg/kg/day and 10/397 in the control group; statistically significant only on LD 6–LD 8) and a significant increase in F_1 female pup deaths with 30 mg/kg/day on post-weaning days 2–8. F_2 generation pup survival was unaffected. In pregnant Sprague-Dawley rats dosed with 0, 3, 10, or 30 mg/kg/day from GD 4 to LD 21, one dam at 3 mg/kg/day and two dams at 30 mg/kg/day delivered small litters (3–6 pups/litter compared with 12–19 pups/litter in the control group); however, statistical significance was not indicated, and given the small sample size (5 dams/group), the biological significance of this finding is unclear (Hinderliter et al., 2005) (Figure 3-70).



Endpoint	Study Name	Study Design	Observation Time	Animal Description
Full Litter Resorption (%)	Li et al., 2018, 5084746	developmental (GD1-17)	GD18	P0 Mouse, Kunming (♀, N=10)
	Lau et al., 2006, 1276159	developmental (GD1-17)	GD18	P0 Mouse, CD-1 (♀, N=9-45)
	Wolf et al., 2007, 1332672	developmental (GD1-17)	PND1	P0 Mouse, CD-1 (♀, N=25-39)
Resorptions, Mean/Litter	Blake et al., 2020, 6305864	developmental (GD1.5-11.5)	GD11.5	P0 Mouse, CD-1 (♀, N=11)
		developmental (GD1.5-17.5)	GD17.5	P0 Mouse, CD-1 (♀, N=11)
Resorptions, Early	Chen et al., 2017, 3981369	developmental (GD1-7)	GD7	P0 Mouse, Kunming (♀, N=6)
Resorptions, Late	Chen et al., 2017, 3981369	developmental (GD1-13)	GD13	P0 Mouse, Kunming (♀, N=6)
Resorptions, Percent Dams	Abbott et al., 2007, 1335452	developmental (GD1-17)	GD17	P0 Mouse, 129S1/SvImJ (♀, N=5-22)
				P0 Mouse, 129S4/SvJae PPARα null (♀, N=4-23)
Prenatal Loss (% per Live Litter)	Lau et al., 2006, 1276159	developmental (GD1-17)	GD18	P0 Mouse, CD-1 (♀, N=5-42)
Fetal Survival	Li et al., 2018, 5084746	developmental (GD1-17)	GD18	P0 Mouse, Kunming (♀, N=10)
Fetuses, Dead	Blake et al., 2020, 6305864	developmental (GD1.5-11.5)	GD11.5	P0 Mouse, CD-1 (♀, N=11)
		developmental (GD1.5-17.5)	GD17.5	P0 Mouse, CD-1 (♀, N=11)
Fetuses, Live (No. per Live Litter)	Lau et al., 2006, 1276159	developmental (GD1-17)	GD18	P0 Mouse, CD-1 (♀, N=5-42)
Stillborn Pups, Mean/Litter	Butenhoff et al., 2004, 1291063	reproductive (84d)	PND0	F1 Rat, Crl:CD(SD)IGS BR (♂♀, N=27-29)
		reproductive (GD1-PND106)	PND0	F2 Rat, Crl:CD(SD)IGS BR (♂♀, N=29-30)
Dams with Whole Litter Loss (%)	Wolf et al., 2007, 1332672	developmental (GD1-17)	PND1	P0 Mouse, CD-1 (♀, N=28-48)
Litter Loss (% per Live Litter)	Wolf et al., 2007, 1332672	developmental (GD1-17)	PND1	P0 Mouse, CD-1 (♀, N=24-38)
Litter Loss (%)	Abbott et al., 2007, 1335452	developmental (GD1-17)	GD17	P0 Mouse, 129S1/SvImJ (♀, N=5-22)
				P0 Mouse, 129S4/SvJae PPARα null (♀, N=4-23)
	Wolf et al., 2007, 1332672	developmental (GD15-17)	PND22	P0 Mouse, CD-1 (♀, N=4-10)
Live Pups Born (No. per Live Litter)	Wolf et al., 2007, 1332672	developmental (GD1-17)	PND1	P0 Mouse, CD-1 (♀, N=25-39)
Offspring Survival	Song et al., 2018, 5079725	developmental (GD1-17)	PND0	F1 Mouse, Kunming (♂♀, N=10)
			PND21	F1 Mouse, Kunming (♂♀, N=10)
	Lau et al., 2006, 1276159	developmental (GD1-18)	PND0	F1 Mouse, CD-1 (♂♀, N=8)
			PND22	F1 Mouse, CD-1 (♂♀, N=8)
	Wolf et al., 2007, 1332672	developmental (GD1-17)	PND4	F1 Mouse, CD-1 (♂♀)
			PND22	F1 Mouse, CD-1 (♂♀)
		developmental (PND1-22)	PND4	F1 Mouse, CD-1 (♂♀)
			PND22	F1 Mouse, CD-1 (♂♀)
		developmental (GD1-PND22)	PND4	F1 Mouse, CD-1 (♂♀)
			PND22	F1 Mouse, CD-1 (♂♀)
		developmental (GD7-17)	PND1-22	F1 Mouse, CD-1 (♂♀, N=7-13)
		developmental (GD10-17)	PND1-22	F1 Mouse, CD-1 (♂♀, N=7-13)
		developmental (GD13-17)	PND1-22	F1 Mouse, CD-1 (♂♀, N=7-10)
		developmental (GD15-17)	PND1-22	F1 Mouse, CD-1 (♂♀, N=3-10)
Pre-Weaning Mortality (%)	Butenhoff et al., 2004, 1291063	reproductive (84d)	PND1-22	F1 Rat, Crl:CD(SD)IGS BR (♂♀, N=27-29)
Viability Index	Butenhoff et al., 2004, 1291063	reproductive (84d)	PND5	F1 Rat, Crl:CD(SD)IGS BR (♂♀, N=27-29)
		reproductive (GD1-PND106)	PND5	F2 Rat, Crl:CD(SD)IGS BR (♂♀, N=29-30)
	NTP, 2020, 7330145	chronic (GD6-PND21)	PND4	F1 Rat, Sprague-Dawley (♂♀, N=31-90)
Lactation Index	Butenhoff et al., 2004, 1291063	reproductive (84d)	PND22	F1 Rat, Crl:CD(SD)IGS BR (♂♀, N=27-29)
		reproductive (GD1-PND106)	PND22	F2 Rat, Crl:CD(SD)IGS BR (♂♀, N=29-30)
	NTP, 2020, 7330145	chronic (GD6-PND21)	PND21	F1 Rat, Sprague-Dawley (♂♀, N=30-86)
Post-Weaning Mortality (%)	Butenhoff et al., 2004, 1291063	reproductive (84d)	PND22-60	F1 Rat, Crl:CD(SD)IGS BR (♀, N=60)

Figure 3-70. Offspring Mortality in Rodents Following Exposure to PFOA[a]

Interactive figure and additional study details available on HAWC.
GD = gestation day; PND = postnatal day; P_0 = parental generation; F_1 = first generation; F_2 = second generation; d = day.
[a] Lau et al. (2006) exposed pregnant mice from GD 1 to GD 19, but some of the mice were sacrificed and examined on GD 18. Based on data from the pregnant mice sacrificed on GD 18, all litters from dams administered 40 mg/kg/day were resorbed, and therefore no offspring were available for postnatal assessments.

3.4.4.2.4 Offspring Body Weight

Available studies of oral gestational PFOA exposure to mice report significant decreases in offspring body weight in prenatal evaluations at doses ≥5 mg/kg/day and postnatal evaluations at dose levels as low as 0.5 mg/kg/day (Blake et al., 2020; Li et al., 2018a; Tucker et al., 2014; Hu et al., 2012; Suh et al., 2011; White et al., 2011; Hu et al., 2010; Yahia et al., 2010; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006). Offspring weight deficits in pups were observed to extend beyond weaning in three studies in CD-1 mice (at 1, ≥3, and 5 mg/kg/day, respectively) (Tucker et al., 2014; White et al., 2011; Lau et al., 2006) and in a multigeneration rat study at doses of 30 mg/kg/day (Butenhoff et al., 2004a). In some studies, decreased fetal and/or pup body weight was observed in the absence of maternal body weight effects.

3.4.4.2.4.1 Mice, Prenatal Evaluations

Blake et al. (2020) reported significantly decreased GD 17.5 fetal weight with 5 mg/kg/day PFOA following gestational exposure in CD-1 mice, despite significantly increased maternal body weight gain. Lau et al. (2006) reported a significant decrease in GD 18 fetal body weights after gestational exposure of CD-1 mice to 20 mg/kg/day PFOA. In pregnant Kunming mice, gestational exposure was associated with significantly decreased GD 18 fetal weights at 5–40 mg/kg/day (Li et al., 2018a). Suh et al. (2011) reported a significant decrease in GD 16 fetal weights at doses ≥10 mg/kg/day after exposure of pregnant CD-1 mice to 0, 2, 10, or 25 mg/kg/day from GD 11 to GD 16. Body weights of GD 18 ICR mouse fetuses were significantly decreased following gestational exposure to 5 or 10 mg/kg/day PFOA (Yahia et al., 2010).

3.4.4.2.4.2 Mice, Postnatal Evaluations

Wolf et al. (2007) reported that CD-1 mouse pup body weights were significantly decreased after gestational exposure to 5 mg/kg/day PFOA from GD 1 to GD 17. The authors also exposed pregnant mice to 20 mg/kg/day from GD 15 to GD 17 and to 5 mg/kg/day for different lengths of time (GD 7–GD 17, GD 10–GD 17, GD 13–GD 17, or GD 15–GD 17). After exposure to 5 mg/kg/day from GD 7 to GD 17 or GD 10 to GD 17 and to 20 mg/kg/day from GD 15 to GD 17, male pup body weights were significantly decreased. Additionally, with 5 mg/kg/day PFOA, male and female pup body weights were significantly decreased throughout lactation in all exposure groups, and the magnitude of the effect increased with increasing number of exposure days. Body weight deficits in male pups that had been exposed from GD 7 to GD 17 or GD 10 to GD 17 persisted for 10–11 weeks.

Hu et al. (2010) exposed C57BL/6N pregnant mice with 0.5 or 1.0 mg/kg/day PFOA in drinking water from GD 6 through GD 17. At PND 2, litter weights were significantly reduced in the PFOA treatment groups (7%—12% less than the controls). At PND 7 and 14, the 0.5 mg/kg/day group litter weight was equivalent to the controls, but the 1.0 mg/kg/day group was still significantly less than the controls (14% and 5%, respectively, by time point).

Body weights of live pups born to pregnant ICR mice dosed with 5 or 10 mg/kg/day during gestation were significantly reduced (Yahia et al., 2010). At ≥3 mg/kg/day, a dose-related trend in growth retardation (body weight reductions of 25%–30%) was observed in neonates at weaning; body weights reached control levels by 6 weeks of age for females and by 13 weeks of age for males (Lau et al., 2006). Exposure of pregnant C57BL/6N mice to 2 mg/kg/day from mating through lactation resulted in significantly decreased pup weights (32.6% lower than controls, on average) from PND 1 to PND 21 (there were no effects on maternal body weights) (Hu et al., 2012). Song et al. (2018) observed significantly increased body weights in PND 21 male offspring after gestational exposure to 2.5 or 5 mg/kg/day PFOA (female data not provided). However, the authors did not report controlling for litter size in this study; the significantly decreased litter size in the 5 mg/kg/day group could potentially result in increased body weight in those pups due to reduced competition for maternal resources.

In a study in which pregnant 129S1/SvImJ wild-type and PPARα-null mice were orally exposed from GD 1 to GD 17 to dose levels ranging from 0.1 to 20 mg/kg/day (Abbott et al., 2007), decreased offspring body weight was seen in wild-type mice at 1 mg/kg/day (highest dose level at which this effect was measured due to extensive litter loss at higher doses) beginning around

PND 6, and this effect achieved statistical significance on PND 9, PND 10, and PND 22 (males) and PND 7–PND 10 and PND 22 (females). No effects were observed on PPARα-null offspring body weights. White et al. (2011) exposed pregnant CD-1 mice to 0, 1, or 5 mg/kg/day from GD 1 to GD 17. A separate group of pregnant mice was dosed with either 0 or 1 mg/kg/day from GD 1 to GD 17 and received drinking water containing 5 ppb PFOA beginning on GD 7. F_1 females and F_2 offspring from the second group continued to receive drinking water that contained 5 ppb PFOA until the end of the study, except during F_1 breeding and early gestation, to simulate a chronic low-dose exposure. F_1 offspring body weight at PND 42 was significantly reduced at 5 mg/kg/day; at PND 63, body weight was significantly reduced for offspring from dams given 1 mg/kg/day plus 5 ppb in the drinking water compared with offspring from dams given only 1 mg/kg/day. For the F_2 pups, a significant reduction in body weight was observed in control plus 5 ppb drinking water PFOA offspring on PND 1, but there was no difference by PND 3. F_2 offspring from the 1 mg/kg/day and 1 mg/kg/day plus 5 ppb drinking water PFOA groups had increased body weights compared with controls on PND 14, PND 17, and PND 22. Female CD-1 mice that had been exposed gestationally to 1 mg/kg/day had significantly decreased "net" body weights (i.e., absolute body weight minus absolute liver weight) at PND 21 and PND 35 but not at PND 56 (Tucker et al., 2014); the absolute body weights of female offspring were not altered due to gestational PFOA treatment. Macon et al. (2011) found no effects on offspring body weights following exposure of pregnant CD-1 mice to PFOA from GD 1 to GD 17 with doses up to 1 mg/kg/day or from GD 10 to GD 17 with doses up to 3 mg/kg/day. Similarly, Cope et al. (2021) exposed CD-1 dams to 0.1 or 1.0 mg/kg/day PFOA via oral gavage from GD 1.5 to GD 17.5 and did not find treatment-related changes in pup weight at PND 0.5, PND 5, or PND 22.

3.4.4.2.4.3 Rats, Postnatal Evaluations

In two NTP 2-year carcinogenicity studies (NTP, 2020), dietary exposure of pregnant Sprague-Dawley rats to 300 ppm PFOA (approximately 22 mg/kg/day during gestation and 45 mg/kg/day from LD 1 to LD 14) resulted in significantly decreased pup weights throughout lactation (3%–8% lower than controls). In both studies, there were minimal to no effects on maternal body weight.

Significantly decreased F_1 pup weight (8%–11% lower than controls) during lactation was observed following exposure of pregnant Sprague-Dawley rats to 30 mg/kg/day, in the absence of effects on maternal body weight; F_2 pup weight was slightly decreased at 30 mg/kg/day, but the effect was not statistically significant (Butenhoff et al., 2004a). At 30 mg/kg/day, significant decreases in body weight and body weight gain were seen in F_1 male offspring during the juvenile and peripubertal phases and in F_1 female offspring beginning on day 8 postweaning and continuing through pre-cohabitation, gestation, and lactation (along with decreased food consumption) (Figure 3-71).

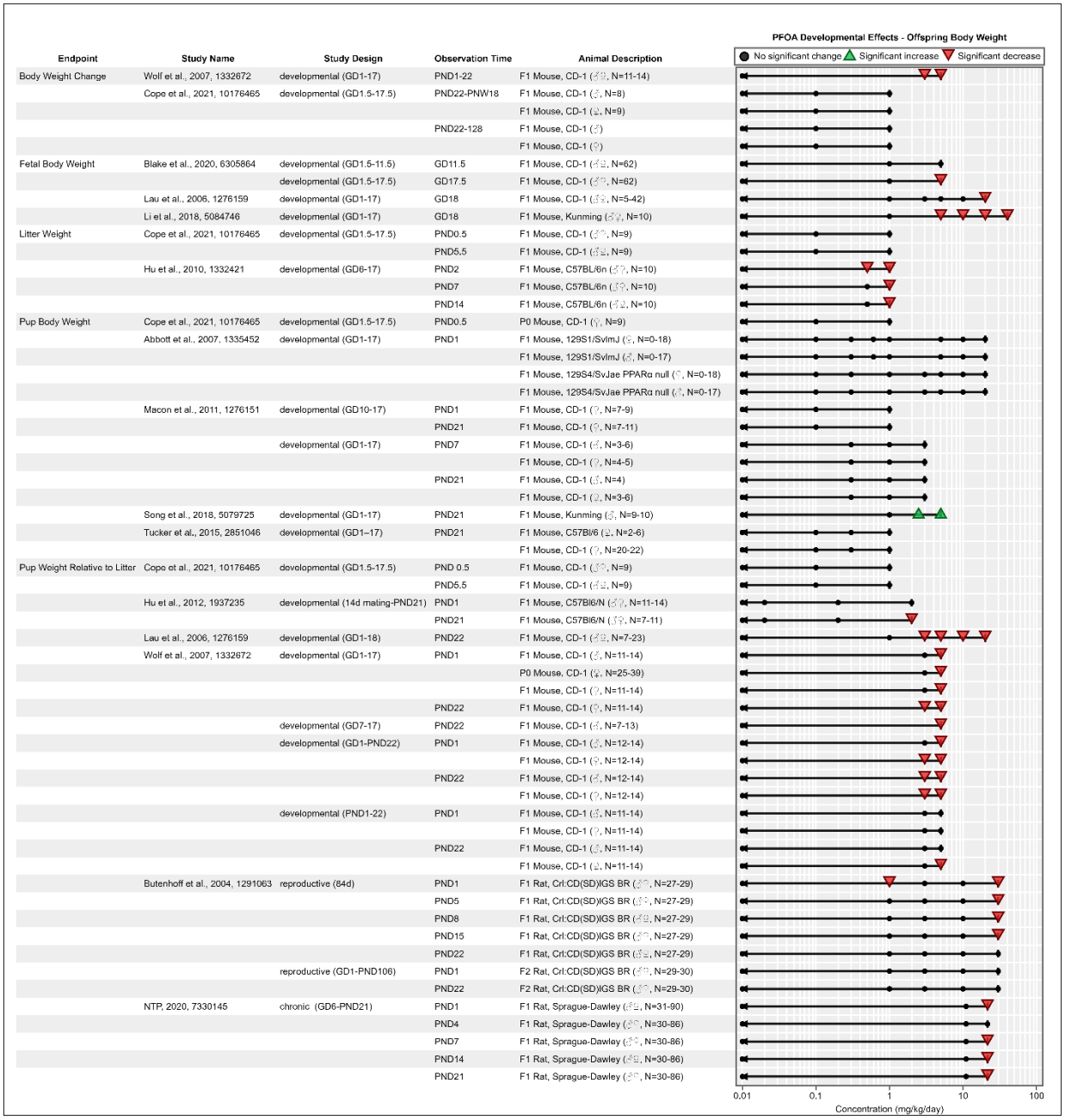


Figure 3-71. Offspring Body Weight in Rodents Following Exposure to PFOA (logarithmic scale)[a]

PFOA concentration is presented in logarithmic scale to optimize the spatial presentation of data. Interactive figure and additional study details available on HAWC.

GD = gestation day; PND = postnatal day; P_0 = parental generation; F_1 = first generation; F_2 = second generation; d = day.

[a] Lau et al. (2006) exposed pregnant mice from GD 1 to GD 19, but some of the mice were sacrificed and examined on GD 18. Based on data from the pregnant mice sacrificed on GD 18, all litters from dams administered 40 mg/kg/day were resorbed, and therefore no offspring were available for postnatal assessments.

3.4.4.2.5 Skeletal and Visceral Alterations

Following exposure of pregnant CD-1 mice to 1, 3, 5, 10, 20, or 40 mg/kg/day PFOA during gestation, Lau et al. (2006) reported decreases in ossification of the forelimb proximal phalanges (significant at all dose levels except 5 mg/kg/day), hindlimb proximal phalanges (significant at all dose levels except 3 and 5 mg/kg/day), calvaria (significant at 1, 3, and 20 mg/kg/day), enlarged fontanel (significant at 1, 3, and 20 mg/kg/day), and supraoccipital bone (significant at 10 and 20 mg/kg/day). Significantly reduced ossification of caudal vertebrae, metacarpals, metatarsals, and hyoid was observed at 20 mg/kg/day. Significant increases in minor limb and/or tail defects were observed in fetuses at ≥5 mg/kg/day (no defects were observed at 0, 1, or 3 mg/kg/day) and significantly increased incidence of microcardia was observed at 10 and 20 mg/kg/day (no incidences were observed in any other groups). Yahia et al. (2010) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day from GD 0 to GD 17 (sacrificed on GD 18) and reported a significant increase in the incidence of cleft sternum and ossification delays (phalanges) in GD 18 fetuses at 10 mg/kg/day. In the same study, some dams were dosed from GD 0 to GD 18 and allowed to give birth, and pup lungs and brains were examined at PND 4; no abnormalities were reported.

3.4.4.2.6 Altered Developmental Timing

Reduced postnatal growth leading to developmental delays was observed in mice. Lau et al. (2006) and Wolf et al. (2007) reported delayed eye opening in CD-1 mice offspring after gestational exposure to ≥5 mg/kg/day PFOA. Additionally, Wolf et al. (2007) observed delayed eye opening following gestational plus lactational exposure to 3 or 5 mg/kg/day. Wolf et al. (2007) also observed delayed body hair emergence following gestational exposure to 5 mg/kg/day or gestational plus lactational exposure to 3 or 5 mg/kg/day. In pregnant 129S1/SvImJ wild-type and PPARα-null mice orally exposed from GD 1 to GD 17 to 0.1–20 mg/kg/day PFOA (Abbott et al., 2007), offspring born to wild-type dams showed a dose-related trend for delayed eye opening compared with controls at 0.6 and 1 mg/kg/day (significant at 1 mg/kg/day; however, extensive litter loss was observed at the higher doses). In PPARα-null offspring, none of the litters from dams exposed to 3 mg/kg/day had eyes open on PND 13, but no significant difference between this group and the control was observed by PND 14. Yahia et al. (2010) dosed pregnant ICR mice with 0, 1, 5, or 10 mg/kg/day PFOA from GD 0 to GD 17 (sacrificed on GD 18) and reported a significant decrease in the percentage of GD 18 fetuses with erupted incisors at 10 mg/kg/day.

3.4.4.2.7 Mammary Gland Development

Altered mammary gland development has been shown to result in later-life functional reproductive consequences, such as reduced lactational efficacy and subsequent pup loss, and has been linked to increased incidence of mammary and breast cancers (Macon and Fenton, 2013; Fenton, 2006; Birnbaum and Fenton, 2003). Studies examining effects of PFOA exposure on mammary gland development in CD-1 mice reported delayed mammary gland development at dose levels as low as 0.01 mg/kg/day (Tucker et al., 2014; Macon et al., 2011). However, no differences in response to a lactation challenge were seen in PFOA-exposed CD-1 mouse dams with delayed mammary gland development, and no significant effects on body weight gain were seen in pups nursing from dams with less fully developed mammary glands (White et al., 2011).

Macon et al. (2011) exposed pregnant CD-1 mice to PFOA from GD 1 to GD 17 (full gestation) or GD 10 to GD 17 (late gestation) to examine effects of PFOA exposure on mammary gland morphology. Mammary gland whole mounts were scored on a 1 to 4 subjective, age-adjusted, developmental scale. Quantitative measures also were made of longitudinal growth, lateral growth, and number of terminal end buds. At all PFOA exposure levels in both experiments (≥0.3 mg/kg/day in the full gestation study and ≥0.01 mg/kg/day in the late-gestation study), significantly stunted mammary epithelial growth was observed in female offspring in the absence of effects on offspring body weight. Additionally, there were significant differences from controls in quantitative measures of longitudinal and lateral growth and numbers of terminal end buds at 1 mg/kg/day in the late-gestation experiment. The delayed development was characterized by reduced epithelial growth and the presence of numerous terminal end buds. Photographs of the mammary gland whole mounts at PND 21 and PND 84 from the full-gestation experiment showed differences in the duct development and branching pattern of offspring from dams given 0.3 and 1 mg/kg/day PFOA (offspring from high-dose dams not pictured). At PND 21, mammary glands from the 1 mg/kg/day late-gestation group had significantly less longitudinal epithelial growth and fewer terminal end buds compared with controls. In the late-gestation experiment, mammary gland development was delayed by exposure to PFOA, especially longitudinal epithelial growth. At PND 21, all treatment groups had significantly lower developmental scores. At the highest dose, poor longitudinal epithelial growth and decreased number of terminal end buds were observed. The quantitative measures were statistically significant only for the high dose compared with the controls, whereas the qualitative scores at all doses were significantly different from controls.

CD-1 mice were dosed with 5 mg/kg/day on GD 7–GD 17, GD 10–GD 17, GD 13–GD 17, or GD 15–GD 17 or with 20 mg/kg/day on GD 15–GD 17 (controls were dosed GD 7–GD 17) and mammary gland effects of this study were published by White et al. (2009). Mammary gland developmental scores for all offspring of dams exposed to PFOA were significantly lower at PND 29 and PND 32. Delayed ductal elongation and branching and delayed appearance of terminal end buds were characteristic of delayed mammary gland development at PND 32. At 18 months of age, mammary tissues were not scored (due to the lack of a protocol applicable to mature animals) but dark foci (composition unknown) in the mammary tissue were observed at a higher frequency in exposed animals compared with controls. There was no consistent response with respect to dosing interval. Qualitatively, mammary glands from treated dams on LD 1 appeared immature compared with control dams (White et al., 2009). The authors also exposed pregnant CD-1 mice to 0, 3, or 5 mg/kg/day from GD 1 to GD 17 and offspring were cross-fostered at birth to create seven treatment groups: control, in utero exposure only (3U and 5U), lactational exposure only (3L and 5L), and in utero + lactational exposure (3U + L and 5U + L). Mammary gland whole mounts from female offspring between PND 22 and PND 63 were scored. With the exception of females of the 3L group, all female offspring of PFOA-exposed dams had reduced mammary gland developmental scores at PND 22. At PND 42, mammary gland scores from females in the 3U + L group were the only ones not statistically different from control scores. This might have been due to inter-individual variance and multiple criteria used to calculate mammary gland development scores. All offspring of dams exposed to PFOA exhibited delayed mammary gland development at PND 63, including those exposed only through lactation (3L and 5L).

White et al. (2011) dosed pregnant CD-1 mice with 0, 1, or 5 mg/kg/day from GD 1 to GD 17. A second group of pregnant mice was dosed with either 0 or 1 mg/kg/day from GD 1 to GD 17 and also received drinking water containing 5 ppb PFOA beginning on GD 7. The F_1 females and F_2 offspring from the second group continued to receive drinking water that contained 5 ppb PFOA until the end of the study, except during F_1 breeding and early gestation, to simulate a chronic low-dose exposure. Only the P_0 dams were given PFOA by gavage. P_0 females were sacrificed on PND 22. F_1 offspring were weaned on PND 22 and bred at 7–8 weeks of age. F_2 litters were maintained through PND 63. Groups of F_1 and F_2 offspring were sacrificed on PND 22, PND 42, and PND 63. A group of F_2 offspring was also sacrificed on PND 10. A lactational challenge experiment was performed on PND 10 with F_1 dams and F_2 offspring to estimate the volume of milk produced during a discrete period of nursing. Mammary glands were evaluated from P_0 dams on PND 22, from F_1 dams on PND 10 and PND 22, and from F_1 and F_2 female offspring on PND 10 (F_2 only), PND 22, PND 42, and PND 63. Mammary gland whole mounts were scored qualitatively. At PND 22, control P_0 dams displayed weaning-induced mammary involution. At PND 22, the mammary glands of all PFOA-exposed P_0 dams, including the dams receiving 5 ppb PFOA via drinking water only, resembled glands of mice at or near the peak of lactation (~PND 10). The F_1 dams examined on PND 10 and PND 22 had significantly lower developmental scores on PND 10, but that was no longer evident at PND 22, except for those exposed in utero to 5 mg/kg/day. In the F_1 female offspring not used for breeding, the mammary glands of all PFOA-exposed mice were significantly delayed in development on PND 22, 42, and 63. For the F_2 female offspring, some differences in mammary gland scores were observed between the groups, but most were not significantly different from controls. No differences in response to a lactational challenge were seen in PFOA-exposed dams with morphologically delayed mammary gland development.

Tucker et al. (2014) orally exposed pregnant CD-1 and C57BL/6 mice to 0, 0.01, 0.1, 0.3, or 1 mg/kg/day from GD 1 to GD 17. After parturition, the number of pups was reduced so that there were ultimately four to eight CD-1 litters and three to seven C57BL/6 litters per treatment. Different treatment blocks monitored for different endpoints at different times. There was a dose-related trend toward decreasing mammary gland developmental scores for both strains of mice. In CD-1 mice, scores were significantly reduced at PFOA doses ≥0.01 mg/kg/day on PND 35 and ≥0.1 mg/kg/day on PND 21. In C57BL/6 mice, scores were significantly reduced at 0.3 and 1.0 mg/kg/day on PND 21. The authors suggest that these differences in responses between strains may be due to increased serum PFOA levels of the CD-1 mice (Tucker et al., 2014). At 5 mg/kg/day, in mammary glands of C57BL/6 mice, there was a significant increase in the number of terminal end buds and stimulated terminal ducts; ductal length was not affected. Mammary gland development was inhibited in C57BL/6 mice dosed with 10 mg/kg/day, with no terminal end buds or stimulated terminal ducts present and very little ductal growth.

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

3.4.4.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse developmental outcomes is discussed in Sections 3.2.6, 3.2.7, 3.3.4, 3.4.1, and 3.4.5 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 19 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA 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 3-72.

Mechanistic Pathway	Animal	Human	In Vitro	Grand Total
Angiogenic, Antiangiogenic, Vascular Tissue Remodeling	1	0	0	1
Big Data, Non-Targeted Analysis	0	6	1	7
Cell Growth, Differentiation, Proliferation, Or Viability	5	1	2	8
Cell Signaling Or Signal Transduction	2	1	0	3
Fatty Acid Synthesis, Metabolism, Storage, Transport, Binding, B-Oxidation	4	0	1	5
Hormone Function	2	0	0	2
Inflammation And Immune Response	0	1	0	1
Oxidative Stress	2	1	0	3
Xenobiotic Metabolism	3	0	1	4
Other	0	0	1	1
Not Applicable/Not Specified/Review Article	1	0	0	1
Grand Total	8	7	4	19

Figure 3-72. Summary of Mechanistic Studies of PFOA 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 PFOA. The mechanistic data are organized by the following outcomes: early survival, general development, and gross morphology; fetal growth and placental effects; metabolism; hepatic development; cardiac development; and neurological development.

3.4.4.3.1 Early Survival, General Development, Gross Morphology

Mechanisms through which PFOA exposure may alter survival and development were studied in several in vivo experimental animal models. In an in vivo mouse developmental study, pregnant NMRI dams exposed to PFOA from GD 5 to GD 9 via intraperitoneal (IP) injection showed increased fetal death in the offspring at the highest dose (20 mg/kg/day) of PFOA, as well as histopathological abnormalities in the brain, liver, and heart, possibly due to the observed

mitochondrial toxicity/dysfunction (e.g., increased mitochondrial swelling, increased mitochondrial membrane potential (MMP) collapse) or oxidative stress (e.g., increased mitochondrial ROS formation) (Salimi et al., 2019). In another mouse developmental study examining lower doses in the dams, embryo survival was not affected at up to 10 mg/kg/day PFOA exposure in dams exposed from GD 1.5 to GD 11.5 or GD 1.5 to 17.5 via oral gavage (Blake et al., 2020). However, 5 and 10 mg/kg exposure via oral gavage from GD 1 GD 17 decreased survival rate in 5-day old pups, possibly due to hepatotoxicity; the authors observed significantly increased liver index in pups and increased reactive oxygen species and changes in liver enzyme function, mediated by the PPARα pathway (Li et al., 2019b).

Several studies using zebrafish as a model organism that were identified in the current assessment were included in a recent review of developmental effects of PFOA (Lee et al., 2020). In general, PFOA exposure was associated with developmental delays, reductions in measures of embryo survival, and increased malformations in the head and tail that may be related to perturbations in gene expression during critical windows of organism development.

The review by Lee et al. (2020) included a zebrafish multigenerational study by Jantzen et al. (2017), in which embryos were exposed to PFOA from 3 to 120 hours post-fertilization (hpf). Embryos were allowed to reach adulthood and breed. Although exposure to PFOA did not decrease survival in the first exposed generation (P_0), there were significantly fewer eggs and viable embryos than the controls in the P_0. Further, F_1 embryos had significant developmental delays and delayed hatching. Gene expression analysis of four solute carrier organic anion transporter family members (*slco1d1*, *slco2b1*, *slco3a1*, and *slco4a1*) and the growth factor transforming growth factor beta 1a (*tgfb1a)* in the P_0 generation showed that PFOA exposure led to decreased expression in *slco2b1*, *slco3a1*, and *slco4a1* and increased expression in *slco1d1*. In the F_1 embryos, there was a significant increase in expression of the protein transporter adaptor related protein complex 1 subunit sigma 1 (*ap1s1).* The authors concluded that alterations in the expression of these genes during development likely contributed to the delayed development and morphologic and toxic effects observed (Jantzen et al., 2017). The elevations in *ap1s1* were in conflict with a prior publication from the same research group that reported decreased *ap1s1* at 120 hpf, which coincided with alterations in morphometric parameters in zebrafish embryos, including increased interocular distance (a metric of cranio-facial development), reduced total body length, and reduced yolk sac area (Jantzen et al., 2016a). Other alterations in gene expression at 120 hpf included elevations in *slco2b1* (transport protein) and transcription factor 3a (*tfc3a;* involved in muscle development), and *c-fos* (transcription factor complex). Altogether, results suggest that alterations in *ap1s1* are unlikely the result of a global upregulation or downregulation of genes and that PFOA may differentially influence genes at certain points in development. However, the current data cannot rule out the possibility that the observed alterations in gene expression are due to a delay or acceleration in development.

In another zebrafish study by Bouwmeester et al. (2016), embryos that were exposed to 10–320 μM PFOA were examined for developmental toxicity and morphological effects. PFOA did not induce embryotoxic effects at the exposure levels in the experiment; however, some epigenome modifications were noted. When locus-specific methylation was assessed, PFOA exposure was associated with hypomethylation on the CpG region of *vasa*, and hypermethylation at CpG1 in *vitellogenin 1* (*vtg1). Vasa* is expressed in the germline and is active during development, and *vtg1* is expressed in the liver of egg-laying vertebrates and encodes for the

estrogen responsive egg-yolk protein vitellogenin, although, interestingly, PFOA was included in this study to demonstrate a "non-estrogenic PPARγ/RXR agonist." These epigenetic modifications early in life and development may play a role in the development of later life adverse health outcomes (Bouwmeester et al., 2016).

In humans, epigenetic modification during development of the fetus can be measured via cord blood at birth. Several human studies evaluated cord blood DNA methylation patterns to understand the epigenetic effects of PFOA exposure. Miura et al. (2018) found that increased PFOA in the cord blood was associated with global hypermethylation in a cohort from Japan; however, two other cord blood studies of global methylation found no associations between PFOA exposure and global methylation changes (Leung et al., 2018; Liu et al., 2018a). Similarly, Kingsley et al. (2017) did not observe associations between PFOA exposure in cord blood and epigenome-wide changes in global methylation status. However, for the high PFOA exposure group, the authors found hypomethylation in seven CpG sites located in several genes, including *RAS P21 protein Activator 3 (RASA3)* and Opioid Receptor Delta 1 *(OPRD1). OPRD1* is involved in weight and obesity, as well as morphine and heroin dependence, and could potentially be a mechanistic pathway linking PFOA and obesity, an association that has previously been reported (Kingsley et al., 2017). Cord blood samples from a prospective cohort in China were used by Liu et al. (2018c) to evaluate potential associations between PFOA exposure and leukocyte telomere lengths (LTLs). There was no association between PFOA exposure and LTLs in this study.

3.4.4.3.2 Fetal Growth and Placental Effects

Fetal growth was assessed in four mouse developmental studies. Blake et al. (2020) found decreased embryonic weights in CD-1 mice at GD 17.5, with concurrent increases in placental weights and placental lesions consistent with labyrinth congestion (Section 3.4.4.2.4.1). Placentas also had higher thyroxine (T4) levels relative to controls, suggesting a possible endocrine mechanistic pathway of effect. In NMRI mice exposed to 0, 1, 10, or 20 mg/kg/day PFOA from GD 5 to 9, Salimi et al. (2019) observed reduced fetal length and weight, and decreased placental diameter at the highest dose group (20 mg/kg/day). The authors note that toxicity was likely mediated through mitochondrial toxicity in the liver (described below), which appeared to be isolated to the mouse fetus rather than the placenta. Li et al. (2019b) reported a dose-dependent reduction in growth and weight gain in Kunming mouse pups exposed to PFOA during gestation (GD 0–17). The authors attribute the stunted growth to hepatotoxicity consequent to increased ROS and changes in liver enzyme function mediated by the PPARα pathway (Li et al., 2019b).

Perturbations in growth and corresponding changes in gene expression of key developmental genes have been observed in several studies in zebrafish. In the multigenerational zebrafish study by Jantzen et al. (2017), P_0 generation fish exposed to PFOA had significantly shorter body length and reduced body weight compared with controls. Offspring of PFOA-exposed fish were significantly developmentally delayed and had increased expression in the protein transport gene *ap1s1* at 48 hpf, possibly leading to the changes in growth (Jantzen et al., 2017). In Jantzen et al. (2016a), several morphometric endpoints were measured in zebrafish embryos exposed to 0.02, 0.2, or 2.0 μM PFOA, including interocular distance, total body length, and yolk sac area. The size of all three parameters was reduced in groups exposed to PFOA, indicating slowed embryonic development) at values 5- to 25-fold below previously calculated median lethal

concentration (LC_{50}) values. The authors also evaluated gene expression at 120 hpf and 14 days post-fertilization (dpf). At 120 hpf, *slco2b1* (transport protein), *tfc3a* (involved in muscle development), and *c-fos* (transcription factor complex) were upregulated, while *ap1s* (involved in protein transport) was downregulated. At 14 dpf, *slco2b1* and *Tcf3a* (involved in muscle development) were upregulated (Jantzen et al., 2016a).

Gorrochategui et al. (2014) evaluated cytotoxicity and aromatase activity in a placental cell line (JEG-3 cells). PFOA exposure was found to induce cytotoxicity and inhibit aromatase (CYP19) activity (Gorrochategui et al., 2014). In a rhesus monkey trophoblast cell line, PFOA treatment showed significant differences in gene expression, with possible affected diseases/biological functions including cell movement, epithelial tissue growth, and vasculogenesis. Pathways included cysteine metabolism, interleukin signaling, Toll-like receptor, TGF-β, PDGF, PPAR, NFKB, MAPK, Endothelin 1, TNRF2, tight junctions, cytokines including IFNY and IFNα, and possible FOS signaling (Midic et al., 2018). A result from the Kingsley et al (2017) study in human cord blood mentioned above was methylation changes to the *RASA3* gene associated with exposure to PFOA (high exposure group, which could result in impaired cell growth and differentiation, contributing to reduced fetal growth and birth weight.

Lastly, a longitudinal study by Ouidir et al. (2020) examined global methylation in the placenta at birth in women for whom PFOA levels in the plasma were determined in the first trimester. The authors did not find any associations between PFOA exposure and DNA methylation status of the placenta (Ouidir et al., 2020).

3.4.4.3.3 Metabolism

van Esterik et al. (2015) examined metabolic effects of developmental exposure to 3–3,000 µg/kg PFOA exposure in C57BL/6JxFVB hybrid mice. The authors found that PFOA exposure during gestation and lactation resulted in reduction in weight that persisted to adulthood. The weight loss was attenuated by a high-fat diet (from 21—25 days) in males, but not females, suggesting that the weight reductions were mediated through metabolic mechanisms that may exhibit a female bias. There were no significant changes in metabolic parameters (i.e., glucose homeostasis, basal glucose, energy expenditure, uncoupling protein 1 (*ucp1;* also known as *thermogenin*) expression in brown adipose tissue) in either sex. However, in females, cholesterol and triglycerides showed a dose-dependent decrease. The authors suggest that these changes in lipid metabolism could be mediated by PPARα activation (van Esterik et al., 2015). Li et al. (2019b) examined PPARα activation pathways as a mechanism of PFOA-induced liver and metabolic toxicity during development in mice. The authors found that female mice exposed gestationally to PFOA had significantly downregulated gene expression of *PPARα* in the 2.5 and 5 mg/kg/day groups, but not the highest dose group (i.e., 10 mg/kg/day). PFOA exposure also increased gene expressions of *Acot1* and *Acox1* (downstream regulatory genes of PPARα), indicating that early PFOA exposure causes lasting changes in the PPARα pathway. PPARα regulates fatty acid oxidative metabolism and energy consumption, through peroxisome and mitochondrial β-oxidation and microsome ω-oxidation (Li et al., 2019b). PFOA has been described as a weak PPARα ligand, but the role of PPARα in mediating the developmental toxicity associated with PFOA exposure is not yet clear (Peraza et al., 2006).

Metabolomic profiles in relation to PFOA exposure were analyzed in a human study. 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 PFOA concentrations. They found that PFOA exposure was associated with several lipid and amino acid metabolism pathways, including that of arginine, proline, aspartate, asparagine, and butanoate (Kingsley et al., 2019).

3.4.4.3.4 Hepatic Development

Three developmental mouse studies examined the effect of PFOA on liver development and function. van Esterik et al. (2015) found that developmental exposure to PFOA resulted in increased liver weights and abnormal liver histopathology, with toxicity possibly mediated through the PPARα pathway. Salimi et al. (2019) exposed pregnant mice to PFOA from GD 5 to 9 and observed mitochondrial disruption in the fetal liver, including mitochondrial swelling and mitochondrial membrane potential collapse. These effects significantly increased at the highest (20 mg/kg/day) exposure group. Measures of oxidative stress (hydrogen peroxide production) in the liver were also significantly higher in groups exposed to 10 or 20 mg/kg/day PFOA in comparison to control animals. Li et al. (2019b) hypothesized that PFOA accumulation in pup liver may promote oxidative stress via PPARα activation pathways that contribute to liver and metabolic toxicity in mice. The authors found that female mice exposed gestationally to PFOA had increased liver weight and dose-responsive morphological changes in the liver including swollen hepatocytes, blurred architecture, and vacuolar degeneration. Liver enzymes (AST and ALT) were increased in the serum, and oxidative stress biomarkers (Catalase (CAT), Superoxide dismutase (SOD), and 8-OHdG) were increased. Liver histone acetyltransferase (HAT) activity was reduced, and histone deacetylase (HDAC) activity was increased. Further, histone acetylation in the liver was reduced. These effects suggest that PFOA can alter the epigenetic regulation of liver responses which may contribute to adverse hepatic health outcomes (Section 3.4.1).

3.4.4.3.5 Cardiac Development

Data from one study in mice, one study in zebrafish, and one in vitro study provide insight into the mechanism by which PFOA perturbs cardiac development. In a recent review that covered PFOA toxicity in zebrafish, Lee et al. (2020) reported that PFOA exposure has been consistently associated with increases in pericardial edema and altered heart rates at various stages of development in embryos. An in vivo mouse developmental study by Salimi et al. (2019) also found that PFOA exposure was associated with cardiotoxicity in offspring. In this study, pregnant dams were treated with PFOA, and fetuses were studied for tissue abnormalities. Groups treated with PFOA showed increased histopathological abnormalities in the fetal heart, including hepatomegaly. Mitochondrial swelling in mitochondrial suspension of fetal heart tissue was also observed along with increased mitochondrial membrane potential collapse. Measures of oxidative stress in the fetal heart were also significantly higher in exposed versus control animals (Salimi et al., 2019). An in vitro experiment by Zhou et al. (2017a) examined the ability of mouse embryonic stem cells to differentiate into myocardiocytes following exposure to 2.5, 5, 10, 20, 40, 80, or 160 μg/mL PFOA. Differentiation was determined by the contractility (i.e., contract rate) of the cells, as well as the upregulation of *myh6*, which is a regulatory gene that is essential for cardiac muscle development. No effects on differentiation or *myh6* expression were observed below 20 μg/mL.

3.4.4.3.6 Neurological Development

Salimi et al. (2019) also reported teratogenic effects in the brain of fetal mice following maternal exposures up to 20 mg/kg/day PFOA via IP injection from GD 5 to 9. The histopathological abnormalities in the brain included anencephaly, microcephaly, and hydrocephaly, all at the highest (20 mg/kg/day) exposure. Mitochondrial swelling in mitochondrial suspension of fetal brain tissue was also observed along with increased mitochondrial membrane potential collapse. Higher mitochondrial disruption was observed at lower concentrations in the brain tissue than other fetal tissues (i.e., heart and liver), suggesting that the brain was more susceptible to mitochondrial toxicity/dysfunction. Measures of oxidative stress in the brain were also significantly higher in exposed animals in comparison to controls.

The effects of PFOA on neurodevelopment and behavior in zebrafish were examined in two studies. In the aforementioned zebrafish embryo assay by Jantzen et al. (2016a), embryonic exposure to 0.02, 0.2, or 2.0 micromolar (μM) PFOA during the first five dpf resulted in hyperactive locomotor activity in larvae as evidenced by increased swimming velocity, possibly mediated through altered expression of development-associated genes (*c-fos, tfc3a*, *slco2b1*, and *ap1s*). Stengel et al. (2018) developed a neurodevelopmental toxicity test battery using zebrafish embryos. PFOA did not produce any changes in acetylcholinesterase (AChE) inhibition, nor the neuromast assay, olfactory, or retinal toxicity assays (Stengel et al., 2018).

3.4.4.3.7 Conclusion

In the context of the available mechanistic studies, it appears that several mechanisms may be involved in PFOA-driven developmental toxicity. In general, the observed effects suggest that the developing liver, developing heart, and placenta may be affected by PFOA at the molecular level (e.g., differential methylation of genes, gene expression changes), which may be reflected in developmental health effects described in Section 3.4.4. The effects tend to vary by sex and developmental timepoint of outcome evaluation. More research is needed to strengthen the association between PFOA exposure to any one of the several possible contributing factors, including fluctuations in transporter gene expression, epigenetic changes, oxidative stress, and PPARα pathway activation, particularly in the placenta.

3.4.4.4 Evidence Integration

The evidence of an association between PFOA and developmental effects in humans is *moderate* based on the recent epidemiological literature. As noted in the fetal growth restriction summary, there is evidence that PFOA may impact fetal growth restriction across a variety of BWT-related measures. Comparing the postnatal growth results in infants with birth-related measures is challenging due to complex growth dynamics including rapid growth catch-up periods for those with fetal restriction. Nonetheless, the evidence for postnatal weight deficits was comparable to that seen for BWT. Collectively, the majority of LBW studies were supportive of an increased risk with increasing PFOA exposures. Five *medium* or *high* confidence studies on LBW showed increased risks with increased PFOA levels. Several meta-analyses also support evidence of associations between maternal or cord blood serum PFOA and BWT or BWT-related measures (Steenland et al., 2018a; Negri et al., 2017; Verner et al., 2015; Johnson et al., 2014) (see Appendix A, (U.S. EPA, 2024a)).

Overall, there was mixed evidence of inverse associations between PFOA and both gestational age (7 of the 18 studies) and preterm birth (6 of 11 studies). Most of the associations for either of these gestational duration measures were reported in *medium* or *high* confidence studies. For example, five of six studies were increased odds of PTB were *high* confidence. Few other patterns were evident that explained any between study heterogeneity. For example, five of the null studies were rated as having adequate sensitivity, and one was rated deficient. There was a preponderance of associations related to sample timing possibly related to pregnancy hemodynamic influences on the PFOA biomarkers, as five of the seven studies reporting inverse associations were sampled later in pregnancy (i.e., trimester two onward).

There was less consistent evidence of PFOA impacts on rapid growth measures, postnatal height and postnatal adiposity measures up to age 2. There was less evidence available for other endpoints such as fetal loss and no evidence of associations in recent studies of PFOA and birth defects such as cryptorchidism or hypospadias. Similarly, there was less consistent evidence of an impact of PFOA exposure on gestational duration measures i.e., as many of studies did not show inverse associations for gestational age measures or for an increased risk of preterm birth.

However, as noted previously, considerable uncertainty remains as to what degree the evidence may be impacted by pregnancy hemodynamics factors related to sample timing may result in either confounding or reverse causality and explain some of the observed birth weight deficits (Steenland et al., 2018a). Additional uncertainty exists due to the potential for confounding by other PFAS, and considerations for potential confounding by co-occurring PFAS are described in Section 5.1. Very few of the existing studies performed multipollutant modeling in comparison with single-pollutant estimates of PFOA associations. The multipollutant modeling results were often mixed from single-pollutant estimates with some estimates increasing and some decreasing. Unlike other PFAS, PFOA was chosen amongst dimension-reducing statistical approaches from models with various PFAS and or other environmental contaminants adjusted for two different studies (Starling et al., 2017; Lenters et al., 2016). Although these results are smaller in magnitude, they appear coherent with single exposure model results. There is some concern that controlling for other highly correlated co-exposures in the same model may amplify the potential confounding bias of another co-exposure rather than removing it (Weisskopf 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 of an association between PFOA and developmental toxicity is *robust* based on 13 *high* or *medium* confidence animal toxicological studies, in concordance with the data in humans, supporting that the developing fetus is a target of PFOA toxicity. Specifically, several studies in rodents show decreased fetal and pup weight with gestational PFOA exposure, similar to the evidence of LBW seen in infants. Oral studies in rodents consistently show that gestational PFOA exposure results in pre- and postnatal effects on offspring, as well as maternal effects in dams. Notably, mice appear to be more sensitive to developmental toxicity as a result of gestational exposure compared with rats. In addition, studies in both rats and mice show that effects on offspring (e.g., decreases in body weight, survival) occur at lower dose levels than those that produce maternal body weight effects.

Evidence from mechanistic studies that relates to observed developmental effects of PFOA is limited. Decreased survival in the offspring of pregnant mice exposed to PFOA was potentially related to hepatotoxicity induced by PPARα activation, as discussed in detail in Section 3.4.1.3. In human cord blood samples, evidence of epigenetic alterations within genes that are involved in cell growth and differentiation and obesity was observed; however, these epigenetic alterations were not evaluated in the context of postnatal outcomes and are inconsistent; two other studies found no association between PFOA exposure and changes to the epigenome. In zebrafish studies, the expression of several genes that are related to growth and development (e.g., *tfc3a*, which is involved in muscle development) was altered by PFOA exposure, with variable magnitude and, in some cases, the direction of change according to the timepoint measured. Oxidative stress was observed in the developing brain and heart of mice exposed to PFOA in utero, suggesting toxicity of PFOA during development. Overall, the data demonstrate that PFOA may alter the expression of genes involved in growth and development, although additional studies in mammals are needed to confirm such. Additionally, evidence exists that PFOA can alter the epigenome, although the functional effects of the epigenetic effects are not clear.

3.4.4.4.1 Evidence Integration Judgment

Overall, considering the available evidence from human, animal, and mechanistic studies, the *evidence indicates* that PFOA exposure is likely to cause developmental toxicity in humans under relevant exposure circumstances (Table 3-15). This conclusion is based primarily on evidence of decreased birth weight from epidemiologic studies in which PFOA was measured during pregnancy, primarily with median PFOA ranging from 1.1 to 5.2 ng/mL. The conclusion is supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth, birth length), as well as consistent findings of dose-dependent decreases in fetal weight and other developmental effects observed in animal models gestationally exposed to PFOA at doses as low as 0.5 mg/kg/day. Although there is available mechanistic information that provides support for the biological plausibility of the phenotypic effects observed in exposed animals, the data are too limited to sufficiently support the human relevance of the animal findings.

Table 3-15. Evidence Profile Table for PFOA 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)* *Primary basis and cross-stream coherence*: Evidence consisted of decreased birth weight from epidemiologic studies in which PFOA was measured during pregnancy. This is supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth, birth length) and consistent findings of dose-dependent decreases in fetal weight observed in animal models gestationally exposed to PFOA. *Human relevance and other inferences:* Although there is available mechanistic information that provides support for the biological plausibility of the phenotypic effects observed in exposed animals, the data are too limited to sufficiently support the human relevance of the animal findings.
Fetal growth restriction 26 *High* confidence studies 25 *Medium* confidence studies 13 *Low* confidence studies 3 *Mixed* confidence studies	Some deficits in mean birth weight were observed in most studies (30/42) in the overall population. The majority of studies on changes in standardized birth weight measures reported inverse associations (10/18), with most (7/10) of these being *high* and *medium* confidence. Similarly, most studies (12/17) observed either an increased risk of low birth weight or SGA. Deficits in birth weight were supported by adverse findings for related FGR outcomes such as decreased birth length and head circumference in the overall population or across sexes.	• *High* and *medium* confidence studies • *Consistent* direction of effects for most outcomes • *Coherence* of findings across different measures of FGR	• *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* Epidemiological evidence for developmental effects is based on consistent adverse effects for FGR and post-natal growth. Consistent deficits in birth weight and standardized birth weight were observed in many *high* and *medium* confidence cohort studies. Birth weight findings were supported by adverse results reported for other measures of FGR, including birth length and head circumference, and adverse effects on gestational duration. Some uncertainties remain regarding the	

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**	
Gestational duration 13 *High* confidence studies 13 *Medium* confidence studies 7 *Low* confidence studies	In *medium* and *high* confidence studies, inverse effects were observed on gestational age (10/20). An increased risk of preterm birth was also observed in *medium* and *high* confidence studies (9/18).	• *High* and *medium* confidence studies	• *Potential bias* due to hemodynamic difference noted in studies using samples from later pregnancy	shape of the exposure-response relationship, and the potential impact of hemodynamics in later pregnancy due to use of biomonitoring samples from the second and third trimester or post-partum.	
Fetal Loss 2 *High* confidence studies 6 *Medium* confidence studies 1 *Low* confidence study	A significantly increased risk of fetal loss was reported in one *high* (1/2) and one *medium* (1/6) confidence study. The response in the *high* confidence study was monotonic across exposure quartiles. Other *medium* confidence studies (5/6) reported mixed results, differing by the exposure comparison. One study reported a	• *High* and *medium* confidence studies • *Good* or *adequate* sensitivity • *Consistent* magnitude of effect • *Exposure-response* relationship	• 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**	
	decreased risk of fetal loss, but the study was considered *low* confidence.				
Post-natal growth 6 *High* confidence studies 7 *Medium* confidence studies 3 *Low* confidence studies	Five *medium* and *high* confidence studies (5/11) reported inverse associations with infant weight and two studies (2/11) reported positive associations, while the remaining studies were mixed by sex or timepoint. Similarly, inverse associations with BMI were observed in five *medium* and *high* confidence studies (5/8),	• *High* and *medium* confidence studies • *Good* or *adequate sensitivity* for most studies	• *Inconsistent* timing of follow-up evaluation		
	and increased risk of rapid growth rate was observed in only one study (1/5). Two *medium* and *high* confidence studies (2/8) observed increased infant length or height and one study reported an inverse association, while				

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**	
	other studies were null or mixed by sex.				
Birth Defects 4 *Medium* confidence studies 2 *Low* confidence studies	Two *low* confidence studies and two *medium* confidence studies reported mixed results for total or combined birth defects. No association with cryptorchidism was reported in one study; one study reported decreased odds of septal defects, conotruncal defects, and total congenital heart defects.	• No factors noted	• *Low* confidence studies • *Limited number* of studies examining individual defects		
Evidence from In Vivo Animal Toxicological Studies (Section 3.4.4.2)					
Maternal body weight 2 *High* confidence studies 6 *Medium* confidence studies	Many rodent studies observed a change in maternal body weight or weight gain following PFOA exposure (5/8). The direction of this change was not consistent among studies, with some rodent studies observing a decrease in weight (3/5), and some mouse studies	• *High* and *medium* confidence studies	• *Inconsistent direction* of effects	⊕⊕⊕ *Robust* Evidence based on 13 *high* or *medium* confidence animal toxicological studies indicates that the developing fetus is a target of PFOA toxicity. Several studies in rodents show decreased fetal	

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**	
	observing an increase (2/5).			and pup weight with gestational PFOA exposure, similar to the evidence of FGR seen in human infants. Oral studies in rodents consistently show that gestational PFOA exposure results in pre- and postnatal effects on offspring, as well as maternal effects in dams. Notably, mice appear to be more sensitive to developmental toxicity as a result of gestational exposure compared with rats. In addition, studies in both rats and mice show that effects on offspring (e.g., decreases in body weight, survival) occur at lower dose levels than those that produced maternal body weight effects.	
Offspring body weight 2 *High* confidence studies 10 *Medium* confidence studies	Many rodent studies observed changes in fetal or pup body weight following PFOA exposure (9/12). Most of these show a decrease in offspring weight (8/9). One study observed an increase in offspring body weight, but only in male mice. Three mouse studies showed no change in offspring body weight (3/12).	• *High* and *medium* confidence studies • *Consistent direction* of effects	• No factors noted		
Offspring mortality 2 *High* confidence studies 7 *Medium* confidence studies	Many rodent studies observed increases in offspring mortality following PFOA exposure (6/9). A rat study observed increased post-weaning mortality in female pups but no pre-weaning mortality or change in stillborn pups. Five mouse studies found increased offspring mortality	• *High* and *medium* confidence studies • *Consistent* direction of effects	• 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**	
	including increased resorption (4/4), decreased live fetuses or live pups born (2/4), and decreased postnatal survival (2/3). Two studies found no change in offspring mortality or survival (2/8). No change in litter size was observed in any rat or mouse study (3/3).				
		•	•		
Placenta effects 2 *Medium* confidence studies	Two mouse studies noted a decrease in relative placenta weight following gestational PFOA exposure. In these studies, lesions on the placenta and	• *Medium* confidence studies	• *Limited number* of studies examining outcomes		

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Studies and Interpretation	**Summary and Key Findings**	**Factors that Increase Certainty**	**Factors that Decrease Certainty**	**Evidence Stream Judgment**	
	other histopathological changes were observed including changes to the labyrinth (e.g., atrophy, decreased area, congestion, necrosis) and early fibrin clot. Fewer placentas were determined to be within normal limits (1/1).				
Offspring liver weight 3 *Medium* confidence studies	Increases in offspring relative liver weight were noted in three mouse studies following gestational PFOA exposure (3/3).	• *Medium* confidence studies	• *Limited number* of studies examining outcomes		
Developmental timing 2 *Medium* confidence studies	Delayed eye opening (2/2) and delayed body hair development (1/1) were observed in both sexes of mice.	• *Medium* confidence studies	• *Limited number* of studies examining outcomes		
Structural abnormalities 1 *Medium* confidence study	One mouse study found structural abnormalities (e.g., reduced skeletal ossification) after developmental exposure to PFOA.	• *Medium* confidence study	• *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**	
Mammary gland development 2 *Medium* confidence studies	Two mouse studies (2/2) found abnormal mammary gland development in animals exposed to PFOA during gestation (e.g., decreases in terminal end buds, mammary gland developmental score).	• *Medium* confidence study	• *Limited number* of studies examining outcomes		
Lactation index 2 *High* confidence studies	Of the two rat studies that evaluated lactation index, one noted a decrease following PFOA (1/2).	• *High* confidence studies	• *Limited number* of studies examining outcomes		

Mechanistic Evidence and Supplemental Information (Section 3.4.4.3)	
Summary of Key Findings, Interpretation, and Limitations	**Evidence Stream Judgment**
Key findings and interpretation: • Decreased survival in mice offspring exposed to PFOA in utero related to PPARα-related hepatotoxicity. • Alterations to the expression of genes related to growth and development in vivo in zebrafish. • Inconsistent results for PFOA-related alterations to DNA methylation in human cord blood. **Limitations:** • Very limited database. • 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 limited evidence demonstrates that PFOA 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: DNA = deoxyribonucleic acid; FGR = fetal growth restriction; mRNA = messenger ribonucleic acid; PPARα = peroxisome proliferator-activated receptor alpha; SGA = small-for-gestational-age.

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 Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* is either *suggestive* of associations or *inadequate* to determine associations between PFOA and the health effects described (U.S. EPA, 2021c). 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 PFOA 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 28 (29 publications[16]) epidemiological and 5 animal toxicological studies that investigated the association between PFOA and cancer. Of the epidemiological studies, 12 were classified as *medium* confidence, 12 as *low* confidence, 2 were considered *uninformative,* and 2 were *mixed* confidence (1 *medium*/*low* and 1 *low/uninformative* confidence) (Section 3.5.1). Of the animal toxicological studies, 2 were classified as *high* confidence, 1 as *medium* confidence, and 2 as *low* confidence (Section 3.5.2). Though *low confidence* studies are considered qualitatively in this section, they were not considered quantitatively for the dose-response assessment (Section 4).

3.5.1 Human Evidence Study Quality Evaluation and Synthesis

3.5.1.1 Introduction

There are 10 epidemiological studies (11 publications[17]) from the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and cancer effects. Study quality evaluations for these 10 studies are shown in Figure 3-73.

The 2016 PFOA HESD (U.S. EPA, 2016c) concluded there was suggestive evidence of carcinogenic effects of PFOA for kidney and testicular cancer, based on two C8 Health Project studies and two occupational cohorts (Figure 3-73). Specifically, two studies involving participants in the C8 Health Project showed a positive association between PFOA levels (mean at enrollment 24 ng/mL) and kidney and testicular cancers (Barry et al., 2013; Vieira et al., 2013). There is some overlap in the cases included in these studies. As part of the C8 Health

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

Project, the C8 Science Panel (C8 Science Panel, 2012b) concluded that a probable link existed between PFOA exposure and testicular and kidney cancer. Two occupational cohorts in Minnesota and West Virginia (Raleigh et al., 2014; Steenland and Woskie, 2012) also examined cancer mortality. Raleigh et al. (2014) reported no evidence of elevated risk for kidney cancer. In the West Virginia occupational cohort, Steenland and Woskie (2012) observed significantly elevated risk of kidney cancer deaths in the highest quartile of modeled PFOA exposure (>2,384 ng/mL-years). However, each of these studies is limited by a small number of observed cases (six kidney cancer deaths, 16 incident kidney cancer cases, and five incidence testicular cancer cases in Raleigh et al. (2014); 12 kidney cancer deaths and one testicular cancer death in Steenland and Woskie (2012)). None of the general population studies reviewed for the 2016 PFOA HESD examined kidney or testicular cancer, and no associations were observed in the general population between exposure to PFOA (mean serum PFOA levels up to 86.6 ng/mL) and colorectal, breast, prostate, bladder, or liver cancer (Bonefeld-Jørgensen et al., 2014; Hardell et al., 2014; Innes et al., 2014; Eriksen et al., 2009). In the C8 Health Project cohort, Barry et al. (2013) observed a significant inverse association with breast cancer for both unlagged and 10-year lagged estimated cumulative PFOA serum concentrations. Barry et al. (2013) also observed positive and significant associations between PFOA and thyroid cancer in DuPont workers at the Washington, West Virginia plant, but not in community residents. However, Vieira et al. (2013) found no association between estimated serum concentrations of PFOA with thyroid cancer risk among residents living near the DuPont Teflon-manufacturing plant in Parkersburg, West Virginia.

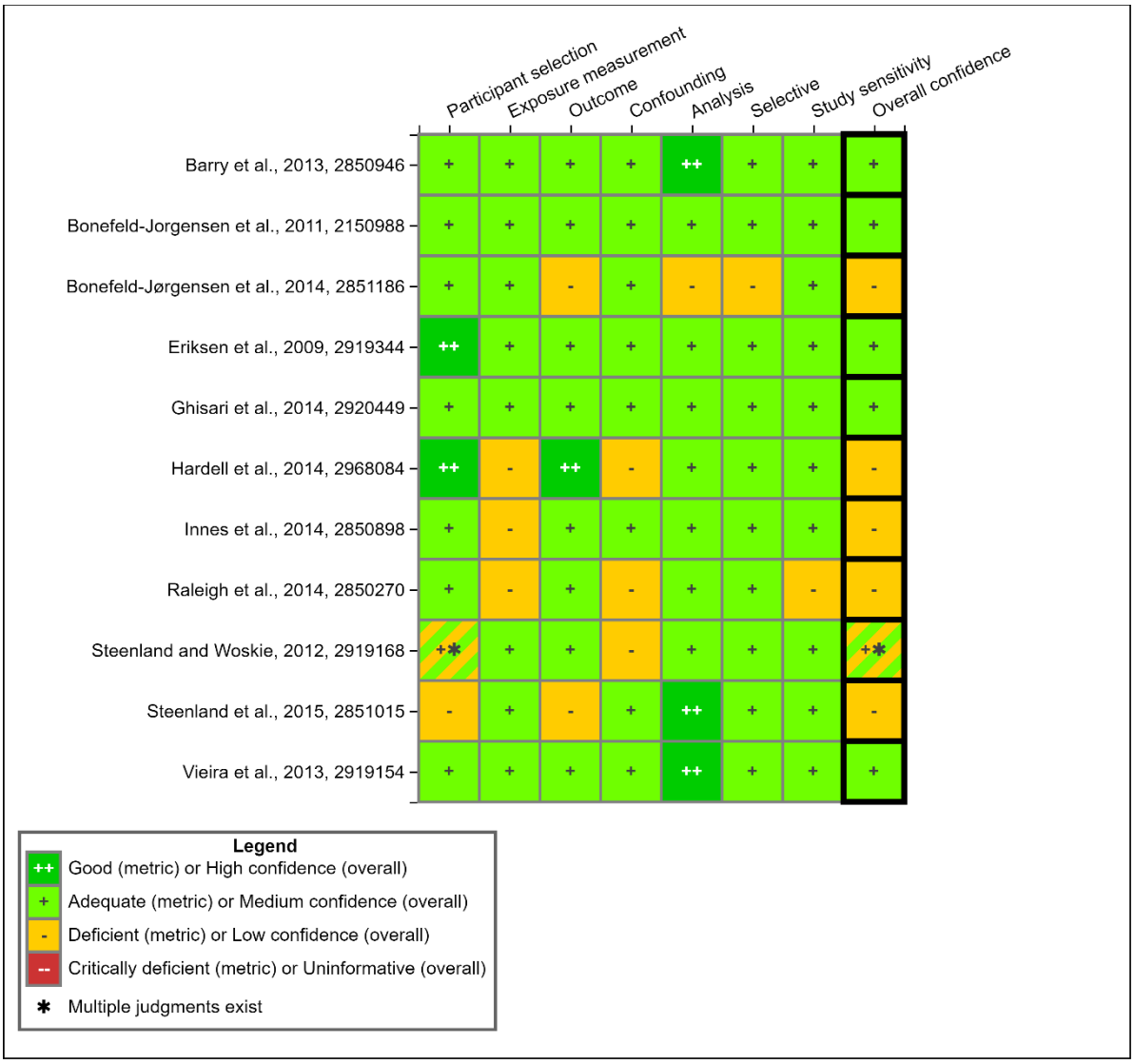


Figure 3-73. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cancer Effects Published Before 2016 (References from 2016 PFOA HESD)

Interactive figure and additional study details available on HAWC.

Since publication of the 2016 PFOA HESD (U.S. EPA, 2016c), 18 epidemiological studies have been published that investigated the association between PFOA and cancer (see Appendix, (U.S. EPA, 2024a)). One of the publications (Girardi and Merler, 2019) was an occupational study and the remainder were conducted on the general population, with one in a high-exposure community (C8 Health Project). Different study designs were also used including four cohort studies (Li et al., 2022; Girardi and Merler, 2019; Fry and Power, 2017; Steenland et al., 2015), 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), Italy (Girardi and Merler, 2019), 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; Steenland et al., 2015). All studies measured PFOA in study subjects' blood components (i.e., serum or plasma) with two exceptions: one study measured PFOA in the maternal serum (Cohn et al., 2020) and one study categorized exposure to any PFAS based on residence near highly contaminated sources of drinking water (Li et al., 2022). Cancers evaluated included bladder (Li et al., 2022; Steenland et al., 2015), 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), colorectal (Li et al., 2022; Steenland et al., 2015), germ cell tumors (Lin et al., 2020b), kidney (Li et al., 2022; Shearer et al., 2021), liver (Cao et al., 2022; Goodrich et al., 2022; Li et al., 2022; Girardi and Merler, 2019), lung (Li et al., 2022; Girardi and Merler, 2019), lymphatic or hematopoietic tissue (Li et al., 2022; Girardi and Merler, 2019), melanoma (Li et al., 2022; Steenland et al., 2015), ovarian (Omoike et al., 2021), prostate (Omoike et al., 2021; Ducatman et al., 2015; Steenland et al., 2015), thyroid (Liu et al., 2021) uterine (Omoike et al., 2021), and any cancer (Li et al., 2022; Girardi and Merler, 2019; Fry and Power, 2017; Christensen et al., 2016).

3.5.1.2 Study Quality

There are 18 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD (U.S. EPA, 2016c) that investigated the association between PFOA and cancer effects. Study quality evaluations for these 18 studies are shown in Figure 3-74.

Of the 18 studies identified since the 2016 PFOA HESD (U.S. EPA, 2016c), eight were considered *medium* confidence, and eight 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; Girardi and Merler, 2019; 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 *uninformative* (Wielsøe et al., 2017) because of concerns about selection bias and exposure assessment. One study included a liver cancer biomarker analysis which was *uninformative* due to lack of information on biomarker measurement methods (Cao et al., 2022). As a result, these two studies and the biomarker analysis will not be further considered in this review. Concerns with the *low* confidence studies included the possibility of outcome misclassification, confounding, or participation selection methods. 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 diagnosis. The two *low* confidence occupational studies (Girardi and Merler, 2019; Steenland et al., 2015) had several potential sources of bias including potential selection bias, outcome measurement limitations which may lead to survival bias, and poor/insufficient study sensitivity due to a small number of deaths. Girardi et al. (2019) had the potential for residual confounding because of use of standardized mortality ratios (SMRs), which

only account for gender, age, and calendar year. Confounders specific for cancer outcomes, besides age and gender, including factors such as smoking or socioeconomic factors were not addressed in the study and behavioral risk factors could have differed by outcome. Although PFOA has a long half-life in the blood, concurrent measurements may not be appropriate for cancers with long latencies. Temporality of exposure in terms of cancer development was noted to be an issue 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 limited sample sizes. Limited 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).

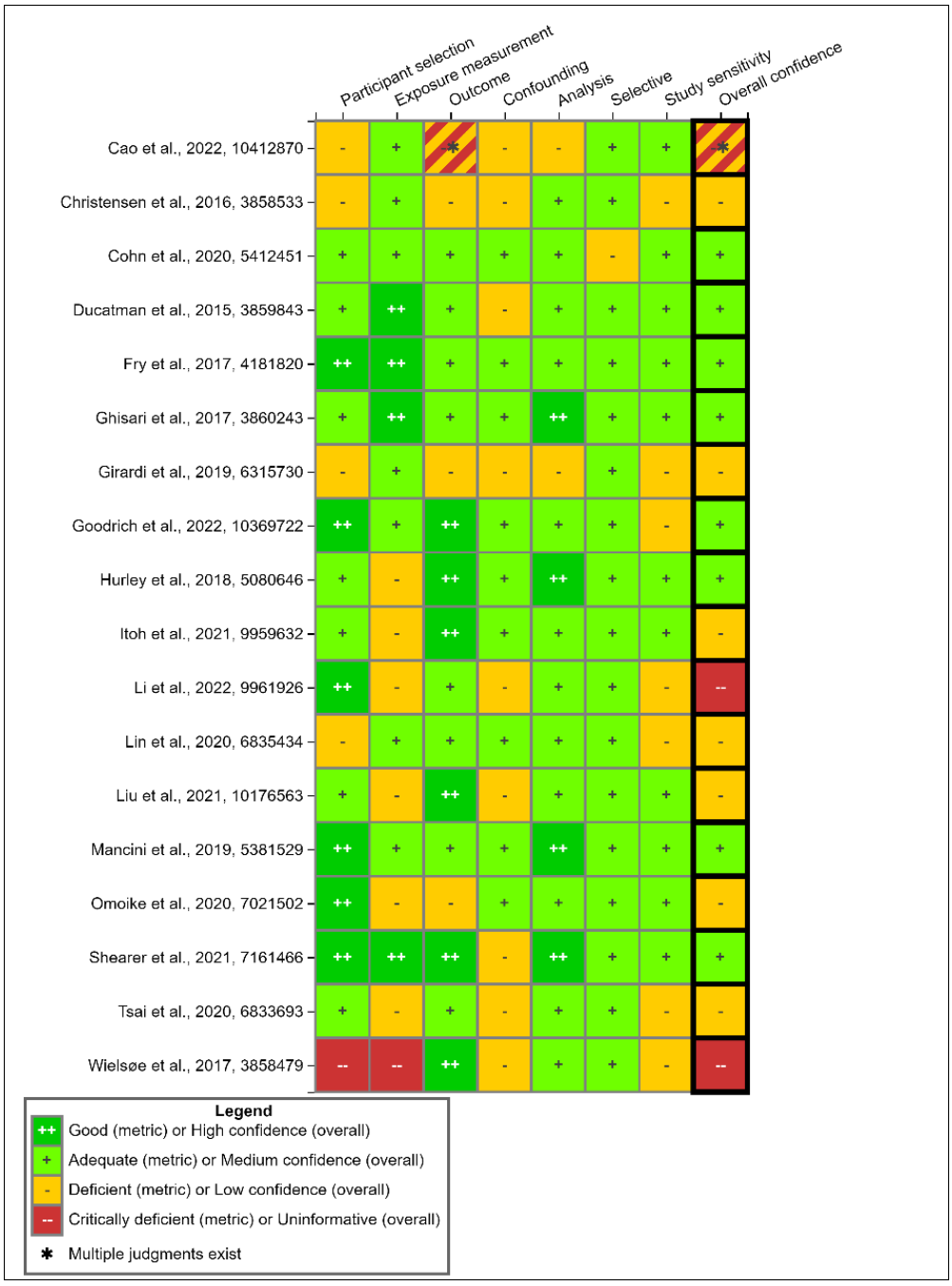


Figure 3-74. Summary of Study Quality Evaluation Results for Epidemiology Studies of PFOA Exposure and Cancer Effects

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 PFOA concentration in 42 pediatric germ cell tumor cases compared with 42 controls in blood samples collected from the children one week after

diagnosis. However, the study did not observe an increase in cancer risk when evaluated on a per ng/mL increase in blood PFOA.

3.5.1.4 Findings From the General Adult Population

PFOA was associated with an increased risk of kidney cancer (i.e., renal cell carcinoma (RCC)) (Shearer et al., 2021). This large *medium* confidence case-control study nested within the National Cancer Institute's (NCI) Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO) reported a statistically significant increase in risk of RCC with pre-diagnostic serum levels of PFOA (OR = 2.63; 95% CI: 1.33, 5.20 for the highest vs. lowest quartiles; p-trend = 0.007, or per doubling of PFOA: OR: 1.71; 95% CI: 1.23, 2.37) (Shearer et al., 2021). Even after adjusting for other PFAS the association remained significant in analyses on a per doubling increase in PFOA. The increase in odds remained across the quartiles and the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS in the highest vs. lowest quartiles), although it was no longer statistically significant. Statistically significant increased odds of RCC were observed in participants ages 55–59 years, and in men and in women, separately (see Appendix D, (U.S. EPA, 2024a)).

Seven general population studies published since the 2016 assessment examined 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). Four were considered *medium confidence* (Cohn et al., 2020; Mancini et al., 2020; Hurley et al., 2018; Ghisari et al., 2017) and had mixed results. All studies were case-control studies (with some nested designs), except for one cross-sectional NHANES-based study (Omoike et al., 2021). Two nested case-control studies did not observe an association between breast cancer and PFOA concentrations measured in maternal serum throughout pregnancy and 1–3 days after delivery ((Cohn et al., 2020); 75th percentile PFOA 0.6 ng/mL) or in in serum after case diagnosis and breast cancer ((Hurley et al., 2018); max concentration of 39.1 ng/mL). Both studies were conducted in California and most breast cancer cases were obtained from the cancer registry. Two nested case-control studies found associations between PFOA and breast cancer, but only in specific genotype or estrogen receptive groups of participants (Mancini et al., 2020; Ghisari et al., 2017). Ghisari (2017) reported an increased risk for breast cancer identified from the cancer registry with increasing PFOA 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 quartile of PFOA (4.8–6.8 ng/mL) compared with the first quartile (1.3–4.8 ng/mL) (OR = 1.86; 95% CI: 1.03, 3.36) in a partially adjusted model (Mancini et al., 2020). Mancini et al. (2020) also reported that the risk for breast cancer (93% verified as pathologically confirmed from medical records after self-reported cancer diagnosis) varied by type of cancer with a statistically significant increase in estrogen receptor negative (ER−) and progesterone receptor negative (PR−) breast cancers in the second quartile of PFOA only. The sample size was small with 26 participants having ER − breast cancers and 57 having PR − breast cancers. No association was observed between PFOA and receptor-positive breast cancer risk.

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, lack of confirmation of control status via examination or medical records (Tsai et al., 2020), and potential for residual confounding due to SES, lifestyle factors and other PFAS.

One *low* confidence study (Tsai et al., 2020) conducted in Taiwan observed an increased risk of breast cancer only in women younger than 50 years (OR = 1.14; 95% CI: 0.66, 1.96). Tsai et al. (2020) also reported an increase in risk in ER+ participants aged 50 years or younger and a decrease in risk for ER− breast cancers in participants aged 50 years or younger, but neither achieved statistical significance. 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 PFOA (OR = 1.089; 95% CI: 1.089, 1.090) and across quartiles of exposure. One *low* confidence case-control study conducted in Japanese women (Itoh et al., 2021) observed a significant inverse association across serum PFOA quartiles with a significant dose-response trend (p-value < 0.0001) (see Appendix D, (U.S. EPA, 2024a)). Median PFOA levels ranged from 3.2 ng/mL in the lowest quartile to 9.3 ng/mL in the highest quartile. The association was null in pre-menopausal women and remained significantly inverse in postmenopausal women in the highest tertile of exposure, with a significant dose-response trend (p-value for trend = 0.005).

Two general population studies published since the 2016 assessment examined liver cancer (Cao et al., 2022; Goodrich et al., 2022) and observed *mixed* results. 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 nonsignificant increase in risk of liver cancer comparing participants with PFOA exposure concentrations above the 85th percentile (8.6 ng/mL) compared with those at or below (OR = 1.20; 95% CI: 0.52, 2.80). There was no association in analyses of continuous PFOA exposure. However, the sample size was small (n = 50 cases and controls each) which likely limited study sensitivity (Goodrich et al., 2022). Elevated risk of liver cancer was also observed in a *low* confidence Chinese case-control study in adults and children (OR per log-ng/mL increase in PFOA exposure = 1.036; 95% CI: 1.002, 1.070) (Cao et al., 2022). However, the confidence in the study results was considered *low* due to limited 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., 20–49-years-old) or older (i.e., 50–69-years-old) men and concurrent mean serum PFOA concentration up to 46 ng/mL. In an NHANES population, Omoike et al. (2021) observed a significantly inverse association with prostate cancer (OR = 0.944; 95% CI: 0.943, 0.944).

Omoike et al. (2021) also observed statistically significant increased odds of ovarian cancer both per ng/mL increase in PFOA (OR = 1.015; 95% CI: 1.013, 1.017) and for the highest versus lowest quartiles of exposure (OR = 1.77; 95% CI: 1.75, 1.79), although the association was significantly inverse for the second and third quartiles of exposure (see Appendix D, (U.S. EPA, 2024a)). A significantly inverse association was also observed for uterine cancer (OR = 0.912; 95% CI: 0.910, 0.914 per ng/mL increase in PFOA) (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 PFOA (p-value for trend < 0.001). The median serum PFOA levels were higher in controls than in cases (10.9 vs. 7.7 ng/mL, p-value < 0.001). However, there is some concern about possible reverse causality. The ability to metabolize PFAS could change when the thyroid becomes cancerous, thereby changing the PFAS concentrations. The abnormality of thyroid hormones may also disturb the PFAS levels.

Two studies examined all cancers together, but collected different information on cancers (i.e., incidence vs. mortality) and obtained the information using different methods. Cancer mortality based on Public-use Linked Mortality Files was observed with PFOA exposure in a *medium* confidence study among subjects over 60 years of age from NHANES 2003–2006 with median PFOA concentration 23.7 ng/g lipid (Fry and Power, 2017). PFOA was associated with an increase in self-reported cancer incidence in a *low* confidence study on male anglers over 50 years (Christensen et al., 2016). Christensen et al. (2016) was considered *low* confidence due to the potential of self-selection because subjects were recruited from flyers and other methods and filled out an online survey including self-reported outcomes.

3.5.1.5 Findings From Occupational Studies

Two *low* confidence occupational studies examined cancer incidence (Steenland et al., 2015) and mortality (Girardi and Merler, 2019). Issues of population selection, outcome measurement and small number of deaths reducing the sensitivity were noted. In a retrospective occupational cohort study based on the same DuPont cohort from West Virginia reported in the 2016 assessment (Steenland and Woskie, 2012), Steenland et al. (2015) observed no significant associations with incidence of cancers of the bladder, colorectal, prostate, and melanoma when compared with the general population (median serum levels in workers was 113 ng/mL in 2005 compared with 4 ng/mL in the general population). There was modest evidence of a positive nonsignificant trend for prostate cancer (across quartiles) and a statistically significant negative exposure-response trend for bladder cancers (p-value = 0.04).

Girardi et al. (2019) conducted a retrospective cohort study at a factory in Italy where PFOA was produced from 1968–2014 and observed statistically significant increases in liver cancer mortality, malignant neoplasms of the lymphatic and hematopoietic tissue, and in all malignant neoplasms with cumulative serum PFOA exposure of >16,956 ng/mL-years. There was no association observed with lung cancer in this occupational cohort. Mortality from cancers in this cohort was low and supplemental data provided mortality for other cancers including kidney, but no risk estimates were calculated.

3.5.2 Animal Evidence Study Quality Evaluation and Synthesis

There are three studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and two studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the association between PFOA and cancer effects in animal models. Study quality evaluations for these five studies are shown in Figure 3-75.

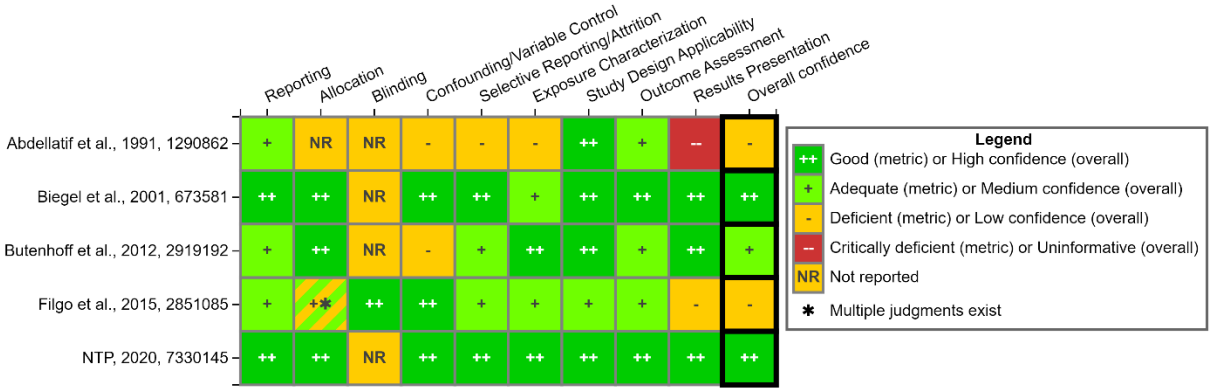


Figure 3-75. Summary of Study Quality Evaluation Results for Animal Toxicological Studies of PFOA Exposure and Cancer Effects

Interactive figure and additional study details available on HAWC.

Three *high* or *medium* confidence animal carcinogenicity studies indicate that PFOA exposure can lead to multiple types of neoplastic lesions including liver adenomas (NTP, 2020; Biegel et al., 2001) or carcinomas (NTP, 2020), Leydig cell tumors (LCTs) (Butenhoff et al., 2012; Biegel et al., 2001), and pancreatic acinar cell tumors (PACTs; adenomas or adenocarcinomas) (NTP, 2020; Biegel et al., 2001) in male Sprague-Dawley rats. Neoplastic lesions were also observed in female Sprague-Dawley rats, but the incidences were not as high as the incidences observed in the males and often did not achieve statistical significance, though there were reported incidences of rare and/or malignant neoplasms of the liver, pancreas, and uterus (NTP, 2020; Butenhoff et al., 2012). Another study (Filgo et al., 2015) assessed hepatic tumor development in three strains of female mice after perinatal exposures to PFOA. This study is not further discussed here because of an inadequate study design to assess lifetime/chronic carcinogenicity (i.e., the study did not include exposure postweaning) and the results were equivocal (i.e., few significant findings that did not display a dose-response relationship) and difficult to interpret due to small sample sizes (n = 6–10 for some strains).

In the three rat carcinogenicity studies (NTP, 2020; Butenhoff et al., 2012; Biegel et al., 2001), rats were fed diets containing similar concentrations of PFOA for approximately 2 years. Butenhoff et al. (2012) analyzed a variety of tissues collected from male and female Sprague-Dawley rats fed diets containing 0, 30, or 300 ppm PFOA (equivalent to 1.3 and 14.2 mg/kg for males and 1.6 and 16.1 mg/kg for females) for 2 years. Similarly, Biegel et al. (2001) analyzed tissues collected from male Crl:CD® BR (CD) rats fed diets containing 0 or 300 ppm PFOA (equivalent to 13.6 mg/kg/day) for 24 months. Using a matrix-type exposure paradigm, NTP (2020) administered PFOA in feed to pregnant Sprague-Dawley (Hsd:Sprague-Dawley® SD®) rats starting on GD 6 and analyzed tissues of male and female offspring also fed postweaning diets containing PFOA for a total of 107 weeks. Dose groups for this report are referred to as "[perinatal exposure level]/[postweaning exposure level]" (e.g., 300/1,000; see further study design details in Section 3.4.4.2.1.2).

Liver adenomas in male rats were observed in the Biegel et al. (2001) study at an incidence of 10/76 (13%) at 13.6 mg/kg/day, compared with 2/80 (3%) in controls. Liver adenomas in male

rats were also significantly increased in the NTP (2020) in the 0/40, 0/80, and 300/80 ppm groups (Table 3-16). Both the 0/0 and 300/0 ppm control groups had no observed liver adenomas. NTP (2020) reported increases in the incidence of hepatocellular carcinomas in the male 300/80 ppm group only and a statistically significant trend of increased incidence with dose in the groups exposed during both perinatal and postnatal periods. Although no liver adenomas were observed in Butenhoff et al. (2012), carcinomas were identified in the male controls (3/49), males in the low-dose group (1.3 mg/kg/day; 1/50), and male (5/50) and female (1/50) rats in the high-dose group (14.2 and 16.1 mg/kg/day, respectively). The differences in carcinoma incidences from controls were not statistically significant in the Butenhoff et al. (2012) study.

Table 3-16. Incidences of Liver Tumors in Male Sprague-Dawley Rats as Reported by NTP (2020)

Perinatal Dose	Postweaning Dose			
	0 ppm	20 ppm	40 ppm	80 ppm
Hepatocellular Adenomas				
0 ppm	0/50 (0%)***	0/50 (0%)	7/50 (14%)*	11/50 (22%)**
300 ppm	0/50 (0%)***	1/50 (2%)	5/50 (10%)	10/50 (20%)**
Hepatocellular Carcinomas				
0 ppm	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
300 ppm	0/50 (0%)*	0/50 (0%)	0/50 (0%)	4/50 (8%)

Notes:
*Statistically significant compared with the respective control group (0/0 or 300/0 ppm) at $p \leq 0.05$.
**Statistically significant compared with the respective control group (0/0 or 300/0 ppm) at $p \leq 0.01$.
***Statistically significant trend of response at $p \leq 0.001$.

Nonneoplastic/preneoplastic liver lesions were identified by Butenhoff et al. (2012) in males and females at the 1- and 2-year sacrifices. An increased incidence of diffuse hepatomegalocytosis and hepatocellular necrosis occurred in the high-dose groups. At the 2-year sacrifice, hepatic cystic degeneration (characterized by areas of multilocular microcysts in the liver parenchyma) was observed in males. Hyperplastic nodules in male livers were increased in the 14.2 mg/kg/day group. NTP (2020) similarly reported a variety of nonneoplastic and preneoplastic liver lesions in both male and female rats including increased incidences of liver necrosis and mixed-cell foci, hepatocyte hypertrophy, and focal inflammation. These lesions were more pronounced in males than females and were observed at both the 16-week interim and 107-week final necropsies.

Testicular LCTs were identified in both the Butenhoff et al. (2012) and Biegel et al. (2001) studies. The tumor incidence reported by Butenhoff et al. (2012) was 0/50 (0%), 2/50 (4%), and 7/50 (14%) for the 0, 1.3, and 14.2 mg/kg/day dose groups, respectively. The Biegel et al. (2001) study included one dose group (13.6 mg/kg/day); the tumor incidence was 8/76 (11%) compared with 0/80 (0%) in the control group. LCT incidence at similar dose levels was comparable between the two studies (11% and 14%). NTP (2020) analyzed testicular tissue for LCTs but did not observe increased incidence due to PFOA treatment. The authors noted that this inconsistency with other carcinogenicity studies could be a result of differences in exposure concentrations or stock of Sprague-Dawley rat (i.e., CD vs. Hsd:Sprague-Dawley).

PACTs were observed in both the NTP (2020) and Biegel et al. (2001) studies. NTP (2020) reported increased incidences of pancreatic acinar cell adenomas in males in all treatment groups compared with their respective controls (Table 3-17). NTP (2020) observed increases in pancreatic acinar cell adenocarcinoma incidence in males in multiple dose groups and slight increases in the incidence of combined acinar cell adenoma or carcinoma in females from the 300/1,000 ppm dose group, though these increases did not reach statistical significance (Table 3-17 and Table 3-18). In male rats, the incidence of PACTs in the Biegel et al. (2001) study was 8/76 (11%; 7 adenomas, 1 carcinoma) at 13.6 mg/kg/day while none were observed in the control animals. In a peer-reviewed pathological review of male pancreatic tissue collected by Butenhoff et al. (2012), Caverly Rae et al. (2014) identified 1/47 carcinomas in the 300 ppm group (compared with 0/46 in the control and 30 ppm groups) and no incidence of adenomas with any treatment. Pancreatic acinar hyperplasia was observed in males of the control, 1.3, and 14.2 mg/kg/day groups at incidences of 3/46 (7%), 1/46 (2%), and 10/47 (21%), respectively. Butenhoff et al. (2012) also reported increased incidences of acinar atrophy in males (6/46 (13%), 9/46 (20%), and 11/49 (22%) in 0, 1.3, and 14.2 mg/kg/day dose groups, respectively), though this lesion was not discussed in the peer-reviewed pathology report (Caverly Rae et al., 2014). NTP (2020) similarly reported increased incidences of acinus hyperplasia in males at incidence rates of 32/50 (64%), 37/50 (74%), 31/50 (62%) in the 0/20, 0/40, 0/80, and 27/50 (54%), 38/50 (76%), and 33/50 (66%) in the 300/20, 300/40, and 300/80 groups. The incidences in controls were 18/50 (36%) and 23/50 (46%) in the 0/0 and 300/0 groups, respectively. There were also low occurrences of acinus hyperplasia in the exposed female groups, though not as frequently observed as in males. However, the authors concluded that the incidence of pancreatic acinar cell neoplasms in males increased confidence that the occurrence in females was due to PFOA exposure.

Table 3-17. Incidences of Pancreatic Acinar Cell Tumors in Male Sprague-Dawley Rats as Reported by NTP (2020)

Perinatal Dose	Postweaning Dose			
	0 ppm	20 ppm	40 ppm	80 ppm
Pancreatic Acinar Cell Adenomas				
0 ppm	3/50 (6%)**	28/50 (56%)**	26/50 (52%)**	32/50 (64%)**
300 ppm	7/50 (14%)**	18/50 (36%)*	30/50 (60%)**	30/50 (60%)**
Pancreatic Acinar Cell Adenocarcinomas				
0 ppm	0/50 (0%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
300 ppm	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)

Notes:
*Statistically significant compared with the respective control group (0/0 or 300/0 ppm) at $p \leq 0.05$.
**Statistically significant compared with the respective control group (0/0 or 300/0 ppm) at $p \leq 0.001$. Asterisks on the control group denotes a statistically significant trend of response.

Table 3-18. Incidences of Pancreatic Acinar Cell Tumors in Female Sprague-Dawley Rats

as Reported by NTP (2020)

Perinatal Dose	Postweaning Dose		
	0 ppm	300 ppm	1,000 ppm
Pancreatic Acinar Cell Adenomas			
0 ppm	0/50 (0%)	0/50 (0%)	1/49 (2%)
150 ppm	–	0/50 (0%)	–
300 ppm	–	–	3/50 (6%)
Pancreatic Acinar Cell Adenocarcinomas			
0 ppm	0/50 (0%)	0/50 (0%)	1/49 (2%)
150 ppm	–	0/50 (0%)	–
300 ppm	–	–	2/50 (4%)

NTP (2020) observed increased incidences of uterine adenocarcinomas in female Sprague-Dawley rats during the extended evaluation (i.e., uterine tissue which included cervical, vaginal, and uterine tissue remnants). Incidence rates for this lesion are reported in Table 3-19. The accompanying incidences of uterine hyperplasia did not follow a dose-response relationship. Butenhoff et al. (2012) identified mammary fibroadenomas and ovarian tubular adenomas in female rats, though there were no statistical differences in incidence rates between PFOA-treated dose groups and controls.

Table 3-19. Incidences of Uterine Adenocarcinomas in Female Sprague-Dawley Rats from the Standard and Extended Evaluations (Combined) as Reported by NTP (2020)

Perinatal Dose	Postweaning Dose		
	0 ppm	300 ppm	1,000 ppm
0 ppm	1/50 (2%)	5/49 (10%)	7/48 (15%)*
150 ppm	–	3/50 (6%)	–
300 ppm	–	–	5/48 (10%)

Notes:
*Statistically significant compared with the control group (0/0 ppm) at p = 0.050.

NTP concluded that under the exposure conditions presented, there was *clear evidence* of carcinogenic activity of PFOA in male Sprague-Dawley rats based on increased incidences of hepatocellular neoplasms (predominately hepatocellular adenomas) and acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas (NTP, 2020). In females, NTP concluded there was *some evidence* of carcinogenic activity of PFOA based on increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms. The study authors also noted that the higher incidence of hepatocellular carcinomas and adenocarcinomas of the uterus may have been related to exposure (NTP, 2020).

3.5.3 Mechanistic Evidence

Mechanistic evidence linking PFOA exposure to adverse cancer outcomes is discussed in Sections 3.1.2, 3.2.9, 3.3.1, 3.4.2, 3.4.3, 3.4.4, and 4.2 of the 2016 PFOA HESD (U.S. EPA, 2016c). There are 42 studies from recent systematic literature search and review efforts conducted after publication of the 2016 PFOA HESD that investigated the mechanisms of action of PFOA that lead to cancer effects. A summary of these studies is shown in Figure 3-76.

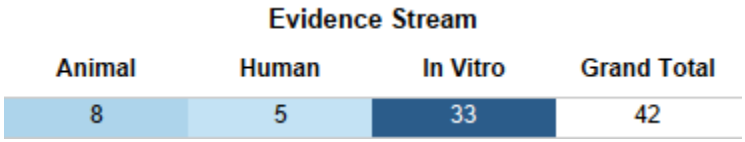


Figure 3-76. Summary of Mechanistic Studies of PFOA and Cancer Effects

Interactive figure and additional study details available on HAWC.

In 2016, 10 key characteristics of carcinogens were selected by a multidisciplinary 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 PFOA, the studies containing mechanistic data were organized by the proposed key characteristics of carcinogens for the following section. Evidence related to eight of the 10 key characteristics of carcinogens was identified in the literature included in this assessment: 'Is Genotoxic,' 'Induces Epigenetic Effects,' 'Induces Oxidative Stress,' 'Induces Chronic Inflammation,' 'Is Immunosuppressive,' 'Modulates Receptor Mediated Effects,' 'Alters Cells Proliferation, Cell Death, and Nutrient Supply,' and 'Causes Immortalization.' No studies from the 2016 PFOA HESD (U.S. EPA, 2016c) and recent systematic literature search and review efforts were identified for the following key characteristics: 'Is Electrophilic or Can Be Metabolically Activated to Electrophiles' (key characteristic #1) and 'Alters DNA Repair and Causes Genomic Instability' (key characteristic #3).

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. Overall, the evidence suggests that PFOA does not induce mutations or operate through a genotoxic mechanism, with the majority of the study data demonstrating a lack of genotoxic effect of PFOA in both in vitro and in vivo assays. A notable exception is aneuploidy and DNA fragmentation of sperm significantly associated with PFOA exposure in humans. The genotoxicity evidence is detailed below.

3.5.3.1.1 Gene Mutation

All of the studies identified in this assessment that investigated the mutagenic potential of PFOA were conducted in in vitro models. Of the available studies, most found that PFOA exposure did not induce mutagenicity (Table 3-20). Studies involving Chinese hamster ovary (CHO) K-1 cell lines presented primarily negative results. Sadhu (2002) reported PFOA exposure did not induce gene mutations in CHO K-1 cells when tested with or without metabolic activation. Zhao et al. (2011) also observed that PFOA did not induce mutagenesis in human-hamster hybrid (A_L) cells, which contain a standard set of CHO-K1 chromosomes and a single copy of human chromosome 11, at sub-cytotoxic concentrations (<200 μM). A subsequent experiment using DMSO to quench oxidative stress found that PFOA was not mutagenic in the presence of DMSO, suggesting that an increase in reactive oxygen species production may be required for PFOA-induced mutagenicity (Section 3.5.3.3).

Of the six publications that tested PFOA mutagenicity in *Salmonella typhimurium (S. typhimurium)* or *Escherichia coli (E. coli)* (NTP, 2019; Buhrke et al., 2015; Butenhoff et al., 2014; Fernández Freire et al., 2008; Lawlor, 1996, 1995), two reported exposure-associated mutagenicity (NTP, 2019; Butenhoff et al., 2014) (Table 3-20). Mutation was observed in *S. typhimurium* following exposure to cytotoxic concentrations of PFOA in the presence of S9 metabolic activation (Butenhoff et al., 2014). NTP (2019) reported PFOA exposure caused a slight increase in mutation in *S. typhimurium* TA98 cells, and Lawlor (1996) reported that one plate of *S. typhimurium* had a significant amount of mutagenicity in the absence of S9 metabolic activation. However, neither of these results were reproducible.

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

Two studies reported on the genotoxic potential of PFOA exposure in humans (Table 3-21). Franken et al. (2017) measured blood PFOA concentrations in adolescents (14–15 years of age) that resided for >5 years within industrial areas of Belgium (near a stainless-steel plant or a shredder factory). These data were then compared with age-matched controls. A significant increase in DNA damage associated with PFOA exposure was observed, as evidenced by an alkaline comet assay performed on the same blood samples. Urinary 8-hydroxydeoxyguanosine (8-OHdG) was used as a biomarker for oxidative DNA damage. While there was no significant change observed, a positive dose-response relationship with increasing PFOA concentrations was noted. The authors attributed the DNA damage to oxidative stress, but noted that urinary 8-OHdG can also indicate DNA repair. 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 (PFOA was detected in 75% 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

Studies of the genotoxicity related to PFOA exposure were conducted in rat and mouse models (Table 3-21). All of the studies presented data from micronucleus tests of bone marrow, peripheral blood, and splenocytes, with the exception of one study of DNA strand breaks. 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). With the exception of one micronucleus assay, there was no evidence for PFOA-induced genotoxic effects after acute or subchronic exposures (Figure 3-16).The single study of DNA strand breakage used a comet assay in tissues from male C57Bl/6 mice administered ≤5 mg/kg/day for five weeks (Crebelli et al., 2019). Analysis of the liver and testis following exposure indicated there was no change in DNA fragmentation. Acute and subchronic PFOA exposures in mouse studies found no evidence of micronuclei formation, a measure of genotoxic damage to DNA in proliferating cells and spindle formation (Hayashi, 2016), in either peripheral blood cells or splenocytes (Crebelli et al., 2019) or within erythrocytes of the bone marrow (Butenhoff et al., 2014; Murli, 1996c, 1995). 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.

A subchronic study in Sprague-Dawley rats noted that PFOA exposure induced a slight increase in micronuclei formation in peripheral blood cells of male rats administered 10 mg/kg/day; however, the micronuclei level was within the historical control range, and there was no effect in females) (NTP, 2019).

3.5.3.1.2.2 In Vitro Evidence

3.5.3.1.2.2.1 Chromosomal Aberrations

Measurements of chromosomal aberrations have been performed using human and animal cell lines, and predominantly found that PFOA exposure does not cause alterations (Table 3-22). In human lymphocytes, PFOA did not induce chromosomal aberrations in the presence of S9 activation (3 hours) or without the addition of S9 (≤46 hours) at concentrations up to 600 µg/mL (Butenhoff et al., 2014). This evidence corroborates previous studies of human lymphocyte cells that found similar results using non-cytotoxic concentrations of PFOA (NOTOX, 2000; Murli, 1996b) as reported in the 2016 PFOA HESD (U.S. EPA, 2016c).

In contrast, Butenhoff et al. (2014) observed chromosomal aberrations after PFOA exposure (≥750 µg/ml) with S9 metabolic activation in CHO cells. These results corroborate with previously reported studies in S9 activated CHO cells (Murli, 1996a, d). Butenhoff et al. (2014) and Murli (1996a) also reported PFOA-induced chromosomal aberrations in CHO cells without S9 metabolic activation but were unable to replicate their own results.

3.5.3.1.2.2.2 DNA Double Strand Breaks

Evaluation of DNA strand breakage using comet assays and histological analysis of phosphorylated H2AX (γH2AX) yielded positive results in all of the studies reviewed (Table 3-22). PFOA exposure caused DNA breakage in a dose-dependent manner in human lymphocytes exposed to ≥250 ppm PFOA for two hours (Yahia et al., 2016) and in HepG2 cells exposed to ≥100 µM PFOA for 24 hours in one study (Yao and Zhong, 2005), ≥10 µM PFOA for 24 hours in another study (Wielsøe et al., 2015), and at 10 and 200 µM PFOA (but not 50 or 100 µM PFOA) for 24 hours in a third study (Florentin et al., 2011). *Paramecium caudatum* (*P.*

caudatum), a unicellular protozoa, exhibited DNA damage after exposure to 100 µM PFOA (Kawamoto et al., 2010). Peropadre et al. (2018) observed a 4.5-fold higher level of double strand breaks in human keratinocyte cells (HaCaT) exposed to 50 µM PFOA for 24 hours, compared with controls, as evidenced by γH2AX. Eight days post-exposure, γH2AX levels were twice that of the controls, indicating that double strand breaks were not fully repaired. In contrast, a study conducted in Syrian hamster embryo (SHE) cells demonstrated no change in DNA strand breaks by the comet assay at 4.1×10^{-5} to 300 µM PFOA for 5 or 24 hours (Jacquet et al., 2012).

3.5.3.1.2.2.3 Micronuclei Formation

Three studies measured micronucleus formation in cells exposed to PFOA (Table 3-22). Buhrke et al. (2013) demonstrated that PFOA exposure (10 µM, 24 hours) did not induce micronuclei formation in Chinese hamster lung cells (V79). Studies conducted in human HepG2 cells reported conflicting results: in one study, PFOA induced micronuclei formation at concentration of ≥100 µM after 24 hours (Yao and Zhong, 2005), while another study reported no difference in micronuclei frequency in HepG2 cells exposed to concentrations of PFOA up to 400 µM for 24 hours compared with controls (Florentin et al., 2011). The micronucleus assays were performed according to the same method (Natarajan and Darroudi, 1991).

Table 3-20. Mutagenicity Data from In Vitro Studies

Reference	Cell Line or Bacterial Strain	Results		Concentration (Duration of exposure)
		S9-Activated	Non-Activated	
NTP (2019)	*Salmonella typhimurium* (TA98, TA100)	Equivocal[a] (Not reproducible)	Equivocal[a] (Not reproducible)	100–5,000 µg/plate
	*Escherichia coli (*WP2*uvr*A/pkM101)	Negative	Negative	100–10,000 µg/plate
Zhao et al. (2011)	Human-hamster hybrid (A_L) cells	N/A	Positive[b]	1–200 µM (1–16 d)
	Mitochondrial DNA-deficient human-hamster hybrid (p^0A_L) cells	N/A	Negative	1–200 µM (1–16 d)
Sadhu (2002)	CHO K-1	Negative	Negative	≤39 µg/mL (5 or 17 hr)
Butenhoff et al. (2014)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537)	Positive[c]	Negative	20–1,000 µg/plate
Buhrke et al. (2015)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Negative	Negative	5 µM
Fernández Friere et al. (2008)	*Salmonella typhimurium* (TA98, TA100, TA102, TA104)	Negative	Negative	100 or 500 µM
Lawlor (1995)	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537)	Negative	Negative	100–5,000 µg/plate

Reference	Cell Line or Bacterial Strain	Results		Concentration (Duration of exposure)
		S9-Activated	Non-Activated	
	Salmonella typhimurium (TA98, TA100, TA1535, TA1537)	Negative	Negative	100–5,000 µg/plate
	*Escherichia coli (*WP2*uvr*A*)*	Negative	Negative	100–5,000 µg/plate
	Escherichia coli (WP2uvrA)	Negative	Negative	6.67–5,000 µg/plate

Notes:
[a] Mutagens were present in 1 of 3 TA98 replicate plates only.
[b] Mutagens were present in cells that were exposed only to 200 µM for 16 days.
[c] Mutagenicity found at cytotoxic concentrations only.

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

Reference	Species, Strain (Sex)	Tissue	Results	PFOA Concentration (Dosing Regimen)
DNA Strand Breakage				
Franken et al. (2017)	Human (Male and Female)	Peripheral Blood Cells	Positive	Average Blood Concentration of 2.55 µg/L
Governini et al. (2015)	Human (Male)	Semen	Positive	Average Seminal Plasma Concentration of 7.68 ng/g f.w.
Crebelli et al. (2019)	Mouse, C57BL/6 (Male)	Liver, Testis	Negative	0.1–5 mg/kg/day (daily via drinking water for 5 wk)
Micronuclei Formation				
Crebelli et al. (2019)	Mouse, C57BL/6 (Male)	Peripheral Blood Cells, Splenocytes	Negative	0.1–5 mg/kg/day (daily via drinking water for 5 wk)
Butenhoff et al. (2014)	Mouse, Crl:CD-1 (Male and Female)	Bone Marrow	Negative	250–1,000 mg/kg (single dose via gavage)
NTP (2019)	Rat, Sprague-Dawley (Male and Female)	Peripheral Blood Cells	Positive[a]	6.25–100 mg/kg/day (daily via gavage for 28 d)
Murli (1995)	Mouse	Bone Marrow	Negative	1,250–5,000 mg/kg (Single dose delivered via gavage)
	Mouse	Bone Marrow	Negative	498–1,990 mg/kg (Single dose delivered via gavage)

Notes: f.w. = formula weight.
[a] A slight increase in micronuclei in the male 10 mg/kg/day group was within the historical control range. No change in females.

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

Reference	In Vitro Model	Results		Concentration (Duration of exposure)
		S9 Activated	Non-Activated	
Chromosomal Aberrations				

Reference	In Vitro Model	Results		Concentration (Duration of exposure)
		S9 Activated	Non-Activated	
Butenhoff et al. (2014)	Human Lymphocytes	Negative	Negative	12.4–600 µg/mL (3–46 hr)
	Chinese Hamster Ovarian Cells	Positive	N/A	50–1,500 µg/mL (3 hr)
	Chinese Hamster Ovarian Cells	N/A	Positive (Not reproducible)	25–1,000 µg/mL (3–41.8 hr)
NOTOX (2000)	Human Lymphocytes	Negative	Negative	≤Cytotoxic concentration[a]
Murli (1996b)	Human Lymphocytes	Negative	Negative	125–4,010 µg/mL (3–43.3 hr)
	Chinese Hamster Ovarian Cells	Positive	Negative	100–2,750 µg/mL (3–41.8 hr)
	Chinese Hamster Ovarian Cells	Positive	Positive (Not reproducible)	125–5,000 µg/mL (3 hr)
Cell Transformation				
Jacquet et al. (2012)	Syrian Hamster Embryo Cells	N/A	Negative	3.7×10^{-4}–37 µM (6 d)
Garry and Nelson (1981)	C3H10T½	N/A	Negative	0.1–200 µg/mL (24 hr)
DNA Strand Breakage				
Peropadre et al. (2018)	Human Keratinocyte HaCaT cells	N/A	Positive	50 µM (24 hr)
Yahia et al. (2016)	Human Lymphocytes	N/A	Positive	125–500 ppm (2 hr)
Florentin et al. (2011)	Human HepG2 Cells	N/A	Positive[b]	5–400 µM (1 or 24 hr)
Wielsøe et al. (2015)	Human HepG2 Cells	N/A	Positive	0.2– 20 µM (24 hr)
Yao and Zhong (2005)	Human HepG2 Cells	N/A	Positive	50–400 µM (24 hr)
Kawamoto et al. (2010)	Paramecia	N/A	Positive	10–100 µM (1–24 hr)
Micronuclei Formation				
Buhrke et al. (2013)	Chinese Hamster Lung Fibroblast Cells	Negative	Negative	10 µM (3 hr)
Florentin et al. (2011)	Human HepG2 Cells	N/A	Negative	5–400 µM (1 or 24 hr)
Yao and Zhong (2005)	Human HepG2 Cells	N/A	Positive[c]	50–400 µM (24 hr)

Notes: N/A = not applicable.
[a] Findings based on the 2016 EPA's Health Effects Support Document (U.S. EPA, 2016c), concentration(s) unknown.
[b] Slight increase was observed at 10 and 200 µM in a non-dose-dependent manner after 24-hour exposure only.
[c] Micronuclei were present in cells that were exposed only to ≥100 µM for 16 days.

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). Overall, the evidence demonstrates that PFOA exposure can lead to cancer-relevant changes in DNA methylation at both the global and gene-specific level, across human, animal, and in vitro studies. The evidence related to epigenetic alterations is detailed below.

3.5.3.2.1.1 In Vivo Evidence

3.5.3.2.1.2 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. No change in CpG island methylation was correlated with PFOA levels, although changes in this epigenetic alteration were found to be significantly correlated with several other toxicants in the blood samples. Two other studies evaluated global DNA methylation patterns in cord blood. Miura et al. (2018) found that increased PFOA in the cord blood was associated with a global DNA hypermethylation in a cohort from Japan. Kingsley et al. (2017) did not observe associations between PFOA exposure in cord blood and epigenome-wide changes in methylation status. However, the authors found significant changes in methylation in seven CpG sites located in several genes, including *RAS P21 Protein Activator 3 (RASA3)* and Opioid Receptor Delta 1 *(OPRD1).* Three studies reviewed herein found no association between maternal PFOA exposure and global methylation changes in offspring (Leung et al., 2018; Liu et al., 2018a) or placenta (Ouidir et al., 2020).

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 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 PFOA concentrations. Serum concentrations of PFOA significantly decreased between enrollment (2005–2006) and follow-up (2010). However, methylation of long interspersed nuclear elements (LINE-1) transposable DNA elements in peripheral blood leukocytes was not associated with PFOA exposure at any timepoint.

Several studies detail the influence of PFOA exposure on the epigenome in humans. Specifically, in prenatal studies, PFOA exposure was associated with mixed results of increased methylation in cord blood but not in placenta. However, consistently, studies found alterations in methylation patterns in genes associated with fetal growth. 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).

3.5.3.2.1.3 Animals

An in vivo analysis of epigenetic modifications in an oral PFOA study (1–20 mg/kg/day; 10 days) was performed in female CD-1 mice (Rashid et al., 2020). Measurement of 5-methylcytosine (5mc) and 5-hydroxymethylcytosine (5hmc) indicated no alteration of global CpG methylation levels in the kidneys. Downregulation of DNA methyltransferase 1 (*Dnmt1*) mRNA was observed at ≤5 mg/kg/day PFOA, while *Dnmt1* expression increased by 4- and 7-fold at doses of 10 and 20 mg/kg/day, respectively. Levels of *Dmnt3a* decreased at all doses, and *Dnmt3b* expression increased at the highest dose (20 mg/kg/day). mRNA expression of

translocation (Tet) 1/2/3 methylcytosine dioxygenases was decreased at low doses of PFOA exposure compared with controls, with no change at higher doses.

3.5.3.2.2 In Vitro Evidence

In vitro PFOA exposures have yielded mixed results with evidence of both hyper- and hypomethylation of DNA. Data presented here are categorized by global DNA methylation and gene-specific modifications.

3.5.3.2.2.1 Global DNA Methylation

5mC expression can be used to indicate global DNA methylation. Pierozan et al. (2020) treated MCF-10A cells with PFOA (100 μM, 72 hours) and found elevated global methylation levels in the first daughter cell subculture. However, methylation levels returned to baseline after the second passage. This study contrasts with the results of Wen et al. (2020) in a study conducted in HepG2 cells (20–400 μM PFOA, 48 hours), and Liu and Irudayaraj (2020) in a study of MCF7 cells (20–400 μM PFOA, 24–48 hours). Both studies found dose-dependent reductions in 5mC after PFOA exposure.

3.5.3.2.2.2 Modification to Gene Expression

Assays evaluating gene expression modified by enzymes that regulate DNA methylation levels, such as DNMT and TET enzymes, and histone modifications have been used to assess the impact of PFOA on the epigenome. Liu and Irudayaraj (2020) reported significantly lower levels of DNMT1 protein after PFOA exposure in both MCF7 (≥100 μM) and HepG2 (≥200 μM) cells. However, DNMT3A expression was increased in a dose-dependent manner in MCF7 cells (≥200 μM). Authors attributed PFOA-induced global demethylation to alterations of DNMT3A and subsequent enzymatic activity of DNMT. Levels of DNMT3B did not change significantly in either cell line. Wen et al. (2020) found no significant changes to *DNMT1/3A/3B* gene profiles after PFOA exposure (20–400 μM, 48 hours) in HepG2 cells. Further analysis found PFOA (200 μM) decreased *TET1* expression, which is strongly associated with DNA methylation, but increased *TET2 and TET3*. Pierozan et al. (2020) noted that PFOA-exposed MCF-10A cells and the direct daughter cell passages contained decreased levels of histone 3 lysine 9 dimethylation (H3K9me2). H3K9me2 is a silencing epigenetic marker; thus, a decrease in H3K9me2 is indicative of transcriptional activation, and has been associated with altered gene expression in breast cancer transformation.

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). Overall, the evidence supports that oxidative stress can result from PFOA exposure, based on animal and in vitro studies. The evidence related to oxidative stress is detailed below and in the referenced sections.

3.5.3.3.1 In Vivo Evidence

3.5.3.3.1.1 Humans

Franken et al. (2017) measured urinary 8-OHdG to evaluate DNA damage induced by oxidative stress, in adolescents (14–15 years of age) that resided for >5 years in industrial areas of Belgium and compared their findings to blood PFOA concentrations. While no significant change was observed in urinary 8-OHdG in the subjects when compared with that of age-matched controls, a positive dose-response relationship with increasing PFOA concentrations was noted. The authors attributed the DNA damage to oxidative stress but noted that elevated 8-OHdG could also reflect aberrant DNA repair.

3.5.3.3.1.2 Animals

Several in vivo analyses of PFOA exposure in rodents found evidence that PFOA exposure caused increased oxidative stress and markers of oxidative damage in a tissue-specific manner.

Takagi et al. (1991) performed a two-week subchronic study (0.02% powdered PFOA in the diet) in male Fischer 344 rats and evaluated the levels of 8-OHdG in the liver and kidneys after exposure. While a significant increase was noted in liver and kidney weights, elevated levels of 8-OHdG was observed only in the liver. A second subset of animals were given a single IP injection of PFOA (100 mg/kg) and sacrificed at days 1, 3, 5, and 8. Results were comparable to that of the dietary exposure study, as PFOA significantly increased liver (by day 1) and kidney (on days 3 and 8) weights with elevated liver 8-OHdG levels (by day 3).

Minata et al. (2010) exposed wild-type (129S4/SvlmJ) and *Pparα*-null (129S4/SvJae-Pparα^{tm1Gonz}/J) mice to PFOA (≤50 µmol/kg/day) for four weeks. Levels of 8-OHdG were evaluated in the liver. No increase in oxidative stress levels was noted in exposed wild-type mice. In contrast, *Pparα*-null mice demonstrated a dose-dependent increase in 8-OHdG levels, with a significant increase at 50 µmol/kg/day when compared with controls. The correlation between PFOA exposure and 8-OHdG was associated with increased tumor necrosis factor α (*TNF-α*) mRNA levels.

In a developmental toxicity study, Li et al. (2019b) exposed pregnant Kunming mice to PFOA (≤10 mg/kg/day) on gestational day (GD) 1–17. Female mice were sacrificed on postnatal day (PND) 21 and livers were assessed for oxidative damage by quantification of 8-OHdG, catalase, and superoxide dismutase (SOD). Findings indicate the PFOA caused a dose-dependent increase in oxidative DNA damage levels, which were significantly elevated after 2.5 mg/kg/day. These results were associated with increased superoxide dismutase and catalase protein levels. Together, these findings suggest that the livers of exposed mice were producing antioxidant enzymes to counteract PFOA-induced elevated oxidative stress.

The testes are particularly susceptible to oxidative stress due to high energy demand and abundance of polyunsaturated fatty acids. Liu et al. (2015) exposed male Kunming mice to ≤10 mg/kg/day of PFOA for 14 days and examined oxidative stress in the testis and epididymis. A dose-dependent increase in lipid peroxidation and oxidative stress was observed with a significant increase at ≥5 mg/kg/day relative to controls. In contrast to the results of Li et al. (2019b), levels of the antioxidant enzymes SOD and carnitine acyltransferase (CAT), and *Nrf2* expression (an oxidative stress response gene) decreased as PFOA exposure doses increased.

Several other studies measuring oxidative stress in the liver have found that PFOA induces damage through hydrogen peroxide production (Salimi et al., 2019) and through PPARα activation pathways (Li et al., 2019b). For additional information that PFOA induces oxidative stress in the liver, please see the hepatic mechanistic section (Section 3.4.1.3; refer to the interactive HAWC visual for additional supporting information and study details).

Evidence that PFOA induces oxidative stress in the immune system has been reported. Wang et al. (2014) observed that the spleens of mice treated with PFOA had mitochondrial swelling and cavitation as well as swollen and ruptured cristae, which suggests impaired oxidative processes. For additional information that PFOA induces oxidative stress in immune cells, please see the immune mechanistic section (Section 3.4.2.3; refer to the interactive HAWC visual for additional supporting information and study details).

Mechanistic studies noted PFOA exposure increased oxidative stress in the heart and brain. For additional information, please see the developmental (Section 3.4.4.3) and cardiovascular (Section 3.4.3.3) mechanistic sections (refer to the interactive HAWC for additional supporting information and study details).

3.5.3.3.2 In Vitro Evidence

The ability of PFOA to induce oxidative stress has been assessed in vitro in several human, nonhuman primate, and animal cell lines.

PFOA exposure caused a dose-dependent increase in 8-OHdG in human lymphoblast cells (TK6), with significant results noted at ≥250 ppm (2 hours) (Yahia et al., 2016). A similar relationship was noted in HepG2 cells with significant increase in 8-OHdG levels found at PFOA concentrations ≥100 µM (3 hours) (Yao and Zhong, 2005). Yao and Zhong (2005) measured ROS using a 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) assay and observed a dose-dependent increase associated with elevated 8-OHdG levels. Peropadre et al. (2018) found 8-OHdG levels were nonsignificantly elevated in human HaCaT cells following 24-hour exposure to PFOA (50 µM). However, measurements taken 8 days following exposure found levels to be significantly elevated by 50%.

Panaretakis et al. (2001) observed the peak in ROS generation three hours following PFOA exposure in HepG2 cells exposed to concentrations of 200 and 400 µM. Both concentrations significantly increased hydrogen peroxide and superoxide anions. Wielsøe et al. (2015) noted nonsignificant elevated levels of ROS after HepG2 cells were exposed to PFOA (0.2–20 µM) for 24 hours. Additionally, total antioxidant capacities were reduced after exposure to 0.02–2,000 µM. These studies contrast with the findings of Florentin et al. (2011), which found no change in ROS using a DCFH-DA test in HepG2 cells exposed to 5–400 µM PFOA for 1 or 24 hours.

Kidney cells isolated from the African green monkey (Vero) were used in a DCFH-DA assay to measure ROS production (Fernández Freire et al., 2008). Authors reported a dose-dependent increase in ROS production that reached significance at 500 µM after 24 hours. Vero cells also displayed fragmentation of mitochondrial reticulum at ≥50 µM, a morphological change consistent with defective metabolism, indicating that irregular metabolic activity may play a role in ROS production in this model and exposure scenario.

ROS production was significantly higher in *Paramecium caudatum* exposed to PFOA (100 μM) for 12 or 24 hours, while 8-OHdG was not affected by PFOA (Kawamoto et al., 2010). Addition of the antioxidant glutathione attenuated the PFOA-induced ROS production but not DNA damage (as measured by a comet assay), indicating that the PFOA-induced DNA damage was not associated with oxidative stress in *P. caudatum*.

Hocevar et al. (2020) exposed mouse pancreatic acinar cells to PFOA (≤100 μg/mL; 6 or 24 hours) and observed an increase in intracellular calcium-induced activation of the unfolded protein response (UPR) in the endoplasmic reticulum at concentrations ≥50 μg/mL. This is a well-established oxidative stress-inducing pathway.

Zhao et al. (2011) exposed human-hamster hybrid (A_L) cells to PFOA (1–200 μM; 1–16 days) and found significantly increased intracellular ROS, NO, and $O_2^{\cdot -}$ levels at all timepoints exposed to ≥100 μM. These increases correlated with cytotoxicity, which was significant at all timepoints at 100 and 200 μM. DNA mutagenicity was only significant at the highest concentration at the longest exposure (16 days). Effects were reversed when previously PFOA-exposed cells were treated with oxidative stress inhibitors dimethyl sulfoxide (DMSO) and NG-methyl-L-arginine (L-NMMA). When repeating the study using a mitochondrial deficient cell line (p^0A_L), authors reported no mutagenesis, indicating that if the increase in DNA mutation after PFOA exposure is related to ROS generation, the association is mitochondria dependent.

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. Overall, the evidence demonstrates that PFOA exposure is related to increased markers of inflammation in animal and in vitro studies. The evidence related to chronic inflammation is detailed below.

3.5.3.4.1 In Vivo Evidence

Increased inflammation and/or inflammatory markers (i.e., inflammatory cytokines) has been reported in animal toxicological studies of acute, subchronic, and chronic exposures to PFOA. NTP (2020) used a matrix-type exposure paradigm. Pregnant Sprague-Dawley rats were administered PFOA via gavage beginning on GD 6 and exposure was continued in offspring postweaning for a total of 107 weeks. Dose groups for this report are referred to as (perinatal exposure level (ppm))/(postweaning exposure level (ppm)) and ranged from 0/0–300/300 ppm in males and 0/0–300/1,000 ppm in females. At the 16-week interim sacrifice, incidences of chronic active inflammation of the glandular stomach submucosa was significantly higher in the male 0/300 ppm group compared with the control group. No effects were seen in female rats at the interim sacrifice. At the 2-year evaluation, females in the 0/1,000 and 300/1,000 ppm groups exhibited increased incidences of ulcer, epithelial hyperplasia, and chronic active inflammation of the submucosa of the forestomach when compared with controls.

Histopathological analysis of animals exposed to PFOA (0.625–10 mg/kg) by oral gavage for 28 day exhibited nasal respiratory epithelium inflammation in both males and females, though

these effects did not follow a linear dose response (NTP, 2019). Similarly, olfactory epithelial inflammation and degeneration were observed in females. Increases in nasal and olfactory hyperplasia were thought to be a result of the observed epithelial degradation and/or inflammation.

Activation of the NF-κB signaling pathway plays an important role in the regulation of inflammation, including through expression of proinflammatory cytokines (Shane et al., 2020; Zhong et al., 2020; Lee et al., 2017a; Zhang et al., 2014a). Modification to NF-κB expression has been observed in adult zebrafish after 7, 14, and 21 days of PFOA exposure (Zhong et al., 2020; Zhang et al., 2014a) and in female BALB/c mice dermally exposed to PFOA for 14 days (Shane et al., 2020). Additionally, proinflammatory cytokines IL-1β, TNF-α, and others were upregulated by PFOA exposure at doses ranging from 0.002% w/w in the diet and 2.5–10 mg/kg/day by gavage for 10 or 14 days in various tissues across several mouse studies (Liu et al., 2016; Wang et al., 2014; Yang et al., 2014; Qazi et al., 2010).

3.5.3.4.2 In Vitro Evidence

Saejia et al. (2019) noted that PFOA (1 nM, 72 hours) significantly increased activation of NF-κB in FTC133 cells. Furthermore, translocation of the phosphorylated version of NF-κB to the nucleus from the cytosol, a crucial step in inflammation cytokine production, was observed. Inhibition of NF-κB activation was found to reduce invasive characteristics of cells, likely through reduced expression of MMP-2 and MMP-9. PFOA increased the levels of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8, in a dose-responsive manner in IgE-stimulated rat mast cells (RBL-2H3 cell line) (Lee et al., 2017a). 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.

Several studies have identified the potential of PFOA to increase inflammation within various testing systems. For additional information, please see the immune (Section 3.4.2.3), hepatic (Section 3.4.1.3), 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). Overall, the evidence demonstrates that PFOA exposure can alter and impair immune and inflammatory response and function in both humans and animals, as detailed briefly in the following paragraph and in further detail in the referenced section.

Studies have identified the immunosuppressive potential of PFOA in in vivo and in vitro testing systems. The pleotropic immunomodulatory effects of PFOA, including impaired vaccine response in humans and reduction in B and T cell populations in the thymus and spleen in laboratory animals, may reflect perturbed function of B and/or T cells. At the molecular level, dysregulation of the NF-κB pathway may contribute to the immunosuppressive effects of PFOA. The NF-κB pathway facilitates initial T cell responses by supporting proliferation and regulating

apoptosis, participates in the regulation of CD4+ T cell differentiation, and is involved in mediating inflammatory responses. Dysregulation of the NF-κB pathway by PFOA, potentially consequent to the induction of oxidative stress, may be a key component of the underlying mechanism of PFOA-mediated immunosuppression. Reduced NF-κB activation and consequent elevation of apoptosis is consistent with increased apoptosis in multiple cell types, the reduction of pre/pro B cell numbers, and dysregulation of pro-inflammatory cytokines and mediators of inflammation. 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). Overall, the animal and in vitro evidence demonstrates that PFOA activates several nuclear receptors: PPARα, CAR/PXR, ERα, and HNF4α, as detailed briefly in the following paragraphs and in detail in the referenced sections.

3.5.3.6.1 In Vivo Evidence

Yan et al. (2015a) exposed adult male Balb/c mice to PFOA (0.08–20 mg/kg/day) via oral gavage for four weeks. Livers were isolated and mRNA levels of several peroxisome proliferator-activated receptors (PPARs) were evaluated using RT-PCR. PPARα was found to be increased by 50% in the 0.08 and 0.31 mg/kg/day dose groups. This trend was not consistent as PPARα levels diminished at higher doses. PPARγ was found to increase in a dose-dependent manner that reached significance at 1.25 mg/kg/day PFOA. No differences were observed in PPARβ/δ mRNA expression after exposure.

Data from studies conducted in rodent models have demonstrated PPARα activation as a mechanism for PFOA-induced hepatotoxicity, due to the association between hepatic lesions and/or increased liver weight and peroxisome proliferation downstream of PPARα activation. There is also growing evidence the PFOA activates other nuclear receptors (e.g., CAR/PXR, ERα, HNF4α) in tandem with PPARα to enact its effects. For additional information, please see the hepatic (Section 3.4.1.3) and cardiovascular (Section 3.4.3.3) mechanistic sections (refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.6.2 In Vitro Evidence

PPARα and PPARγ gene expression was assessed in hepatocellular carcinoma cells (Hepa 1-6) exposed to PFOA (50–200 μM; 72 hours) (Yan et al., 2015a). While no significant changes were observed for these genes, PPARα target genes were significantly increased, indicating that PPARα was activated by PFOA.

Available mechanistic evidence demonstrates that PFOA has the potential to dysregulate hormone levels in hepatic cells, particularly regarding thyroid function. Furthermore, rodent and human hepatocytes treated with PFOA demonstrated a concentration-dependent decrease in lipid accumulation that was associated with PPARα activation. 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).

3.5.3.7 Key Characteristic #9: Causes Immortalization

Immortalization leads to tumorigenesis when cells continue to divide after sustaining DNA damage and/or shortened telomeres, events that cause cells to cease to divide in healthy or normal states (i.e., the Hayflick limit). Immortalization is a key characteristic typically observed in and associated with human DNA and RNA viruses, such as human papillomaviruses and hepatitis C virus, among others. In vitro cell transformation assays have been historically used to test carcinogenic potential of both genotoxic and non-genotoxic compounds (Creton et al., 2012), and is recognized as an assay related to key characteristic #9 (Smith et al., 2020). Overall, the limited evidence demonstrates that PFOA does not alter cell transformation or cause immortalization, as detailed in the following paragraph.

In the case of PFOA, two studies reported no change in cell transformation in vitro in cells exposed to PFOA relative to controls. Jacquet et al. (2012) exposed SHE cells to PFOA at concentrations ranging from 3.7×10^{-4} to 37.2 µM for 6 days with or without pre-treatment with the tumor initiator benzo-α-pyrene (BaP). PFOA exposure alone did not induce cell transformation, but PFOA did significantly induce transformation in BaP-sensitized cells, indicating that PFOA does not alone initiate cell transformation, but may have tumor promoter-like activity. A second in vitro cell transformation assay reported no evidence of transformation in C3H 10T-1/2 mouse embryo cells exposed to 0.1–200 µg/mL PFOA in a 14-day colony assay for transformation nor in a 38-day foci transformation assay (Garry and Nelson, 1981).

3.5.3.8 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). Overall, the evidence demonstrates that PFOA exposure can increase cell proliferation in animals and in cell models, and results are conflicting on the ability of PFOA to induce or inhibit apoptosis. The evidence related to cell proliferation, cell death, and migration (cancer cell invasiveness) is detailed below.

3.5.3.8.1 In Vivo Evidence

To determine if PFOA exposure induced proliferation in cancer cells, Ma et al. (2016) xenografted human endometrial adenocarcinoma (Ishikawa cell line) cells into the flanks of six-week-old female BALB/c mice. Animals were then treated with PFOA (20 mg/kg/day) by oral gavage daily for three weeks beginning the same day of the xenograft. Tumor volume was measured after five weeks, and data indicated that PFOA caused tumors to nearly triple in size. Additionally, levels of proliferating cell nuclear antigen (PCNA) and vimentin protein were both upregulated by PFOA, suggesting increased cell proliferation and invasion. E-cadherin expression was downregulated after PFOA exposure, indicating that cells were more likely to migrate and form metastases.

Treatment effects on apoptosis and cell cycle have also been observed in immune system cells of animals exposed to PFOA. Wang et al. (2014) exposed BALB/c mice to PFOA (5–20 mg/kg/day, 14 days) via gavage and reported that the percent of apoptotic cells increased in

the spleen (10–20 mg/kg/day) and in the thymus (20 mg/kg/day). Yang et al. (2002b) reported significant reductions in the proportion of thymocytes in the S and G2/M phases and significant increases in the G0/G1 phases of mice treated with PFOA, effects that were PPARα-dependent.

Additional mechanistic studies, detailed elsewhere, noted PFOA exposure alters the number of various B and T cell subsets in primary and secondary lymphoid organs, which may impact immune system development, including dysregulation of proliferation, differentiation, and/or apoptosis. 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).

3.5.3.8.2 In Vitro Evidence

PFOA has been demonstrated to increase cell proliferation and apoptosis evasion in vitro. Evidence presented here is organized into three categories: induced proliferation, apoptosis evasion, and modification of cellular migration.

3.5.3.8.2.1 Proliferation

Exacerbation of proliferation in cancer cell lines is of particular concern to the development and prognosis of cancer. Several studies have utilized MTT assays to measure cellular metabolic activity to determine cell proliferation and cytotoxicity rates.

PFOA exposure (5–50 μM) increased cellular proliferation in MCF-7 human breast cancer cells and HepG2 human hepatoma (nontumorigenic) cells (Liu and Irudayaraj, 2020; Buhrke et al., 2015; Buhrke et al., 2013). However, predictably, proliferation rates decreased at cytotoxic concentrations (≥100 μM PFOA) (Wen et al., 2020; Buhrke et al., 2015; Buhrke et al., 2013). Similar results were observed in the breast epithelial (nontumorigenic) cell line MCF-10A, in which PFOA exposure (50 and 100 μM; 24–72 hours) increased cell proliferation, whereas proliferation rates decreased as the PFOA concentration was increased to a cytotoxic level (250 μM) (Pierozan et al., 2018). A subsequent study by Pierozan et al. (2020) reported that PFOA-induced (100 μM, 72 hours) proliferation persisted in MCF-10A daughter subcultures that were not exposed to PFOA. PFOA exposure (1–100 nM) in colorectal cancer cells (DLD-1) has also been shown to modify the cell cycle by causing more cells to enter S-phase and less in G_1 of mitosis (Miao et al., 2015).

Several studies of the effects of low exposure to PFOA found no evidence of modification to cell proliferation rates. These studies include ovarian cancer cell line A2780 (1–200 nM, 48 hours) (Li et al., 2018b) Ishikawa human endometrial adenocarcinoma cells (50 nM, 48 hours) (Ma et al., 2016), and human colorectal cancer cell line DLD-1 (1–10,000 nM, 72 hours) (Miao et al., 2015).

Insulin growth factor 1 (IGF-1) expression has been implicated in governing proliferation in cancer cells. A series of experiments performed by Gogola et al. (2020a; 2020b, 2019) used COV434 and KGN cells exposed to PFOA (0.02 ng/mL–2 μg/mL; 72 hours). All studies found increased proliferation in both cell lines. Proliferation was highest in COV434 and KGN cells at 0.02 ng/mL and 2 ng/mL, respectively. Interestingly, proliferation returned to baseline levels in both cell lines at PFOA concentration of 2 μg/mL, indicating a bell-shaped dose response. These experiments were repeated after inhibition of IGF-1 caused normalization in both cell lines after

PFOA exposure. Together, these studies demonstrate the potential pathway in which PFOA induces proliferation in cancer cells.

HepG2 cells were exposed to non-cytotoxic concentrations of PFOA for 24 hours before SHP-2, a tumor suppressor protein, was immunoprecipitated from the cell lysates (Yang et al., 2017). PFOA (100 μM) slightly lowered SHP-2 mRNA expression and decreased SHP-2 enzyme activity in a concentration-dependent manner. SHP-2 protein levels were increased only at 140 μM exposure, and unchanged at other concentrations. These results indicate that PFOA inhibits SHP-2 by reducing enzyme activity, not protein content.

Rainieri et al. (2017) evaluated the effects of PFOA on cell proliferation by quantifying the distribution of cells in different stages of the cell cycle in a human macrophage cell line (TLT cells). Significantly more cells were in G2M phase following exposure to PFOA (50–500 mg/L; 12 hours) in parallel with a lower proportion of cells in the G0/G1 phase, suggesting increased cell proliferation. For additional evidence of the effect of PFOA on cell death and cell proliferation in the immune system, please see the immune mechanistic section (Section 3.4.2.3; refer to the interactive HAWC visual for additional supporting information and study details).

3.5.3.8.2.2 Apoptosis

Evasion of programmed cell death is a characteristic of cancer cells, allowing them to continue proliferating, which can be enhanced by PFOA exposure. Dairkee et al. (2018) evaluated several human breast cancer cell lines for apoptosis following PFOA exposure (1 or 100 nM; 7 days). Using fluorescence activated cell sorting (FACS) of Annexin V-FITC, PFOA concentrations were found to be inversely correlated with apoptosis rates. However, in HepG2 cells, PFOA exposure was found to increase metabolically induced BAX apoptosis in a dose-dependent manner (Wen et al., 2020). Apoptosis was also found to increase in HepG2 cells after PFOA exposure (200 or 400 μM; ≤24 hours) and was associated with an increase in caspase-9 activation after 5 hours of exposure (Panaretakis et al., 2001). Additionally, the murine spermatogonial cell line GC-1 exhibited a dose-dependent increase in apoptosis after exposure to PFOA (≥250 μM) for 24 hours that reached significance at ≥500 μM (Lin et al., 2020d).

Caspase protease enzymes are essential in apoptotic cell death and are frequently used to assess apoptosis. Gogola at al. (2020a; 2020b) found that PFOA (0.2–20 ng/mL; 72 hours) caused no changes to caspase 3/7 expression in COV434 and KGN cells. Additionally, PFOA (≤100 μM) had no effect on caspase 3/7 activity in HepG2 cells. Lin et al. (2020d) reported a dose-dependent increase in caspase-3 activity that correlated with apoptosis rates in GC-1 cells. Additionally, apoptosis and caspase activity were inversely correlated with Bcl-2/Bax ratios. These results indicate that PFOA may induce apoptosis through an increase in BAX expression. Hu and Hu (2009) also suggested that PFOA could induce apoptosis by overwhelming the homeostasis of antioxidative systems, increasing ROS, impacting mitochondria, and changing expression of apoptosis gene regulators, based on their findings in studies with HepG2 cells. Overall, data are conflicting on the ability of PFOA to induce or inhibit apoptosis, with the variation likely dependent upon dose and duration of exposure.

3.5.3.8.2.3 Modulation of Migration

Cancer cells are invasive in nature due to their ability to increase mobility, reduce attachment to neighboring cells, and express proteins that break down the extracellular matrix of tissues. Wound healing assays are a common and reproducible way to inflict a ‘wound’ on a monolayer

plate of cells and measure the time for the cells to re-establish confluency. Two independent studies concluded PFOA exposure increased the rate at which Ishikawa cells (50 nM, 48 hours) (Ma et al., 2016) and A2780 cells (≥100 nM, 72 hours) (Li et al., 2018b) were able to re-establish confluency in a dose-dependent manner.

Assays of migration and invasion measure the ability of a cell to travel either without inhibition or through the extracellular matrix of plated cells, respectively. Two studies investigated cellular migration after PFOA exposure and found no change after FTC133 cells were exposed to 1 nM (72 hours) (Saejia et al., 2019) or 0–1 mM (24–72 hours) (Pierozan et al., 2018), while an increase in migration was found at 100 nM (72 hours) in MCF-10A cells (Pierozan et al., 2018). All studies reviewed found an increase in the invasive nature of cancer cells lines FTC133 (1 nM, 72 hours) (Saejia et al., 2019), Ishikawa (≥50 nM) (Ma et al., 2016), MCF-10A (100 nM, 72 hours) (Pierozan et al., 2018), A2780 (≥100 nM, 72 hours) (Li et al., 2018b), and DLD-1 (1 nM–1 μM, 72 hours) (Miao et al., 2015) after PFOA exposure.

Pierozan et al. (2020) exposed MCF-10A cells to PFOA (100 μM, 72 hours) and found that invasion and migration of daughter cell passages was elevated when compared with control.

Several reports noted cell invasion and upregulated MMP2 and MMP9 expression levels, which help to break down the extracellular matrix allowing cells to move freely, indicating that cancer cells could be more likely to become invasive or metastasize after exposure to PFOA (Saejia et al., 2019; Li et al., 2018b; Miao et al., 2015).

Additional mechanistic studies have identified the potential of PFOA to induce aberrant cellular proliferation rates and increase apoptosis within in vitro testing systems. For additional information, please see the immune (Section 3.4.2.3) and hepatic (Section 3.4.1.3) mechanistic sections (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 PFOA has been documented in both epidemiological and animal toxicological studies. The evidence from *medium* confidence epidemiological studies is primarily based on the incidence of kidney and testicular cancer, as well as some evidence of increased breast cancer incidence in susceptible subpopulations. Other cancer types have been observed in humans, although the evidence for these is generally limited to *low* confidence studies. The evidence of carcinogenicity in animal models is provided in three *high* or *medium* confidence chronic oral animal bioassays in Sprague-Dawley rats which together identified neoplastic lesions of the liver, pancreas, and testes. The available mechanistic data suggest that multiple MOAs could play a role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models.

3.5.4.1.1 Evidence From Epidemiological Studies

The strongest evidence of an association between PFOA exposure and cancer in human populations is from studies of kidney cancer. Two *medium* confidence studies of the C8 Health Project population reported positive associations between PFOA levels (mean at enrollment 0.024 μg/mL) and kidney cancer among the residents living near the DuPont plant in

Parkersburg, West Virginia (Barry et al., 2013; Vieira et al., 2013). Vieira et al. (2013) reported elevated risk of kidney cancer in residents of the Little Hocking water district of Ohio (OR: 1.7, 95% CI: 0.4, 3.3; n = 10) and the Tuppers Plains water district of Ohio (OR: 2.0, 95% CI: 1.3, 3.1; n = 23). Barry et al. (2013) extended this work, and found increased risk of kidney cancer (HR: 1.10, 95% CI: 0.98, 1.24; n = 105), though the levels did not reach statistical significance. The high-exposure occupational study by Steenland and Woskie (2012) evaluated kidney cancer mortality in workers from West Virginia and observed significant elevated risk of kidney cancer death in the highest exposure quartile. As part of the C8 Health Project, the C8 Science Panel (2012a) concluded a probable link between PFOA exposure and kidney cancer (Steenland et al., 2020).

The findings of another recently published *medium* confidence study add support to the previous evidence of an association between PFOA and kidney cancer (Shearer et al., 2021). Shearer et al. (2021) is a multicenter case-control study nested within the National Cancer Institute (NCI) Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial (n = 326). The authors reported a statistically significant increase in risk of renal cell carcinoma (RCC) with pre-diagnostic serum levels of PFOA (OR = 2.63; 95% CI: 1.33, 5.20 for the highest vs. lowest quartiles; p-trend = 0.007, or per doubling of PFOA: OR: 1.71; 95% CI: 1.23, 2.37). The association remained significant in analyses on a per doubling increase in PFOA after adjusting for other PFAS. The increase in the highest exposure quartile remained and the magnitude was similar (i.e., OR = 2.63 without adjusting for other PFAS vs. 2.19 after adjusting for other PFAS), but it was no longer statistically significant. Statistically significant increased odds of RCC were observed in a subgroup of participants ages 55–59 years, and in men and in women, analyzed separately. A recent critical review and meta-analysis of the epidemiological literature concluded that there was an increased risk for kidney tumors (16%) for every 10 ng/mL increase in serum PFOA (Bartell and Vieira, 2021). Although the authors concluded that the associations were likely causal, they noted the limited number of studies and therefore, additional studies with larger cohorts would strengthen the conclusion. Taken together, the recent pooled analysis of the NCI nested case-control study (Shearer et al., 2021) of 324 cases and controls and the C8 Science Panel Study (Barry et al., 2013) of 103 cases and 511 controls provide evidence of concordance in kidney cancer findings from studies of the general population and studies of high-exposure communities (Steenland et al., 2022). CalEPA (2021) similarly concluded, "[t]here is evidence from epidemiologic studies that exposure to PFOA increases the risk of kidney cancer."

There is also evidence of associations between PFOA serum concentrations and testicular cancer in humans, though no new epidemiological studies reporting these associations have been published since the studies described in the 2016 PFOA HESD (U.S. EPA, 2016c). Similar to their results for kidney cancer, Vieira et al. (2013) reported an increased adjusted OR for testicular cancer (OR: 5.1, 95% CI: 1.6, 15.6; n = 8) in residents of the Little Hocking water district of Ohio. Barry et al. (2013) also found significantly increased testicular cancer risk with an increase in estimated cumulative PFOA serum levels (HR: 1.34, 95% CI: 1.00, 1.79; n = 17). The C8 Science Panel (2012a) concluded that a probable link also exists between PFOA exposure and testicular cancer (Steenland et al., 2020). A recent critical review and meta-analysis of the epidemiological literature concluded that there was an increased risk for testicular tumors (3%) for every 10 ng/mL increase in serum PFOA (Bartell and Vieira, 2021) (see Appendix A, (U.S. EPA, 2024a)). In their review of the available epidemiological data, IARC (2016)

concluded that the evidence for testicular cancer was "considered credible and unlikely to be explained by bias and confounding, however, the estimate was based on small numbers." Similarly, CalEPA (2021) concluded, "[o]verall, the epidemiologic literature to date suggests that PFOA is associated with testicular cancer."

The majority of epidemiological studies examining the carcinogenicity after PFOA exposure reported on breast cancer risk. Two nested case-control studies found associations between PFOA exposure and breast cancer, but only in participants with known genetic susceptibility (e.g., specific genotype or tumor estrogen receptor (ER) type) (Mancini et al., 2020; Ghisari et al., 2017). In Taiwan, Tsai et al. (2020) observed an increased risk of breast cancer only in all women 50 years old or younger (including ER+ and ER– participants), and in ER+ participants aged 50 years or younger, along with a decrease in risk for ER– breast cancers in participants aged 50 years or younger. Significantly increased odds of breast cancer were also observed in an NHANES population across serum PFOA quartiles with a significant dose-response trend (Omoike et al., 2021). Two nested case-control studies did not report an association between breast cancer and PFOA concentrations measured in maternal serum throughout pregnancy and 1–3 days after delivery (Cohn et al., 2020) or in serum after case diagnosis and breast cancer (Hurley et al., 2018). One nested case-cohort study did not report an association between breast cancer and PFOA concentrations measured in a group of predominantly premenopausal women (Bonefeld-Jørgensen et al., 2014). In the C8 Health Project cohort, Barry et al. (2013) observed a significant inverse association with breast cancer for both unlagged (i.e., concurrent) and 10-year lagged (i.e., cumulative exposures occurring 10 years in the past) estimated cumulative PFOA serum concentrations. Similarly, a recent study in a Japanese population reported an inverse association across serum PFOA quartiles with a significant dose-response trend (Itoh et al., 2021). Overall, study design differences, 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 PFOA and breast cancer. However, there is suggestive evidence that PFOA exposure may be associated with an increased breast cancer risk based on studies in populations with specific genetic polymorphisms conferring increased susceptibility and for specific types of breast tumors.

3.5.4.1.2 Evidence From Animal Bioassays

In addition to the available epidemiological data, two multidose bioassays and one single-dose chronic cancer bioassay are available that investigate the relationship between dietary PFOA exposure and carcinogenicity in male and female rats (NTP, 2020; Butenhoff et al., 2012; Biegel et al., 2001). Increased incidences of neoplastic lesions were primarily observed in male rats, though results in females are supportive of potential carcinogenicity of PFOA. Testicular Leydig cell tumors (LCTs) were identified in both the Butenhoff et al. (2012) and Biegel et al. (2001) studies. LCT incidence at similar dose levels was comparable between the two studies (11% and 14%). Pancreatic acinar cell tumors (PACTs) were observed in both the NTP (2020) and Biegel et al. (2001) studies. NTP (2020) reported increased incidences of pancreatic acinar cell adenomas and adenocarcinomas in males in all treatment groups compared with their respective controls (Table 3-17). These pancreatic tumor types were also observed in female rats in the highest dose group, a rare occurrence compared with historical controls (0/340), though these increases did not reach statistical significance. Biegel et al. (2001) similarly reported increases in the incidence of PACTs in male rats treated with PFOA, with zero incidences observed in control animals. In addition, NTP (2020) reported dose-dependent increases in the incidence of liver

adenomas and carcinomas in male rats (Table 3-16) and Biegel et al. (2001) also observed increased incidence of adenomas in male rats. Overall, NTP concluded that in their 2-year feeding studies, there was *clear evidence* of carcinogenic activity of PFOA in male Sprague-Dawley rats and *some evidence* of carcinogenic activity of PFOA in female Sprague-Dawley rats based on the observed tumor types (NTP, 2020).

The report from NTP (2020) provides evidence that chronic oral exposure accompanied by perinatal exposure (i.e., exposure beginning at gestation day 5 through lactation) to PFOA does not increase cancer risk when compared with chronic exposure scenarios beginning during the postnatal (i.e., exposure initiated after weaning) stage. The incidences of all tumor types examined did not differ significantly between the treatment groups administered PFOA during both perinatal and postweaning periods compared with the postweaning-only treatment groups (see further study design details in Section 3.4.4.2.1.2). Lifestage-dependent sensitivity to the carcinogenic effects of PFOA exposure was previously assessed in the study by Filgo et al. (2015) which exposed two mouse strains during gestation only (i.e., prenatal exposure with no comparisons to mice exposed through adulthood). Filgo et al. (2015) observed a nonmonotonic increase in hepatocellular adenomas in the female offspring of one strain (CD-1) and hepatocellular adenoma incidence in approximately 13% of all PFOA-exposed peroxisome proliferator-activated receptor (PPAR) α-knockout mice. However, these results are not conclusive due to the study's limited sample size and study design.

3.5.4.2 Mode of Action Analysis

In the 2016 PFOA HESD (U.S. EPA, 2016c), the EPA concluded that the induction of tumors was likely due to multiple MOAs, specifically noting interactions with nuclear receptors, perturbations in the endocrine system, interruption of intercellular communication, mitochondrial effects, and/or perturbations in the DNA replication and cell division processes. Since that time, the available mechanistic data continue to suggest that multiple MOAs could play role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models. The few available mechanistic studies focusing on PFOA-induced renal toxicity highlight several potential underlying mechanisms of PFOA exposure-induced renal tumorigenesis, including altered cell proliferation and apoptosis, epigenetic alterations, and oxidative stress. However, due to data limitations, it is difficult to distinguish which mechanism(s) are operative for PFOA-induced kidney cancer. Similarly for testicular cancer, the available literature highlights several potential MOAs by which PFOA exposure may result in increased incidence of LCTs in animals, though it is unclear whether these MOAs are relevant to testicular cancers associated with PFOA exposure in humans.

As described in the following subsections, the available mechanistic data continue to suggest that multiple MOAs could play role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models.

3.5.4.2.1 Mechanistic Evidence for Renal Tumors

As discussed in Section 3.5.13.4.5, there is convincing evidence for an association between renal carcinogenesis and serum PFOA concentrations in epidemiological studies from both the general population and residents of high-exposure communities (Shearer et al., 2021; Barry et al., 2013). However, there is limited mechanistic information from epidemiological studies explaining the observed renal carcinogenicity. Additionally, many animal models are limited in their ability to

replicate kidney damage due to PFOA exposure that is observed in humans (Li et al., 2017a). One factor that may be driving this inconsistency between humans and animals is the difference in renal clearance rates between human and animal models. Regardless of elimination differences, both animal toxicological studies and the limited available human biomonitoring data suggest that the kidneys may be a site of enrichment upon PFOA exposure and subsequent distribution (Shearer et al., 2021).

The few available studies focusing on PFOA-induced renal toxicity highlight several potential underlying mechanisms of PFOA exposure-induced renal tumorigenesis, including altered cell proliferation and apoptosis, epigenetic alterations, and oxidative stress. However, due to data limitations, it is difficult to distinguish what mechanism(s) are the most relevant for PFOA-induced kidney cancer. The renal-specific evidence supporting multiple mechanisms involved in tumorigenesis is described in the subsections below, which are all key characteristics of carcinogens and may be related to PFOA-induced renal cell carcinoma.

3.5.4.2.1.1 Altered Cell Death, Cell Proliferation, or Nutrient Supply

There is evidence that relative kidney weight, particularly in male rats, is increased after PFOA treatment (see Appendix C, (U.S. EPA, 2024a)) (NTP, 2020, 2019; Butenhoff et al., 2004a). However, these increases in kidney weight and presumably increases in cell proliferation may be due to increased need for renal transporters and not necessarily an indicator of the initial stages of carcinogenesis (U.S. EPA, 2016a). Though there is conflicting evidence of alterations in relative kidney weight in female rats, NTP (2020) reported increased hyperplasia of urothelium that lines the renal papilla in female rats from the 0/1,000 and 300/1,000 ppm (63.4 and 63.5 mg/kg/day, respectively) dose groups at the interim sacrifice timepoint (16 weeks) and in female rats from the 0/300 (18.2 mg/kg/day), 0/1,000, and 300/1,000 ppm dose groups at the terminal sacrifice (107 weeks). These changes were accompanied by increased incidence of renal papilla necrosis at terminal sacrifice in both 1,000 ppm postweaning groups. Though NTP (2020) did not explore the mechanisms of toxicity underlying the observed renal effects, they note that prolonged exposure and relatively high dose levels along with the enhanced efficiency of excretion and increased urinary concentrations of PFOA in female rats (compared with males) may have resulted in cytotoxicity and hyperplasia of the papilla.

Evidence of cytotoxicity and cell cycle disruption was also provided by a single *in vitro* study in Vero cells (cell line derived from monkey kidney epithelial cells) (Fernández Freire et al., 2008). Fernández Freire et al. (2008) assessed potential cytotoxic effects and alterations in cell cycle progression in Vero cells treated with PFOA at concentrations of 50–500 μM for 24 hours. Cells treated with PFOA exhibited decreases in viability and proliferation, as indicated by alterations in mitochondrial metabolism (MTT assay) and the total number of cells (Bradford/TPC assay), though both assays exhibited a plateau in cytotoxicity at PFOA concentrations of approximately 200 μM and higher. The study also reported dose-dependent increases in the percentage of apoptotic cells with increasing PFOA concentrations. Flow cytometric analysis demonstrated G0/G1 cell cycle arrest in Vero cells treated with the maximum concentration of 500 μM PFOA. The percentage of cells in the G0–G1 stage were increased whereas the percentages of cells in the S and G2-M stages were decreased. The authors hypothesized that the observed cell cycle arrest may be linked to increased ROS and oxidative stress, further described below.

3.5.4.2.1.2 Oxidative Stress

The increases in cytotoxicity and apoptosis in Vero cells treated with up to 500 μM PFOA for 24 hours observed by Fernández Freire et al. (2008) were accompanied by a dose-dependent increase in ROS which was statistically significant in the cells treated with 500 μM. The authors noted that severe oxidative stress could induce cell cycle arrest and apoptosis, as described previously (Fernández Freire et al., 2008). However, in the only available animal toxicological study assessing oxidative damage in the kidney, levels of 8-hydroxydeoxyguanosine (8-OH-dG) DNA damage in the kidney were unchanged in male Fischer 344 rats administered PFOA via the diet (0.02% for 2 weeks) or by IP injection (100 mg/kg single injection) (Takagi et al., 1991). Though the renal-specific evidence of PFOA-induced oxidative stress is limited, further discussion on oxidative stress in other organ systems is discussed below, as well as in Section 3.5.3.

3.5.4.2.1.3 Epigenetics

Rashid et al. (2020) investigated epigenetic markers that could contribute to the kidney dysfunction associated with PFOA exposure. CD-1 mice were orally exposed to 1–20 mg/kg/day PFOA for 10 days and kidney tissues were evaluated for epigenetic alterations (DNA methylation and histone acetylation). Though no PFOA-induced changes in global methylation were noted (by measurements of 5-methyl cytosine and 5-hydroxy methylation levels), the study reported specific methylation changes with reduced representation bisulfite sequencing (RRBS). Overall, 879 genes were differentially methylated in in the 20 mg/kg/day dose group versus control. PFOA exposure also altered mRNA expression of several proteins that regulate DNA methylation, including DNA methyl transferases and translocation enzymes, as well as mRNA expression of several histone deacetylases. Combined, these results suggest that PFOA exposure triggered epigenetic alterations, including DNA methylation changes and potentially histone modifications, in the kidney (Rashid et al., 2020). However, further study is needed to explore connections between the observed epigenetic changes and subsequent regulation of genes associated with kidney tumorigenesis.

3.5.4.2.2 Mode of Action for Testicular Tumors

There is both epidemiological evidence and evidence from animal bioassays of an association between increased PFOA serum concentrations or doses and testicular carcinogenesis. Testicular cancer was observed in epidemiological studies from the C8 Health Project (Barry et al., 2013; Vieira et al., 2013). In addition, a recent meta-analysis concluded that there is a 3% increase in risk for testicular cancer with every 10 ng/mL increase in serum PFOA concentrations (Bartell and Vieira, 2021). In animal models, testicular tumors (Leydig cell tumors (LCTs)) were reported in two chronic studies in male Sprague-Dawley rats (Butenhoff et al., 2012; Biegel et al., 2001). Combined, these results indicate that the testes are a common site of PFOA-induced tumorigenesis.

The available literature highlights several potential MOAs by which PFOA exposure may result in increased incidence of LCTs in animals, though it is unclear whether these MOAs are relevant to testicular cancers associated with PFOA exposure in humans. In a review of LCTs published by Clegg et al. (1997), a workgroup identified seven nongenotoxic hormonal MOAs, (i.e., androgen receptor antagonism; testosterone biosynthesis inhibition; 5α-reductase inhibition; aromatase inhibition; estrogen agonism; GnRH agonism; and dopamine agonism), five of which were considered relevant to humans, and the majority of which involved downstream increases

in luteinizing hormone (LH) levels and subsequent Leydig cell hyperplasia/tumorigenesis. The working group noted that sensitivity for the initiating events in these MOAs varies across species, with rodents being more sensitive relative to humans. It has also been proposed that PPARα agonism potentially mediates these effects, though the evidence supporting this claim is not as strong as for other tumor types (i.e., hepatic tumors) (Klaunig et al., 2012; Klaunig et al., 2003). However, CalEPA noted that "PFOA appears to act through multiple MOAs, and the PPARα MOA does not adequately explain the incidences of pancreatic and testicular tumors reported" (CalEPA, 2021).

The testes-specific evidence for the six human-relevant MOAs are described in the subsections below, though, as described in Section 3.5.3, PFOA generally exhibits evidence of multiple key characteristics of carcinogens that may also be relevant to the MOA for testicular cancers associated with increased serum PFOA concentrations in humans.

3.5.4.2.2.1 Hormone-Mediated MOAs

Clegg et al. (1997) identified five human-relevant MOAs for LCTs that involve alterations in hormone balances, steroid receptor activity, or enzymes involved in steroid metabolism (5α-reductase inhibition, androgen receptor antagonism, aromatase inhibition, estrogen agonism, testosterone biosynthesis inhibition). In addition, some compounds have been shown to influence Leydig cell function, including steroidogenesis, via hormone-mediated MOAs that are initiated upon PPARα activation (Klaunig et al., 2003; Gazouli et al., 2002). Klaunig et al. (2003) described two proposed hormone-mediated MOAs and key events by which PPARα agonists could induce LCTs in rats: one MOA which is secondary to liver PPARα induction and one MOA which involves direct inhibition of testosterone biosynthesis in the testes. These two MOAs involve associative key events such as increased aromatase activity, increased serum estradiol (E2) levels, increased TGFα levels, decreased testosterone levels, increased LH levels, and/or Leydig cell proliferation. Evidence for the key events involved in the human-relevant MOAs for testicular tumors in rodents exposed to PFOA is summarized in the paragraphs below and in Table 3-23, Table 3-24, Table 3-25, and Table 3-26. There was no evidence of PFOA treatment resulting in 5α-reductase inhibition in the identified literature, and the majority of the limited available *in vitro* studies for PFOA report that PFOA does not act as an androgen receptor antagonist (McComb et al., 2019; Kang et al., 2016b; Du et al., 2013; Rosenmai et al., 2013). Thus, these two MOAs are not summarized herein.

3.5.4.2.2.1.1 Aromatase Inhibition MOA

In vivo studies in male rats and mice generally found no effect of oral PFOA exposure on testicular aromatase activity or mRNA expression, though there was some evidence for increased hepatic microsomal aromatase activity or mRNA expression (Li et al., 2011; Liu et al., 1996; Biegel et al., 1995). A reduction in serum testosterone is also opposite of the expected key event following aromatase inhibition (increased serum testosterone), further supporting that PFOA does not operate through this MOA. The hepatic aromatase activity provides some support for the MOA that is secondary to liver PPARα induction (Klaunig et al., 2003). Evidence demonstrating the lack of activity for the key events involved in the aromatase inhibition MOA for testicular tumors, as presented in Clegg et al. (1997), in rodents exposed to PFOA is summarized in Table 3-23.

Table 3-23. Evidence of Key Events Associated with the Aromatase Inhibition Mode of Action for Testicular Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: CYP19A1 Inhibition	Key Event 2: Increased Serum T	Key Event 3: Decreased Serum E2	Key Event 4: Increased Serum LH	Key Event 5: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
Dose (mg/kg/day)	**CYP19A1 Activity in Liver**	**Serum T**	**Serum E2**	**Serum LH**	**Leydig Cell Hyperplasia**	**Testicular Tumor**
0.06	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
0.2	– (14 d)	NR	– (14 d)	NR	NR	NR
0.31	NR	– (28 d)	NR	NR	NR	NR
0.64	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
1	– (6 wk)	↓ (6 wk) – (GD1–17) – (14 d)	– (14 d)	– (14 d)	– (6 wk)	– (6 wk)
1.1[b]	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
1.25	NR	↓ (28 d)	NR	NR	NR	NR
1.3	NR	NR	NR	NR	NR	– (105 wk)
1.94	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
2	↑ (14 d)	NR	↑ (14 d)	NR	NR	NR
2.2[b]	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
2.5	NR	↓ (GD1–17)	NR	NR	NR	NR
4.6[b]	↑ (16 wk)	↓ (28 d) ↓ (GD1–17)	NR	NR	NR	– (16 wk)
5	– (6 wk)	↓ (6 wk)	NR	NR	– (6 wk)	– (6 wk)
6.5	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
10	NR	– (14 d)	↑ (14 d)	– (14 d)	NR	NR
13.6	NR	**↑ (26 wk) – (4, 12, 39, 52, 65, 78, 91 wk)[c]**	**↑ (4, 12, 26, 39, 52 wk) – (65, 78, 91 wk)[c]**	**↓ (78 wk) – (4, 12, 26, 39, 52, 65, 91 wk)[c]**	**↑ (104 wk)**	**↑ (104 wk)**
14.2	NR	NR	NR	NR	NR	**↑ (105 wk)**
20	↑ (14 d)	↓ (28 d) ↓ (1, 3, 5 d)	↑ (14 d)	NR	NR	NR
25	↑ (14 d)	– (14 d)	↑ (14 d)	– (14 d)	NR	NR
40	↑ (14 d)	NR	↑ (14 d)	NR	NR	NR
50	NR	– (14 d)	↑ (14 d)	– (14 d)	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; CYP19A1 = cytochrome P-450 19A1 (aromatase); T = testosterone; E2 = β-estradiol; LH = luteinizing hormone; NR = not reported; wk = week(s); d = day(s); GD = gestational day.

Cells in bolded text and 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 Biegel et al. (1995); Biegel et al. (2001); Butenhoff et al. (2012); Cook et al. (1992); Li et al. (2011); Liu et al. (1996); Martin et al. (2007); NTP (2020); Perkins et al. (2004); Song et al. (2018); and Zhang et al. (2014b). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from ad libitum controls. Data from Li et al. (2011) are in a hPPARα model.

[a] Reviewed in Clegg et al. (1997) and Klaunig et al. (2003).

[b] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[c] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.2.1.2 Estrogen Agonism MOA

Although increased aromatase activity was observed, indicating potential increases in the conversion of androgens to estrogens, evidence of estrogen agonism in rodents was not robust. Biegel et al. (2001) reported consistent increases in serum E2 in male rats treated with the same concentration of PFOA that induced LCTs (300 ppm; approximately 13.6 mg/kg/day); however, the estrogen levels were too low to be accurately measured with the radioimmunoassay methods utilized in the study. Cook et al. (1992) observed similar increases in serum E2 concentrations in male rats gavaged with 10, 25, or 50 mg/kg/day PFOA for 14 days, though the authors also used a radioimmunoassay and reported similarly low E2 concentrations. Perkins et al. (2004) additionally reported suggestive increases in serum E2 concentrations in male rats treated with up to 6.5 mg/kg/day PFOA for 13 weeks, though this response was not statistically significant. Overall, there is not sufficient evidence to support estrogen agonism as the MOA for PFOA-induced LCTs. Evidence for the key events involved in the estrogen agonism MOA for testicular tumors, as presented in Clegg et al. (1997), in rodents exposed to PFOA is summarized in Table 3-24.

Table 3-24. Evidence of Key Events Associated with the Estrogen Agonism Mode of Action for Testicular Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Increased CYP19A1 Activity in Liver	Key Event 3: Increased Serum E2	Key Event 4: Increased TGFα in Testis	Key Event 5: Increased Serum LH	Key Event 6: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
Dose (mg/kg/day)	**PPARα Activation in Liver[b]**	**CYP19A1 Activity in Liver**	**Serum E2**	**TGFα in Testis**	**Serum LH**	**Leydig Cell Hyperplasia**	**Testicular Tumor**
0.06	NR	NR	– (4, 7, 13 wk)	NR	– (4, 7, 13 wk)	NR	NR
0.2	NR	– (14 d)	– (14 d)	NR	NR	NR	NR
0.64	NR	NR	– (4, 7, 13 wk)	NR	– (4, 7, 13 wk)	NR	NR
1	NR	– (6 wk)	– (14 d)	NR	– (14 d)	– (6 wk)	– (6 wk)
1.1[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
1.3	NR	NR	NR	NR	NR	NR	– (105 wk)
1.94	NR	NR	– (4, 7, 13 wk)	NR	– (4, 7, 13 wk)	NR	NR
2	NR	↑ (14 d)	↑ (14 d)	NR	NR	NR	NR
2.2[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
4.6[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
5	NR	– (6 wk)	NR	NR	NR	– (6 wk)	– (6 wk)
6.5	NR	NR	– (4, 7, 13 wk)	NR	– (4, 7, 13 wk)	NR	NR
10	NR	NR	↑ (14 d)	NR	– (14 d)	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[d]	NR	↑ (4, 12, 26, 39, 52 wk)	NR	↓ (78 wk)	↑ (104 wk)	↑ (104 wk)

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Increased CYP19A1 Activity in Liver	Key Event 3: Increased Serum E2	Key Event 4: Increased TGFα in Testis	Key Event 5: Increased Serum LH	Key Event 6: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
			– (65, 78, 91 wk)[d]		– (4, 12, 26, 39, 52, 65, 91 wk)[d]		
14.2	NR	NR	NR	NR	NR	NR	↑ (105 wk)
19	↑ (1 ,7, 28 d)	NR	NR	NR	NR	NR	NR
20	– (1, 3, 5 d)	↑ (14 d)	↑ (14 d)	NR	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR	NR
25	NR	↑ (14 d)	↑ (14 d)	↑ (14 d)	– (14 d)	NR	NR
40	NR	↑ (14 d)	↑ (14 d)	NR	NR	NR	NR
50	NR	NR	↑ (14 d)	NR	– (14 d)	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; CYP19A1 = cytochrome P-450 19A1 (aromatase); E2 = β-estradiol; TGFα = transforming growth factor α; LH = luteinizing hormone; NR = not reported; w = week(s); d = day(s).

Cells in bolded text and 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 Biegel et al. (1995); Biegel et al. (2001); Butenhoff et al. (2012); Cook et al. (1992); Elcombe et al. (2010); Li et al. (2011); Liu et al. (1996); Martin et al. (2007); NTP (2020); and Perkins et al. (2004). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from ad libitum controls. Data from Li et al. (2011) are in a hPPARα model.

[a] Reviewed in Clegg et al. (1997) and Klaunig et al. (2003).

[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity in NTP (2020), as hepatic β-oxidation activity in Biegel et al. (2001), as CYP4A1 protein expression and hepatic β-oxidation activity in Elcombe et al. (2010), and as *Cyp4a14*, *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp17a1* gene expression in Martin et al. (2007).

[c] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[d] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.2.1.3 Testosterone Biosynthesis Inhibition MOA

Several of the available studies support an impact of PFOA on testosterone production in male rodents (Eggert et al., 2019; Lu et al., 2019; Song et al., 2018; Zhang et al., 2014b; Li et al., 2011; Martin et al., 2007; Biegel et al., 1995; Cook et al., 1992), as well as in men from the general population or high-exposure communities from epidemiological studies (Cui et al., 2020; Petersen et al., 2018; Lopez-Espinosa et al., 2016). However, neither the subchronic nor the chronic study in male rats that measured serum testosterone reported decreases across multiple time points ranging from 1 to 21 months (Perkins et al., 2004; Biegel et al., 2001) (Table 3-25). Though there is evidence of PFOA-induced inhibition of testosterone biosynthesis, this lack of response in the only study that both observed LCTs and measured testosterone serum levels limits potential conclusions about whether decreased testosterone plays a role in the MOA for LCTs (Biegel et al., 2001). Evidence for the key events involved in the testosterone biosynthesis inhibition MOA for testicular tumors, as presented in Clegg et al. (1997), in rodents exposed to PFOA is summarized in Table 3-25.

Table 3-25. Evidence of Key Events Associated with the Testosterone Biosynthesis Inhibition Mode of Action for Testicular Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: PPARα Activation	Key Event 2: Decreased Testosterone Biosynthesis	Key Event 3: Decreased Serum T	Key Event 4: Increased Serum LH	Key Event 5: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
Dose (mg/kg/day)	**PPARα Activation in Liver[b]**	**Testosterone Biosynthesis[c]**	**Serum T**	**Serum LH**	**Leydig Cell Hyperplasia**	**Testicular Tumor**
0.06	NR	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
0.31	NR	– (28 d)	– (28 d)	NR	NR	NR
0.64	NR	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
1	NR	↓ (6 wk)	↓ (6 wk) – (14 d) – (GD1–17)	– (14 d)	– (6 wk)	– (6 wk)
1.1[d]	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
1.25	NR	↓ (28 d)	↓ (28 d)	NR	NR	NR
1.3	NR	NR	NR	NR	NR	– (105 wk)
1.94	NR	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
2.2[d]	↑ (16 wk)	NR	NR	NR	NR	– (16 wk)
2.5	NR	NR	↓ (GD1–17)	NR	NR	NR
4.6[d]	↑ (16 wk)	↓ (28 d)	↓ (28 d) ↓ (GD1–17)	NR	NR	– (16 wk)
5	NR	↓ (6 wk)	↓ (6 wk)	NR	– (6 wk)	– (6 wk)
6.5	NR	NR	– (4, 7, 13 wk)	– (4, 7, 13 wk)	NR	NR
10	NR	NR	– (14 d)	– (14 d)	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[e]	NR	↑ (26 wk) – (4, 12, 39, 52, 65, 78, 91 wk)[e]	↓ (78 wk) – (4, 12, 26, 39, 52, 65, 91 wk)[e]	↑ (104 wk)	↑ (104 wk)
14.2	NR	NR	NR	NR	NR	↑ (105 wk)
19	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
20	– (1, 3, 5 d)	↓ (28 d)	↓ (28 d) ↓ (1, 3, 5 d)	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
25	NR	NR	– (14 d)	– (14 d)	NR	NR
50	NR	NR	– (14 d)	– (14 d)	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; T = testosterone; LH = luteinizing hormone; wk = week(s); d = day(s); GD = gestational day.

Cells in bolded text and 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 Biegel et al. (1995); Biegel et al. (2001); Butenhoff et al. (2012); Cook et al. (1992); Elcombe et al. (2010); Li et al. (2011); Liu et al. (1996); Martin et al. (2007); NTP (2020); Perkins et al. (2004); Song et al.

(2018); and Zhang et al. (2014b). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from ad libitum controls. Data from Li et al. (2011) are in a hPPARα model.

[a] Reviewed in Clegg et al. (1997) and Klaunig et al. (2003).

[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity in NTP (2020), as hepatic β-oxidation activity in Biegel et al. (2001), as CYP4A1 protein expression and hepatic β-oxidation activity in Elcombe et al. (2010), and as *Cyp4a14*, *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp17a1* gene expression in Martin et al. (2007).

[c] Testosterone biosynthesis provided as gene expression of 3β-HSD, 17-β-HSD, and/or CYP17A1 in Zhang et al. (2014b) and as gene expression of 3β-HSD, 17-β-HSD, and/or CYP17A1 in Li et al. (2011).

[d] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[e] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.2.1.4 PPARα activation MOA

Support for at least partial PPARα mediation of testosterone production inhibition due to PFOA administration is available from one study in mice (Li et al., 2011). Significantly reduced plasma testosterone concentrations were observed in male wild-type PPARα mice and humanized PPARα transgenic mice. These decreases were evident but not statistically significant in PPARα-null mice. In addition, reduced reproductive organ weights and increased sperm abnormalities were also observed in PFOA-treated male PPARα wild-type and humanized PPARα mice but not in PPARα-null mice (Li et al., 2011). However, data are not currently sufficient to demonstrate that the other key steps in the postulated PPARα-mediated MOAs are present in PFOA-treated animals following exposures that lead to tumor formation. Additional studies are needed to demonstrate the increase of GnRH and LH in concert with the changes in aromatase and further study is needed to confirm the potential downstream increases in serum E2. There was also no indication of increased Leydig cell proliferation at the doses that caused adenomas in the Biegel et al. (2001) study. Thus, additional research is needed to determine if the hormone testosterone-E2 imbalance is a key factor in development of LCTs as a result of PFOA exposure. Evidence for the key events involved in the PPARα agonist-induced MOA for testicular tumors in rodents exposed to PFOA is summarized in Table 3-26.

Table 3-26. Evidence of Key Events Associated with PPARα Agonist-Induced Mode of Action for Testicular Tumors[a] in Male Rats and Mice Exposed to PFOA

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Increased CYP19A1 Activity in Liver	Key Event 3: Increased Serum E2	Key Event 4: Increased TGFα in Testis	Key Event 5: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
Dose (mg/kg/day)	**PPARα Activation in Liver[b]**	**CYP19A1 Activity in Liver**	**Serum E2**	**TGFα in Testis**	**Leydig Cell Hyperplasia**	**Testicular Tumor**
0.06	NR	NR	– (4, 7, 13 wk)	NR	NR	NR
0.2	NR	– (14 d)	– (14 d)	NR	NR	NR
0.64	NR	NR	– (4, 7, 13 wk)	NR	NR	NR
1	NR	– (6 wk)	– (14 d)	NR	– (6 wk)	– (6 wk)
1.1[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	– (16 wk)
1.3	NR	NR	NR	NR	NR	– (105 wk)

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Increased CYP19A1 Activity in Liver	Key Event 3: Increased Serum E2	Key Event 4: Increased TGFα in Testis	Key Event 5: Leydig Cell Hyperplasia	Outcome: Testicular Tumor
1.94	NR	NR	– (4, 7, 13 wk)	NR	NR	NR
2	NR	↑ (14 d)	↑ (14 d)	NR	NR	NR
2.2[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	– (16 wk)
4.6[c]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR	– (16 wk)
5	NR	– (6 wk)	NR	NR	– (6 wk)	– (6 wk)
6.5	NR	NR	– (4, 7, 13 wk)	NR	NR	NR
10	NR	NR	↑ (14 d)	NR	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[d]	NR	↑ (4, 12, 26, 39, 52 wk) – (65, 78, 91 wk)[d]	NR	↑ (104 wk)	↑ (104 wk)
14.2	NR	NR	NR	NR	NR	↑ (105 wk)
19	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
20	– (1, 3, 5 d)	↑ (14d)	↑ (14 d)	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
25	NR	↑ (14 d)	↑ (14 d)	↑ (14 d)	NR	NR
40	NR	↑ (14 d)	↑ (14 d)	NR	NR	NR
50	NR	NR	↑ (14 d)	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; CYP19A1 = cytochrome P-450 19A1 (aromatase); E2 = β-estradiol; TGFα = transforming growth factor α; NR = not reported; wk = week(s); d = day(s).

Cells in bolded text and 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 the table were extracted from Biegel et al. (1995); Biegel et al. (2001); Butenhoff et al. (2012); Cook et al. (1992); Elcombe et al. (2010); Li et al. (2011); Liu et al. (1996); Martin et al. (2007); NTP (2020); and Perkins et al. (2004). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from *ad libitum* controls. Data from Li et al. (2011) are in a hPPARα model.

[a] Reviewed in Clegg et al. (1997) and Klaunig et al. (2003).

[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity in NTP (2020), as hepatic β-oxidation activity in Biegel et al. (2001), as CYP4A1 protein expression and hepatic β-oxidation activity in Elcombe et al. (2010), and as *Cyp4a14*, *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp17a1* gene expression in Martin et al. (2007).

[c] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[d] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.3 Mode of Action for Pancreatic Tumors

As discussed in Section 3.5.2, pancreatic acinar cell tumors (PACTs) were identified in male rats in two 2-year chronic cancer bioassays (NTP, 2020; Biegel et al., 2001). In fact, NTP (2020) reported increased incidences of pancreatic acinar cell adenomas in males in all treatment groups, as well as increased incidence, though nonsignificant, in female rats from the highest

dose group. A subchronic drinking water exposure study in the LSL-KRas^{G12D}; Pdx-1 Cre (KC) mouse model for pancreatic cancer also provides evidence that PFOA exposure promotes the growth of pancreatic lesions (Kamendulis et al., 2022).

Two proposed MOAs for PFOA-induced pancreatic tumors in animal models were identified in the literature, including one study that utilizes a transgenic mouse model to mimic the histologic progression of pancreatic cancer in humans (Kamendulis et al., 2022; Klaunig et al., 2012; Klaunig et al., 2003). The proposed MOAs are: 1) changes in bile acids, potentially linked to activation of hepatic PPARα, leading to cholestasis, a positive cholecystokinin (CCK) feedback loop, and acinar cell proliferation; and 2) oxidative stress. However, the existing database is limited in its ability to determine the relationship between PFOA exposure and these MOAs, particularly for the PACTs observed in chronic rat studies. Evidence for the key events involved in the relevant MOAs for pancreatic tumors in rodents exposed to PFOA is summarized in Table 3-27 and Table 3-28.

3.5.4.2.3.1 Gastric Bile Alterations

Gastric bile compositional changes or flow alterations can lead to cholestasis, which is the reduction or stoppage of bile flow. Cholestasis may cause an increase in CCK, a peptide hormone that: stimulates digestion of fat and protein, causes increased production of hepatic bile, and stimulates contraction of the gall bladder. There is some evidence suggesting that pancreatic acinar cell adenomas may result from increased CCK levels resulting from blocked bile flow (Obourn et al., 1997), which may result in a CCK-activated feedback loop that leads to increased proliferation of secretory pancreatic acinar cells.

PFOA may change bile composition by competing with bile acids for biliary transport. Upregulation of MRP3 and MRP4 transporters (Maher et al., 2008) and downregulation of OATPs (Cheng and Klaassen, 2008a) linked to PPARα activation in mice may favor excretion of PFOA from the liver via bile. Minata et al. (2010) found that PFOA levels in bile were much higher in wild-type male mice versus PPARα-null mice, suggesting a link to PPARα. In this study, male mice were dosed with 0, 5.4, 10.8, and 21.6 mg/kg/day PFOA for 4 weeks, resulting in increased total bile acid in PPARα-null mice at the highest dose, which indicated that PFOA-induced activation of PPARα may result in increased PFOA excretion. This may, in turn, result in decreased flow of bile acids that compete for the same transporters. Notably, however, these alterations in male mice occurred at relatively high dose levels compared with those that resulted in PACTs in male rats following 2 years of PFOA exposure (NTP, 2020). In the NTP study, bile acid concentrations were increased greater than twofold in male rats exposed to PFOA in the diet at doses of 15.6 and 31.7 mg/kg/day for 4 weeks compared with the control group. In the same study, serum ALP levels were mildly increased (less than twofold). While these increases may be due to cholestasis, mild increases in ALP (and ALT) activity are also associated with the administration of hepatic microsomal enzyme inducer compounds, including PPARα agonists (NTP, 2020). There was no further evidence of cholestasis reported in the literature. Additionally, CalEPA noted that "PFOA appears to act through multiple MOAs, and the PPARα MOA does not adequately explain the incidences of pancreatic and testicular tumors reported" (CalEPA, 2021).

Additionally, there is no evidence of alterations in CCK associated with PFOA exposure in animal models or human studies. In fact, medical surveillance data from male workers at 3M's

Cottage Grove plant demonstrated a significant negative association between CCK levels and serum PFOA (Olsen et al., 2000; Olsen et al., 1998). Further, cholestasis was not observed in the workers (Olsen et al., 2000). It has been suggested that the lack of a positive association may be due to PFOA levels being too low to increase CCK in humans, although it has been demonstrated that PFOA is not an agonist for the CCKA receptor that activates CCK release (Obourn et al., 1997). Overall, due to limited evidence for altered bile flow in animals that developed tumors and an overall lack of evidence for alterations in CCK levels in PFOA-exposed animals, there is not sufficient evidence to determine whether bile acid alterations contribute to the MOA for PACTs observed in rodents chronically exposed to PFOA. Evidence for the key events involved in the gastric bile acid alteration MOA for pancreatic tumors in rodents exposed to PFOA is summarized in Table 3-27.

Table 3-27. Evidence of Key Events Associated with the Gastric Bile Alterations Mode of Action for Pancreatic Tumors[a] in Male and Female Rats and Mice

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Altered Bile Flow and/or Bile Acid Composition	Key Event 3: Cholestasis	Key Event 4: Increase in CCK Levels	Key Event 5: Acinar Cell Proliferation or Hyperplasia	Outcome: Pancreatic Tumors
Dose (mg/kg/day)	**PPARα Activation in Liver[b]**	**Altered Bile Flow and/or Bile Acid Composition**	**Cholestasis[c]**	**CCK Levels**	**Acinar Cell Proliferation or Hyperplasia**	**Pancreatic Tumors[d]**
1.1[e]	↑ (16 wk)	NR	↑ (16 wk) for ALT, ALP, SDH – (16 wk) for bile acids	NR	↑ (104 wk)	↑ (104 wk)
1.3 (males)/ 1.6 (females)[f]	NR	NR	NR	NR	– (105 wk)	– (105 wk)
2.2[e]	↑ (16 wk)	NR	↑ (16 wk) for ALT, ALP, SDH – (16 wk) for bile acids	NR	↑ (104 wk)	↑ (104 wk)
4.6[e]	↑ (16 wk)	NR	↑ (16 wk) for ALT, ALP, SDH – (16 wk) for bile acids	NR	↑ (104 wk)	↑ (104 wk)
5.4	NR	– (4w)	↑ (4 wk) for ALT ↓ (4 wk) for bilirubin – (4 wk) for AST, bile acid	NR	NR	NR
10.8	NR	– (4w)	↑ (4 wk) for AST, ALT – (4 wk) for bile acid, bilirubin	NR	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[g]	NR	NR	NR	↑ (104 wk)	↑ (104 wk)

Canonical MOA	Key Event 1: PPARα Activation in Liver	Key Event 2: Altered Bile Flow and/or Bile Acid Composition	Key Event 3: Cholestasis	Key Event 4: Increase in CCK Levels	Key Event 5: Acinar Cell Proliferation or Hyperplasia	Outcome: Pancreatic Tumors
14.2 (males)/ 16.1 (females)	NR	NR	NR	NR	– (105 wk)	– (105 wk)
15.6	↑ (16 wk)	NR	↑ (16 wk) for ALP, ALT, SDH, bile acid	NR	NR	NR
18.2 (females)[e]	↑ (16 wk)	NR	– (16 wk) for ALP, ALP, SDH	NR	– (104 wk)	– (104 wk)
19	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
20	– (1, 3, 5 d)	NR	NR	NR	NR	NR
21.6	NR	– (4 wk)	↑ (4 wk) for AST, ALT, bilirubin – (4 wk) for bile acid	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	NR	NR	NR	NR
31.7	↑ (16 wk)	NR	↑ (16 wk) for bile acid, ALP, ALT, SDH	NR	NR	NR
40	NR	↑ (2 d)	NR	NR	NR	NR
63.4 (females)[e]	↑ (16 wk)	NR	↑ (16 wk) for ALT, ALP	NR	– (104 wk)	– (104 wk)
80	NR	↑ (2 d)	NR	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; CCK = cholecystokinin; wk = week(s); NR = not reported; ALT = alanine transaminase; ALP = alkaline phosphatase; SDH = sorbitol dehydrogenase; AST = aspartate transferase; d = day(s).

Cells in bolded text and 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 the table were extracted from: Biegel et al. (2001); Butenhoff et al. (2012); Cheng et al. (2008a); Elcombe et al. (2010); Kamendulis et al. (2022); Martin et al. (2007); NTP (2020); and from wild-type animals in Minata et al. (2010). Doses in mg/kg/day for Minata et al. (2010) were converted from 12.5, 25, and 50 μmol/kg/d as reported in the primary study. Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from *ad libitum* controls.

[a] Reviewed in Klaunig, 2003, 5772415 and Klaunig, 2012, 1289837.

[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity (NTP, 2020), as hepatic β-oxidation activity (Biegel et al., 2001), and as CYP4A1 protein expression and hepatic β-oxidation activity (Elcombe et al., 2010).

[c] Observations consistent with cholestasis include significant increases in serum bile acid concentrations and increased serum liver enzyme activities (e.g., ALP, ALT) in NTP (2020), and increased total bilirubin and ALT in Minata et al. (2010).

[d] Pancreatic tumors reflect increased incidence of acinar cell adenoma and/or adenocarcinoma (combined) in male rats (NTP, 2020; Biegel et al., 2001).

[e] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures in male (20, 40, and 80 ppm, or 1.1, 2.2, and 4.6 mg/kg/day) and female (300 and 1,000 ppm, or 18.2 and 63.4 mg/kg/day) rats in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[f] All data are from male rats with the exception of Butenhoff et al. (2012) and NTP (2020), which include both males and females, as indicated.

[g] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

3.5.4.2.3.2 Oxidative Stress

More recent literature has suggested a potential role for oxidative stress in pancreatic carcinogenesis associated with PFOA exposure. Evidence for the key events involved in the proposed oxidative stress MOA for pancreatic tumors in rodents exposed to PFOA is summarized in Table 3-28. Hocevar et al. (2020) and Kamendulis et al. (2022) suggest that pancreatic cancer is induced through the activation of the UPR pathway, which leads to the activation of nuclear factor erythroid 2–related factor 2 (Nrf2), a regulator of the oxidative stress response, and protein kinase-like endoplasmic reticulum kinase (PERK), a signaler of endoplasmic reticulum (ER) stress, and subsequent upregulation of antioxidant responses (e.g., SOD gene expression). Activation of the UPR pathway can also stimulate ROS production. Activation of Sod1 in the mouse by the Nrf2 or PERK signaling pathways can stimulate cell proliferation through increased production of hydrogen peroxide which can then, in turn, act as a second messenger in mitogen signaling or through its elimination of ROS, leading to prevention of ROS-stimulated apoptosis (Kamendulis et al., 2022). Activation of PERK through the UPR pathway may also result in increased cytosolic calcium levels through activation of the inositol 1,4,5-trisphosphate receptor (IP3R), leading to ER stress and generation of ROS (Hocevar et al., 2020).

Induction of tumors by PFOA through oxidative stress is supported by two studies. Hocevar et al. (2020) evaluated PFOA-induced oxidative stress in mouse pancreatic acinar cells (266-6 cells) treated with 50 µg/mL PFOA for various durations. PFOA-exposed cells exhibited increased ER stress as well as activation of PERK, inositol-requiring kinase/endonuclease 1α (IRE1α), and activating transcription factor 6 (ATF6) signaling cascades of the UPR pathway. Exposure to PFOA at concentrations of 20, 50, or 100 µg/mL was also shown to result in time- and dose-dependent increases in cytosolic calcium levels, an effect that occurred predominantly through activation of IP_3R. Altogether, results in Hocevar et al. (2020) demonstrated that PFOA increased intracellular calcium levels through activation of the IP_3R, leading to ER stress, the generation of ROS and oxidative stress and subsequent PERK-dependent induction of antioxidant genes. The oxidative stress and ROS generated in response to PFOA may serve as a mechanism through which PFOA may induce pancreatic tumors.

Kamendulis et al. (2022) evaluated the ability for PFOA to promote pancreatic cancer using the LSL-KRasG12D;Pdx-1 Cre (KC) mouse model of pancreatic cancer, which has a mutation in the KRas gene, a mutations that is present in over 90% of human pancreatic cancers. This gene mutation in mice results in a histologic progression of pancreatic cancer that mirrors human pancreatic cancer progression, including formation of pancreatic intraepithelial neoplasia (PanIN). KC mice were exposed to 5 ppm PFOA in drinking water for up to 7 months, and increased PanIN was observed after 4 and 7 months of treatment compared with untreated KC mice.

Oxidative stress was also apparent in the PFOA-treated KC mice (Kamendulis et al., 2022). The authors reported increases in Sod enzyme activity at 4 and 7 months, along with threefold increases in Sod1 protein and mRNA levels and increased pancreatic catalase and thioredoxin reductase activities at 4 months relative to control. Pancreatic malondialdehyde, a product of oxidized lipids, was significantly increased at 7 months of exposure relative to untreated mice, but not at 4 months, indicating a potential accumulation of oxidative damage over time. Altogether, the results of Kamendulis et al. (2022) demonstrated that PFOA increased PanIN

area and number at 4 months, indicating early lesion formation. The increased desmoplasia and inflammation (MDA levels) after 7 months of exposure suggest PFOA increased disease severity over time, potentially through prolonged oxidative stress, resulting in pancreatic cancer progression.

Overall, although plausible, there is not sufficient evidence for key events related to an oxidative stress MOA to conclude that the pancreatic tumors in rodents chronically exposed to PFOA are the result of oxidative stress and related molecular events.

Table 3-28. Evidence of Key Events Associated with a Proposed Oxidative Stress Mode of Action Involving the UPR Pathway for Pancreatic Tumors[a] in Male and Female Rats and Mice.

Canonical MOA	Key Event 1: Activation of UPR Pathway	Key Event 2a: Activation of Nrf2 and PERK	Key Event 2b: ROS Production	Key Event 3: Upregulation of Antioxidant Responses	Key Event 4: Increased Production of Hydrogen Peroxide	Key Event 5a: Increased Cell Proliferation	Key Event 5b: Decreased Apoptosis	Outcome: Pancreatic Tumors
Dose (mg/kg/day)	**UPR Pathway**	**Nrf2 and PERK**	**ROS Production**	**Antioxidant Response**	**Hydrogen Peroxide Production**	**Cell Proliferation**	**Apoptosis**	**Pancreatic Tumors[b]**
1.1[c]	NR	NR	NR	NR	NR	↑ (104 wk)	NR	↑ (104 wk)
1.28[d]	NR	NR	↑ (28 wk)	↑ (16 wk)	NR	NR	NR	↑ (16 wk)
1.3 (males)/ 1.6 (females)[e]	NR	NR	NR	NR	NR	– (105 wk)	NR	– (105 wk)
2.2[c]	NR	NR	NR	NR	NR	↑ (104 wk)	NR	↑ (104 wk)
4.6[c]	NR	NR	NR	NR	NR	↑ (104 wk)	NR	↑ (104 wk)
13.6	NR	NR	NR	NR	NR	↑ (104 wk)	NR	↑ (104 wk)
14.2 (males)/ 16.1 (females)	NR	NR	NR	NR	NR	– (105 wk)	NR	– (105 wk)
18.2[c] (females)	NR	NR	NR	NR	NR	– (104 wk)	NR	– (104 wk)
63.4[c] (females)	NR	NR	NR	NR	NR	– (104 wk)	NR	– (104 wk)
50 µg/mL[f]	↑ (in vitro)	↑ (in vitro)	NR	NR	NR	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; UPR = unfolded protein response; MOA = mode of action; ROS = reactive oxygen species; Nrf2 = nuclear factor erythroid 2–related factor 2; PERK = protein kinase-like endoplasmic reticulum kinase; NR = not reported; wk = week(s).

Cells in bolded text and 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 the table were extracted from: Biegel et al. (2001); Butenhoff et al. (2012); Kamendulis et al. (2022); and NTP (2020). Data from Biegel et al. (2001) represent significant differences from pair-fed controls and/or from *ad libitum* controls.

[a] Reviewed in Hocevar et al. (2020) and Kamendulis et al. (2022).

[b] Pancreatic tumors reflect increased incidence of acinar cell adenoma and/or adenocarcinoma (combined) in male rats (NTP, 2020; Biegel et al., 2001).

[c] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures in male (20, 40, and 80 ppm, or 1.1, 2.2, and 4.6 mg/kg/day) and female (300 and 1,000 ppm, or 18.2 and 63.4 mg/kg/day) rats in order to provide a representative set of the available mechanistic data involved in this MOA from

bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[d] Dose from Kamendulis et al. (2022) converted from 5 ppm by summary authors using default assumptions for food consumption, water consumption, and body weight, in the absence of such data in the primary study, which used a Kras mutation model of mouse pancreatic cancer.

[e] All data are from male rats with the exception of Butenhoff et al. (2012) and NTP (2020), which include both males and females, as indicated.

[f] Indicates in vitro evidence from Hocevar et al. (2020), which used mouse pancreatic acinar cells (266-6 cells); data are included here owing to the only available demonstration of two of the key events in the proposed MOA.

3.5.4.2.4 Mode of Action for Hepatic Tumors

Two *high* confidence chronic studies on PFOA reported an increased incidence of hepatocellular adenomas in male rats (NTP, 2020; Biegel et al., 2001), one of which also demonstrated increased incidence of hepatocellular carcinomas specific to male rats exposed to PFOA perinatally. As described in the subsections below, the available mechanistic evidence across different *in vivo* and *in vitro* models establishes that multiple modes of action (MOA) are plausible for PFOA-induced liver cancer, including PPARα activation, activation of other nuclear receptors such as CAR, cytotoxicity, and an oxidative stress-mediated MOA. Evidence for the key events involved in the relevant MOAs for hepatic tumors in rodents exposed to PFOA is summarized in Table 3-29,Table 3-30,Table 3-31,Table 3-32, Table 3-33, and Table 3-34. Evidence related to genotoxicity and other plausible modes of action are also detailed in subsequent sections.

EPA previously concluded that liver tumor development in rats exposed to PFOA was not relevant to human health because it was determined to be mediated through PPARα activation. Evidence exists suggesting that although PPARα activators cause liver tumors in rodents, they may be unlikely to result in liver tumors in humans due to comparatively low hepatic PPARα expression, as well as biological differences between rodents and humans in the responses of events that are downstream of PPARα activation (Corton et al., 2018; U.S. EPA, 2016c). 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). However, there is also evidence that other MOAs are operative in PFOA-induced hepatic tumorigenesis (e.g., cytotoxicity (Felter et al., 2018) and liver necrosis in PFOA-exposed mice and rats; see Section 3.5.2). Recently published data suggest that oxidative stress and other mechanistic key characteristics associated with carcinogens may play a role in liver tumor development, as described further below. The existence of multiple plausible MOAs in addition to PPARα activation suggests that PFOA-induced liver cancer in rats may be more relevant to humans than previously thought.

The available literature on mechanisms related to PFOA-induced hepatic tumor development also supports EPA's prior conclusion that PFOA-induced tumors are likely due to nongenotoxic mechanisms involving nuclear receptor activation, perturbations of the endocrine system, and/or DNA replication and cell division (U.S. EPA, 2016a).

3.5.4.2.4.1 PPARα Activation

Exposure to several PFAS has 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, 2016c). 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 or di-2-ethyl hexyl phthalate), the hepatic tumorigenesis observed in rodents is expected to be infrequent and/or less severe in humans, or not observed at all (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003).

The MOA for PPARα activator-induced rodent hepatocarcinogenesis consists of the following sequence 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 increases in hepatocellular adenomas and carcinomas (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003). Modulating factors in this MOA include increased oxidative stress and activation of NF-κB (Corton et al., 2018), both of which have been demonstrated for PFOA. This MOA 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).

The key events underlying PFOA-induced hepatic tumor development through the PPARα MOA have been demonstrated in both in vivo and in vitro studies and have been discussed in detail previously (U.S. EPA, 2016a), as well as in Sections 3.5.2 and 3.5.3 of this document. A number of studies illustrate the potential of PFOA to activate human and rodent PPARα. For example, Buhrke et al. (2013) demonstrated PPARα activation in human Hep2G cells after 24-hour exposure to PFOA at a concentration of 25 μM. PFOA also activated mouse (Li et al., 2019b; Yan et al., 2015b; Takacs and Abbott, 2007; Maloney and Waxman, 1999) and human PPARα (Takacs and Abbott, 2007) in cell transfection studies. Gene expression analyses showed that PPARα activation was required for most transcriptional changes observed in livers of mice exposed to either PFOA or the known PPARα agonist Wyeth-14,643, demonstrating PFOA's ability to act as a PPARα agonist (Rosen et al., 2008a; Rosen et al., 2008b). Nonneoplastic (or pre-neoplastic) events that are associated with PPARα activation include peroxisome proliferation, hepatocellular hypertrophy, and increases in liver weight. Studies of PFOA exposure in rodents have reported one or more of these nonneoplastic effects (Section 3.5.2). For example, hepatocellular hypertrophy was observed in one of the two available chronic carcinogenicity studies of PFOA in rats (NTP, 2020), and both chronic carcinogenicity studies observed increases in liver weights (NTP, 2020; Biegel et al., 2001).

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 PFOA 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). Minata et al. (2010) observed a correlation between PFOA exposure and increased tumor necrosis factor alpha (*TNF-α*) mRNA levels in the livers of *Pparα*-null (129S4/SvJae-Pparα^{tm1Gonz}/J) mice treated with

PFOA (≤50 μmol/kg/day) for four weeks, while there was no effect of PFOA on wild-type (129S4/SvlmJ) mice in the same study. TNFα is a pro-inflammatory cytokine that can be released upon activation of Kupffer cells (Corton et al., 2018). 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 PFOA.

Studies in both rats and mice have demonstrated (either directly or indirectly) that PFOA induces peroxisome proliferation in the liver, an indication of PPARα activation (Elcombe et al., 2010; Minata et al., 2010; Wolf et al., 2008; Martin et al., 2007; Yang et al., 2001; Pastoor et al., 1987). Gene expression profiling of HepG2 cells exposed to low PFOA concentrations (0.1 and 1 μM) revealed increased expression of cell cycle regulators (e.g., Cyclin D1, Cyclin E1). Higher PFOA concentrations generally had no effect on these genes, but were associated with increased expression of p53, p16, and p21 cell cycle regulators (Buhrke et al., 2013). Evidence for cell proliferation in the form of increased mitotic figures and/or bile duct hyperplasia as observed in PFOA-exposed male mice (Loveless et al., 2008), pregnant mice (Yahia et al., 2010), male rats (Elcombe et al., 2010), and female rats (NTP, 2020). Buhrke et al. (2013) also reported increased proliferation in HepG2 cells exposed to PFOA, in addition to PPARα activation. With respect to inhibition of apoptosis, there are conflicting reports, with some studies reported decreases in apoptosis following PFOA exposure (Son et al., 2008), while others report no effect or an increase in apoptosis (Blake et al., 2020; Elcombe et al., 2010; Minata et al., 2010). There is also evidence to support the clonal expansion key event. In an initiation-promotion study of liver tumors in rats, Abdellatif et al. (1990) reported that PFOA had promoting activity and increased the incidence of hepatocellular carcinomas following tumor initiation with diethylnitrosamine (DEN). Jacquet et al. (2012) exposed SHE cells to PFOA at concentrations ranging from 3.7×10^{-4} to 37.2 μM for 6 days with or without pre-treatment with the tumor initiator benzo-α-pyrene (BaP). PFOA exposure alone did not induce cell transformation, but PFOA did significantly induce transformation in BaP-sensitized cells, indicating that PFOA does not alone initiate cell transformation, but may have tumor promoter-like activity.

Two modulating factors have been proposed as part of the PPARα activation MOA that are relevant to PFOA: increased ROS and activation of NF-κB. Although there is not enough evidence to designate these effects as key events in the MOA, they have the potential to alter the ability of PPARα activators to increase liver cancer and are thus defined as modulating factors. PFOA exposure has been demonstrated to cause oxidative stress (detailed below in Section 3.5.4.2.4.5.2). Evidence for the key events involved in the PPARα activation MOA for hepatic tumors in male and female rodents exposed to PFOA is summarized in Table 3-29 and Table 3-30, respectively.

Table 3-29. Evidence of Key Events Associated with the PPARα Mode of Action for Hepatic Tumors[a] in Male Rats and Mice Exposed to PFOA

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)	**PPARα Activation[b]**	**Altered Cell Growth Signaling**	**Hepatic Cell Proliferation**	**Apoptosis**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors[c]**
1	NR	NR	– (7 d)	NR	NR	NR
1.1[d]	↑ (16, 104 wk)	NR	↑ (16, 104 wk)	NR	NR	– (104 wk)
1.3	NR	NR	– (104 wk)	NR	NR	– (104 wk)
2.2[d]	↑ (16, 104 wk)	NR	↑ (16, 104 wk)	NR	NR	↑ (104 wk)
3	NR	NR	– (7 d)	NR	NR	NR
4.6[d]	↑ (16, 104 wk)	NR	↑ (16, 104 wk)	NR	NR	↑ (104 wk)
5.4	NR	– (4 wk)	NR	– (4 wk)	NR	NR
10	NR	NR	↑ (7 d)	NR	NR	NR
10.8	NR	– (4 wk)	NR	↑ (4 wk)	NR	NR
13.6	↑ (4, 12, 26, 39, 52, 65, 78, 91 wk)[e]	NR	– (4, 12, 26, 39, 52, 65, 78, 91 wk)[e]	NR	NR	↑ (104 wk)
14.2	NR	NR	– (104 wk)	NR	NR	– (104 wk)
19	↑ (1, 7, 28 d)	NR	↑ (1, 7, 28 d)	NR	NR	NR
20	– (1, 3, 5 d)	NR	NR	NR	NR	NR
21.6	NR	– (4 wk)	NR	↑ (4 wk)	NR	NR
23	↑ (1, 7, 28 d)	NR	↑ (1, 7, 28 d)	– (1, 7, 28 d)	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; PPARα = peroxisome proliferator-activated receptor α; NR = not reported; d = day(s); wk = week(s).

Cells in bolded text and 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: Biegel et al. (2001); NTP (2020); Elcombe et al. (2010); Minata et al. (2010) (wild-type); Wolf et al. (2008) (sex of mice not stated); Martin et al. (2007); and Butenhoff et al. (2012).

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

[b] Indirect measurement of PPARα induction provided as CYP4A1 protein expression and hepatic β-oxidation activity (Elcombe et al., 2010), as hepatic acyl-CoA oxidase activity in NTP (2020), as hepatic β-oxidation activity in Biegel et al. (2001), as *Cyp4a14*, *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp17a1* gene expression in Martin et al. (2007).

[c] Hepatic tumors reflect increased incidence of adenoma in Biegel (2001), and carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).

[d] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[e] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

Table 3-30. Evidence of Key Events Associated with the PPARα Mode of Action for Hepatic Tumors[a] in Female Rats and Mice Exposed to PFOA

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)	**PPARα Activation[b]**	**Altered Cell Growth Signaling**	**Hepatic Cell Proliferation[c]**	**Apoptosis[d]**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors[e]**
1	NR	NR	↓ (P_0 GD 1.5–17.5)[f] – (P_0 GD 1.5–11.5)	↑ (P_0 GD 1.5–17.5)[f] – (P_0 GD 1.5–11.5)	NR	NR
1.6	NR	NR	– (104 wk)	NR	NR	– (104 wk)
5	NR	NR	↑ (P_0 GD 1.5–11.5)[f] ↓ (P_0 GD 1.5–17.5)	↑ (P_0 GD 1.5–11.5, P_0 GD 1.5–17.5)[f]	NR	NR
16.1	NR	NR	– (104 wk)	NR	NR	– (104 wk)
18.2 g	↑ (16 wk)	NR	– (104 wk)	NR	NR	– (104 wk)
63.4 g	↑ (16 wk)	NR	– (104 wk)	NR	NR	– (104 wk)

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

Cells in bolded text and 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 (2020); Blake et al. (2020) (dams); and Butenhoff et al. (2012).

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

[b] Indirect measurement of PPARα induction provided as hepatic acyl-CoA oxidase activity in NTP (2020).

[c] Increased hepatic cell proliferation as provided by number of increased mitoses in NTP (2020).

[d] Apoptosis as both apoptosis and single-cell necrosis in Blake et al. (2020).

[e] Hepatic tumors reflect increased incidence of carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).

[f] No statistics were reported for hepatic cell proliferation or for apoptosis in Blake et al. (2020); thus, the arrows indicate direction of increased incidence relative to the control group per the authors' results narrative.

[g] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (300 and 1,000 ppm in female rats, or 18.2 and 63.4 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

3.5.4.2.4.2 Other Nuclear Receptors

In addition to PPARα, there is some evidence that other nuclear receptors, such as CAR, PXR, PPARγ, and ER, can be activated by PFOA. CAR, which has an established adverse outcome pathway of key events similar to that of PPARα, has been implicated in hepatic tumorigenesis in rodents. The key events of CAR-mediated hepatic tumors are: 1) CAR activation; 2) altered gene expression specific to CAR activation; 3) increased cell proliferation; and 4) clonal expansion leading to altered hepatic foci, leading to 5) liver tumors (Felter et al., 2018). Nonneoplastic events associated with this pathway include hypertrophy, induction of CAR-specific CYP enzymes (e.g., CYP2B), and inhibition of apoptosis. There is evidence that PFOA can activate CAR and initiate altered gene expression and associative events (Rosen et al., 2017; Elcombe et al., 2010; Rosen et al., 2008a; Rosen et al., 2008b; Martin et al., 2007). For example, Martin et al. (2007) and Elcombe et al. (2010) observed evidence of activation of CAR-related genes, many of which are also altered by PPARα activation, in rats following PFOA exposure, and Wen et al. (2019c) observed increased CAR activation in PFOA-exposed PPARα knockout mice

compared with PFOA-exposed wild-type mice. Other studies have shown altered gene expression of transcriptional targets of CAR in both wild-type and PPARα knockout mice exposed to PFOA (Rosen et al., 2017; Rosen et al., 2008a; Rosen et al., 2008b). As with PPARα-mediated tumorigenesis, there are claims that CAR-mediated tumorigenesis in animals is not relevant to human risk assessment due to differences in CAR-mediated alterations between species. For example, CAR activators (e.g., phenobarbital) induce cell proliferation and tumors in rodents but not in human cell lines (Elcombe et al., 2014). Hall et al. (Hall et al., 2012) noted that there is evidence that CAR in humans is more resistant to mitogenic effects (e.g., studies showing that human hepatocytes are resistant to induction of replicative DNA synthesis).

There is also evidence that PFOA can activate other nuclear receptors, such as PXR, PPARγ, and ERα. Martin et al. (2007) and Elcombe et al. (2010) observed evidence of PPARγ agonism and/or activation of PXR-related genes in rats following PFOA exposure, and Wen et al. (2019c) reported evidence suggesting increased ERα and PXR activation in PFOA-exposed PPARα knockout mice compared with wild-type. PFOA has also been shown to activate PXR in human HepG2 cells (Zhang et al., 2017). Buhrke et al. (2013) demonstrated PPARγ and PPARδ activation at PFOA concentrations of ≥100 μM in transfected HEK293 cells, and activation of PPARγ by PFOA in HepG2 cells (Buhrke et al., 2015).

There is also evidence that PFOA can suppress hepatocyte nuclear factor alpha (HNF4α) protein, a master regulator of hepatic differentiation. Beggs et al. (2016) observed a decrease in HNF4α in the livers of ten-week-old CD-1 mice exposed to 3 mg/kg/day PFOA once daily by oral gavage for 7 days. HNF4α regulates liver development (hepatocyte quiescence and differentiation), transcriptional regulation of liver-specific genes, and regulation of lipid metabolism. Beggs et al. (2016) also exposed human primary hepatocytes to 0.01–10 μM PFOA for 48 or 96 hours to determine pathways affected by PFOA exposure; after 96 hours of 10 μM PFOA, HNF4α protein expression was significantly decreased. In primary human hepatocytes exposed to 1, 25, or 100 μM PFOA for 24 hours, the number of differentially regulated genes was measured using a human genome gene chip; these microarray data demonstrated that PFOA exposure at 25 and 100 μM inhibited HNF4α function, as evidenced by changes in gene targets of HNF4α using upstream regulator analysis (Buhrke et al., 2015).

An evaluation of high-throughput screening (HTS) assay data from the ToxCast/Tox21 program provides further evidence that PFOA activates other nuclear receptors in addition to PPARα. Chiu et al. (2018) evaluated HTS data for PFOA in the context of the 10 key characteristics of carcinogens as described in Smith et al. (2016b). The assay results demonstrated PFOA activity in four ER assays (ERa, ERE, ERA_LUC, ERa_BLA), seven PPAR and PXR assays (PPARα, PPARγ, PPRE, hRRAg, PXR, PXRE, hPXR), two androgen receptor assays (rAR, AR_LUC), five enzyme assays (hBACE, hTie2, gLTB4, hORL1, hPY2), and six other assays (Nrf2, RXRb, hCYP2C9, AhR, ELG1, and TR LUC Via.) The results suggest a broad range of PFOA-induced receptor-mediated effects that were not exclusively receptor effects.

Many of the above-described nuclear receptors are known to play a role in liver homeostasis and disease and may be driving factors in the hepatotoxicity observed after PFOA exposure; however, their role in hepatic tumorigenesis is less clear. Evidence for the key events involved in the CAR activation MOA for hepatic tumors in male and female rodents exposed to PFOA is summarized in Table 3-31 and Table 3-32.

Table 3-31. Evidence of Key Events Associated with the CAR Mode of Action for Hepatic Tumors[a] in Male Rats and Mice Exposed to PFOA

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)	**CAR Activation[b]**	**Altered Gene Expression[c]**	**Hepatic Cell Proliferation**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors[d]**
1	– (7 d)	↑ (7 d)	NR	NR	NR
1.1[e]	NR	NR	↑ (16, 104 wk)	NR	– (104 wk)
1.3	NR	NR	– (104 wk)	NR	– (104 wk)
2.2[e]	NR	NR	↑ (16, 104 wk)	NR	↑ (104 wk)
3	↑ (7 d)	↑ (7 d)	NR	NR	NR
4.6[e]	NR	NR	↑ (16, 104 wk)	NR	↑ (104 wk)
5.4	NR	– (4 wk)	NR	NR	NR
10	↑ (7 d)	↑ (7 d)	NR	NR	NR
10.8	NR	– (4 wk)	NR	NR	NR
13.6	NR	NR	– (4, 12, 26, 39, 52, 65, 78, 91 wk)[f]	NR	↑ (104 wk)
14.2	NR	NR	– (104 wk)	NR	– (104 wk)
19	↑ (1, 7, 28 d)	NR	↑ (1, 7, 28 d)	NR	NR
20	– (1, 3, 5 d)	NR	NR	NR	NR
21.6	NR	– (4 wk)	NR	NR	NR
23	↑ (1, 7, 28 d)	NR	↑ (1, 7, 28 d)	NR	NR

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

Cells in bolded text and 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: Biegel et al. (2001); NTP (2020); Elcombe et al. (2010); Martin et al. (2007); Minata et al. (2010); Wen et al. (2019c) (wild-type); Rosen et al. (2008a); Rosen et al. (2008b); Rosen et al. (2017); and Butenhoff et al. (2012).

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

[b] Direct and indirect measurement of CAR induction provided CAR gene expression in Wen et al. (2019c), as *Cyp3a1*, *Cyp3a3*, and *Cyp3a9* gene expression in Martin et al. (2007), as *Cyp2b1/2, Cyp3a1*, and *Cyp4a1 g*ene expression in Elcombe et al. (2010), and as CAR gene biomarker set expression in Rosen et al. (2017).

[c] Gene expression as measured by differential expression of CAR target genes by microarray analysis (Rosen et al., 2017) or RT-PCR (Wen et al., 2019c; Rosen et al., 2008b).

[d] Hepatic tumors reflect increased incidence of adenoma (Biegel et al., 2001), and carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).

[e] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, and 80 ppm in male rats, or 1.1, 2.2, and 4.6 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

[f] Biegel et al. (2001) included timepoints at 1, 3, 6, 9, 12, 15, 18, and 21 months, which are represented in the table as 4, 12, 26, 39, 52, 65, 78, and 91 weeks, respectively.

Table 3-32. Evidence of Key Events Associated with the CAR Mode of Action for Hepatic Tumors[a] in Female Rats and Mice Exposed to PFOA

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)	**CAR Activation**	**Altered Gene Expression**	**Hepatic Cell Proliferation[b]**	**Preneoplastic Clonal Expansion**	**Hepatic Tumors**
1	NR	NR	↓ (P_0 GD 1.5–17.5)[c] – (P_0 GD 1.5–11.5)	NR	NR
1.6	NR	NR	– (104 wk)	NR	– (104 wk)
5	NR	NR	↑ (P_0 GD 1.5–11.5)[c] ↓ (P_0 GD 1.5–17.5)[c]	NR	NR
16.1	NR	NR	– (104 wk)	NR	– (104 wk)
18.2[d]	NR	NR	– (104 wk)	NR	– (104 wk)
63.4[d]	NR	NR	– (104 wk)	NR	– (104 wk)

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls unless otherwise noted; MOA = mode of action; CAR = constitutive androstane receptor; NR = not reported; P_0 = parental generation; GD = gestational day; wk = week(s).
Cells in bolded text and 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 (2020); Blake et al. (2020) (dams); and Butenhoff et al. (2012).
[a] Reviewed in Felter, et al. (2018).
[b] Proliferation as provided by number of increased mitoses in Blake et al. (2020), and liver cell proliferation or hyperplasia (no change) in NTP (2020).
[c] No statistics were reported for hepatic cell proliferation for Blake et al. (2020); thus, the arrows indicate direction of increased incidence relative to the control group per the authors' results narrative.
[d] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (300 and 1,000 ppm in female rats, or 18.2 and 63.4 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

3.5.4.2.4.3 Cytotoxicity

There is suggestive evidence that PFOA 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 parallel dose response for cytotoxicity and formation of tumors; and 6) reversibility upon cessation of exposure. As discussed above in the genotoxicity section (Section 3.5.3.1), there is little experimental evidence that PFOA can induce DNA damage, supporting the first key event of the cytotoxicity MOA. Quantitative liver histopathology is available in two studies (NTP, 2020; Butenhoff et al., 2012). Significantly increased single-cell (hepatocyte) death and necrosis in male and female was reported in Sprague-Dawley rats, with a significant dose-response trend. Evidence for the key events involved in the cytotoxicity MOA for hepatic

tumors in male and female rodents exposed to PFOA is summarized in Table 3-33 and Table 3-34.

In vitro results regarding apoptosis are variable. Wielsøe et al. (2015) observed no change in LDH release, a marker for cytotoxicity, in HepG2 cells after 24-hour exposure to PFOA doses as high as $2E^{-5}$M, while Panaretakis et al. (2001) demonstrated that PFOA exposure increased ROS generation, which led to activation of caspase-9 and induction of the apoptotic pathway in HepG2 cells.

Increased cell proliferation or markers of cell proliferation has been reported in vitro. Buhrke et al. (2013) determined that PFOA exposures of 10 μM and 25 μM for 24 hours resulted in increased proliferation of HepG2 cells. Increases in metabolic activity were also detected at 10, 25, and 50 μM exposures. Low PFOA concentrations (0.1 and 1 μM) were associated with increased expression of cell cycle regulators Cyclin D1, Cyclin E1, and Cyclin B1 whereas higher concentrations generally had no effect on these genes (except for increased expression of Cyclin E1 at 100 μM). The higher PFOA concentration of 100 μM was associated with increased expression of p53, p16, and p21 regulators (a nonsignificant increase was observed at 25 μM).

Although Wen et al. (2020) observed decreasing cell viability with increasing PFOA exposure in HepG2 cells after 48 hours of exposure (20 to 600 μM), no change in metabolic activity was observed. Wen et al. (2020) evaluated the impact of PFOA on several genes involved in cell cycle regulation, proliferation, and apoptosis and found that the expression of the *BAX* gene, a regulator of apoptosis, increased at 20, 50, and 150 μM, and decreased at 100 and 200 μM. The expression of cell cycle genes *CCNA2, CCNE1*, and *CCNB1* was altered, as was that of several genes related to cell proliferation (*CDKN1A* and *CDK4*): at lower concentrations (50 μM) of PFOA exposure, a minor increase in expression was observed, while significant decreases in expression was observed in a dose-dependent manner at concentrations >50 μM. Lipid metabolism and transport genes were also altered in the study: increased expression of lipid anabolism gene *ACSL1*, decreased expression of cholesterol synthesis enzyme gene *HMGCR*, decreased expression of fatty acid binding protein gene (*FABP1*), decreased expression *ACOX2*. There was no change in expression in the beta-oxidation acyl-CoA dehydrogenase enzyme encoding genes *ACAD11* and *ACADM.* In addition to the in vitro evidence for the key events in the cytotoxicity MOA for hepatic tumors, data from rodent studies are also available for PFOA. Histopathological and flow cytometric analyses are available for rodent studies, demonstrating hepatocyte cell death (Cope et al., 2021; NTP, 2020; Crebelli et al., 2019; NTP, 2019), increased proliferation in the presence of cell death (NTP, 2020; Loveless et al., 2008), and hyperplasia (NTP, 2020, 2019). Data are also available for increased serum enzymes related to hepatotoxicity in rodents exposed to PFOA (Cope et al., 2021; NTP, 2020; Guo et al., 2019; NTP, 2019; Yan et al., 2014; Butenhoff et al., 2012; Elcombe et al., 2010; Minata et al., 2010; Loveless et al., 2008). Evidence for the key events involved in the cytotoxicity MOA for hepatic tumors in male and female rodents exposed to PFOA is summarized in Table 3-33 and Table 3-34.

Table 3-33. Evidence of Key Events Associated with the Cytotoxicity Mode of Action for Hepatic Tumors[a] in Male Rats and Mice Exposed to PFOA

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)	**Cytotoxicity[b]**	**Serum Enzymes[c]**	**Regenerative Proliferation[d]**	**Hyperplasia and/or Preneoplastic Lesions[e]**	**Hepatic Tumors[f]**
0.08	NR	– (4 wk)	NR	NR	NR
0.10	– (F_1 GD 1.5–17.5) – (5 wk)	– (F_1 GD 1.5–17.5) – (5 wk)	NR	NR	NR
0.30	– (29 d)[g]	NR	– (29 d)[g]	– (29 d)[g]	NR
0.31	NR	– (4 wk)	NR	NR	NR
0.40	NR	– (4 wk)	NR	NR	NR
0.625	NR	↑ (4 wk)	NR	NR	NR
1.0	↑ (29 d)[g] – (F_1 GD 1.5–17.5) – (5 wk)	– (F_1 GD 1.5–17.5) – (5 wk)	– (29 d)[g]	↑ (29 d)[g]	NR
1.1[h]	↑ (16 wk) – (104 wk)	↑ (16 wk)	NR	↓ (104 wk)	– (104 wk)
1.25	NR	↑ (4 wk)	NR	NR	NR
1.3	– (104 wk)	↑ (12, 24, 52, 78 wk) – (104 wk)	NR	– (104 wk)	– (104 wk)
2.0	NR	↑ (4 wk)	NR	NR	NR
2.2[h]	↑ (16, 104 wk)	– (16 wk)	NR	↓ (104 wk)	↑ (104 wk)
2.5	NR	↑ (4 wk)	NR	NR	NR
4.6[h]	↑ (16, 104 wk)	– (16 wk)	NR	↓ (104 wk)	↑ (104 wk)
5.0	↑ (5 wk)	↑ (4 wk) ↑ (5 wk)	NR	NR	NR
5.4	NR	↑ (4 wk)	NR	NR	NR
10	↑ (29 d)[g]	↑ (4 wk)	↑ (29 d)[g]	↑ (29 d)[g]	NR
10.8	NR	↑ (4 wk)	NR	NR	NR
14.2	– (104 wk)	↑ (12, 24, 52, 78, 104 wk)	NR	– (104 wk)	– (104 wk)
15.6[h]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR
19	NR	– (1, 7, 28 d)	↑ (1, 7 d)	↑ (28 d) – (1, 7 d)	NR
20	NR	↑ (4 wk)	NR	NR	NR
21.6	NR	↑ (4 wk)	NR	NR	NR
23	NR	NR	↑ (1, 7, 28 d)	↑ (28 d) – (1, 7 d)	NR
30	↑ (29 d)[g]	NR	↑ (29 d)[g]	↑ (29 d)[g]	NR
31.7[h]	↑ (16 wk)	↑ (16 wk)	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; ↓ = statistically significant decrease in response compared with controls unless otherwise noted; MOA = mode of action; NR = not reported; wk = week(s); F_1 = first generation of offspring; GD = gestational day; d = day(s).

Cells in bolded text and 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); NTP (2020); Elcombe et al. (2010); Minata et al. (2010) (wild-type); Yan et al. (2014); Loveless et al. (2008); Crebelli et al. (2019); Guo et al. (2019); Butenhoff et al. (2012); and Cope et al. (2021) (low-fat diet only; F_1 pups exposed from GD 1.5 to 17.5, and evaluated at postnatal day (PND) 126).

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

[b] Cytotoxicity provided as increased incidence of late apoptosis/necrosis in Crebelli et al. (2019), necrosis in Butenhoff et al. (2012), and as necrosis and/or single-cell necrosis in NTP (2020) and Cope et al. (2021).

[c] Serum enzyme changes provided as changes in alkaline phosphatase (ALP), alanine transaminase (ALT), and/or aspartate transaminase (AST) in Butenhoff et al. (2012), NTP (2020), NTP (2019), and Cope et al. (2021), and as changes in ALT and/or AST in Elcombe et al. (2010), Minata et al. (2010), Guo et al. (2019), and Yan et al. (2014).

[d] Regenerative proliferation provided as increased hepatic S-phase labeling indices (%) and/or increased number of hepatocytes in Elcombe et al. (2010) and as liver proliferation in NTP (2020).

[e] Hyperplasia and/or preneoplastic lesions provided as hepatocellular hyperplasia (qualitative results) in Elcombe et al. (2010); as bile duct hyperplasia in NTP (2020); as hyperplastic nodules in Butenhoff et al. (2012); and as bile duct hyperplasia in rats and mice in Loveless et al. (2008).

[f] Hepatic tumors reflect increased incidence of carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).

[g] No statistics were reported for histopathology results for Loveless et al. (2008); thus, the arrows indicate direction of increased incidence of individual cell necrosis for Key Event (KE)1, mitotic figures for KE3, and bile duct hyperplasia for KE4 relative to the control group.

[h] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (20, 40, 80, 150, and 300 ppm in male rats, or 1.1, 2.2., 4.6, 15.6, and 31.7 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

Table 3-34. Evidence of Key Events Associated with the Cytotoxicity Mode of Action for Hepatic Tumors[a] in Female Rats and Mice Exposed to PFOA

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)	**Cytotoxicity[b]**	**Serum Enzymes[c]**	**Regenerative Proliferation[d]**	**Hyperplasia and/or Preneoplastic Lesions[e]**	**Hepatic Tumors[f]**
0.1	– (F_1 GD 1.5–17.5)	– (F_1 GD 1.5–17.5)	NR	NR	NR
1.0	– (F_1 GD 1.5–17.5, P_0 GD 1.5–11.5, P_0 GD 1.5–17.5)	– (F_1 GD 1.5–17.5, P_0 GD 1.5–11.5, P_0 GD 1.5–17.5)	NR	NR	NR
1.6	– (104 wk)	↓ (78 wk) – (12, 24, 52, 104 wk)	NR	– (104 wk)	– (104 wk)
5.0	– (P_0 GD 1.5–11.5, P_0 GD 1.5–17.5)	↑ (P_0 GD 1.5–17.5) – (P_0 GD 1.5–11.5)	NR	NR	NR
6.25	NR	↑ (4 wk)	NR	NR	NR
12.5	NR	↑ (4 wk)	NR	NR	NR
16.1	– (104 wk)	– (12, 24, 52, 78, 104 wk)	NR	– (104 wk)	– (104 wk)
18.2 g	– (16 wk, 104 wk)	– (16 wk)	– (104 wk)	– (16, 104 wk)	– (104 wk)
25	NR	↑ (4 wk)	NR	NR	NR
50	NR	↑ (4 wk)	NR	NR	NR
63.4 g	↑ (104 wk) – (16 wk)	↑ (16 wk)	– (104 wk)	– (104 wk) – (16 wk)	– (104 wk)
100	NR	↑ (4 wk)	NR	NR	NR

Notes: ↑ = statistically significant increase in response compared with controls; – = no significant response; MOA = mode of action; F_1 = first generation of offspring; GD = gestational day; NR = not reported; P_0 = parental generation; wk = week(s).
Cells in bolded text and 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); NTP (2020); Butenhoff et al. (2012); Blake et al. (2020) (dams); and Cope et al. (2021) (low-fat diet only; F_1 pups exposed from GD 1.5 to 17.5 and evaluated at postnatal day (PND) 126).
[a] Reviewed in Felter et al. (2018).
[b] Cytotoxicity provided as increased incidence of hepatic necrosis in Butenhoff et al. (2012), focal necrosis in Blake et al. (2020), and as single-cell necrosis in NTP (2020) and Cope et al. (2021).
[c] Serum enzyme changes provided as changes in alkaline phosphatase (ALP), alanine transaminase (ALT), and/or aspartate transaminase (AST) in Butenhoff et al. (2012), Blake et al. (2020), Cope et al. (2021), NTP (2020), and NTP (2019). For Butenhoff et al. (2012), only ALP was significantly decreased at 18 months (78 weeks).
[d] Regenerative proliferation provided as liver proliferation in NTP (2020).
[e] Hyperplasia and/or preneoplastic lesions provided as bile duct hyperplasia NTP (2020) and as hyperplastic nodules in Butenhoff et al. (2012).
[f] Hepatic tumors reflect increased incidence of carcinoma and/or adenoma in NTP (2020) and Butenhoff et al. (2012).
[g] NTP (2020) included perinatal (gestation and lactation) and postweaning exposures. This table reports only data from the postweaning exposures (300 and 1,000 ppm in female rats, or 18.2 and 63.4 mg/kg/day) in order to provide a representative set of the available mechanistic data involved in this MOA from bioassays, and because the treatment effects were very similar in the perinatal and postweaning exposure groups. Further study design details are in Section 3.4.4.2.1.2 and study results are in Section 3.5.2.

3.5.4.2.4.4 Genotoxicity

Evidence of PFOA genotoxicity (e.g., chromosomal aberrations, DNA breakage, micronuclei formation) is mixed, whereas most of the evidence for mutagenicity is consistently negative (Table 3-22). In an in vivo study in humans, Franken et al. (2017) observed an increase in DNA damage with increasing PFOA exposure, but the effect did not achieve statistical significance. The authors suggest that the DNA damage may have resulted from induction of oxidative stress. Additionally, Governini et al. (2015) reported that incidence of aneuploidy and diploidy was increased in PFAS-positive semen samples from nonsmokers (PFOA detected in 75% of the samples) compared with PFAS-negative samples. Of the five available animal toxicological studies that evaluated PFOA genotoxicity in vivo, only one yielded a positive result (micronuclei formation in peripheral blood cells from PFOA-exposed rats (NTP, 2019). A number of studies assessing genotoxicity of PFOA in vitro in both animal and human cell lines were reviewed. Results for chromosomal aberrations were negative for PFOA in human lymphocytes both with and without metabolic activation; results in CHO cells were mostly positive, both with and without activation, but the authors reported that the positive results were not reproducible. PFOA exposure induced DNA breakage in all in vitro DNA strand break assays that were reviewed, across three different human cell types. As noted in U.S. EPA (2016c) and Fenton et al. (2021), the clastogenic effects observed in some PFOA studies may arise from an indirect mechanism related to the physical-chemical properties of PFOA (specifically, PFOA is not subject to metabolism, it binds to proteins, it carries a net-negative electrostatic surface charge) and/or as a consequence of oxidative stress.

PFOA is non-mutagenic both with and without activation in several bacterial assays. Although three positive or equivocal results have been reported, these positive results were either exclusively at cytotoxic concentrations or were not reproducible (Table 3-22).

The available evidence suggests that PFOA is not mutagenic, but that PFOA exposure may cause DNA damage, although there is currently no known mechanistic explanation for the direct interaction between PFOA and genetic material. The available in vivo evidence suggests that

exposure to PFOA at levels resulting in cytotoxicity (e.g., hepatotoxicity, bone marrow toxicity) may lead to secondary genotoxicity in target tissues. Although unlikely, genotoxicity cannot be ruled out as a potential key event for PFOA-inducted hepatic tumor formation.

3.5.4.2.4.5 Consideration of Other Plausible Modes of Action

In addition to the evidence supporting modulation of receptor-mediated effects, and potential genotoxicity, PFOA also exhibits several other key characteristics (KCs) of carcinogens (Section 3.5.3), some of which are similarly directly evident in hepatic tissues.

For example, PFOA appears to induce oxidative stress, another KC of carcinogens, particularly in hepatic tissues (Section 3.4.1.3.7). 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.

3.5.4.2.4.5.1 Epigenetics

There is limited in vivo and in vitro evidence that PFOA induces epigenetic changes, (e.g., DNA methylation; Section 3.5.3.2) with very little liver-specific data. Two studies conducted with human cord blood reported associations between PFOA concentration and changes in DNA methylation (Miura et al., 2018; Kingsley et al., 2017), whereas an additional three studies reported no association between maternal PFOA exposure and global DNA methylation changes in the blood of the children or placenta (Ouidir et al., 2020; Leung et al., 2018; Liu et al., 2018a). Leung et al. (2018), however, did report some evidence of changes in methylation at CpG sites associated with PFOA exposure in a subset of a Faroese birth cohort with a mean cord blood PFOA concentration of 2.57 µg/L. Watkins et al. (2014) found no association between DNA methylation and PFOA in adults from the C8 Health Project.

Li et al. (2019b) observed PFOA-associated epigenetic alterations in the liver of female mouse pups following maternal exposure to PFOA. Histone acetyltransferase (HAT) levels were decreased, while histone deacetylase (HDAC) levels were increased at all dose levels. These results suggest that PFOA inhibits HAT and enhances HDAC activity, which was further demonstrated by a dose-dependent decrease in acetylation of histones H3 and H4 in the livers of PFOA-treated mice. The authors proposed that increased HDAC may activate PPARα, based upon known interactions between specific HDACs and PPARα (specifically, the class III HDAC SIRT1 deacetylates PPARα resulting in its activation), representing a regulatory role of an event included in the PPARα MOA.

In vitro studies have yielded mixed results with evidence of both hyper- and hypo-methylation of DNA in response to PFOA exposure (Section 3.5.3.2). For example, Pierozan et al. (2020) observed increased global methylation in the first daughter cell subculture of breast epithelial MCF-10A cells exposed to PFOA, although levels returned to baseline after the second passage. Two other studies found inverse relationships between global methylation and PFOA concentration in HepG2 and MCF7 cell lines (Liu and Irudayaraj, 2020 respectively; Wen et al., 2020).

3.5.4.2.4.5.2 Oxidative Stress

Results vary regarding the effect of PFOA exposure on markers of oxidative stress in in vitro and in vivo studies, both with and without a demonstrated relationship to PPARα activation.

Li et al. (2019b) observed a dose-dependent increase in 8-OHdG, as well as increases in the antioxidants catalase (CAT) and superoxide dismutase (SOD) (also indicative of oxidative stress) in the liver of female offspring of Kunming mice exposed to 1, 2.5, 5, or 10 mg/kg/day PFOA from GD 0 to GD 17, with pups sacrificed at PND 21. Serum AST and ALT levels were significantly increased in the PFOA-treated groups, indicating liver damage. Liver CAT content significantly increased in the 5 and 10 mg/kg/day dose groups. The authors propose that oxidative stress occurred through PPARα activation pathways and demonstrated changes in the mRNA level of PPARα-target genes in the same study. One such target gene is *Acox1*, which was significantly increased in livers of offspring of dams exposed to ≥2.5 mg/kg/day PFOA. Overexpression of *Acox1* has been reported to generate excess ROS, as ACOX1 is involved in fatty acid β-oxidation and produces hydrogen peroxide as a byproduct (Kim et al., 2014). This aligns with oxidative stress being proposed as a modulating factor in the PPARα-activation MOA for rodent hepatic tumors (Corton et al., 2018), as discussed above. Another study observed an increase in hydrogen peroxide in the liver of PFOA-exposed NMRI mice exposed to PFOA in utero (GD 5–9) (Salimi et al., 2019). Although they did not measure PPARα targets or PPARα itself, the type of oxidative stress observed aligns with the modulating factor in the MOA.

In contrast, Minata et al. (2010) did not observe an increase in a biomarker of oxidative stress in wild-type mice exposed to PFOA. The authors treated wild-type (129S4/SvlmJ) and *Pparα*-null (129S4/SvJae-Pparα^{tm1Gonz}/J) mice with PFOA (≤50 μmol/kg/day) for four weeks, after which no changes in 8-OHdG were observed in the wild-type mice. In contrast, a dose-dependent increase in 8-OHdG levels was observed in the *Pparα*-null mice, with a significant increase at 50 μmol/kg/day when compared with controls. The correlation between PFOA exposure and 8-OHdG was associated with increased tumor necrosis factor alpha (*TNF-α*) mRNA levels.

Takagi et al. (1991) performed a two-week subchronic (0.02% powdered PFOA in the diet) in male Fischer 344 rats and evaluated the levels of 8-OHdG in the liver and kidneys after exposure. The 8-OHdG level was significantly higher in the liver of exposed rats relative to controls, while there was no change in the kidneys, despite increased weights of both organs. Another group of rats were administered a single IP injection of PFOA (100 mg/kg) and sacrificed at days 1, 3, 5, and 8. Results were comparable to that of the dietary exposure study, with a significant increase in 8-OHdG levels in the liver (by day 1 following injection) as well as increased liver weight (by day 3).

PFOA exposure caused increases in 8-OHdG, a biomarker of oxidative stress, in human lymphoblast cells (TK6) and HepG2 hepatocytes (Yahia et al., 2016; Yao and Zhong, 2005). Peropadre et al. (2018) observed a slight elevation in 8-OHdG levels in PFOA-exposed human p53-deficient keratinocytes (HaCaT), and significantly elevated levels eight days following cessation of PFOA exposure. Several other in vitro studies reported increases in ROS in PFOA-exposed cells, including HepG2, nonhuman primate kidney, and human-hamster hybrid (AL) cells (Wielsøe et al., 2015; Zhao et al., 2011; Fernández Freire et al., 2008; Panaretakis et al., 2001). In contrast, Florentinet et al. (2011) did not observe increased ROS in HepG2 cells exposed to 5–400 μM PFOA for 24-hours, despite increased cytotoxicity at 200 μM PFOA and higher.

Some of the in vitro studies reported oxidative stress in relation to cell death and/or DNA damage. For example, Panaretakis et al. (2001) investigated ROS, mitochondrial damage, and

caspase-9 following PFOA exposure and determined that PFOA-induced apoptosis involved a ROS- and mitochondria-mediated pathway. ROS generation (H_2O_2 and superoxide anions) was detected in HepG2 cells following exposure to 200 and 400 μM PFOA. PFOA treatment also resulted in depolarization of the mitochondria and loss of mitochondrial transmembrane potential. A population of sub-G0/G2 phase of cell cycle was also observed. PFOA treatment was also associated with an increase in cells undergoing apoptotic DNA degradation. Caspase-9 activation was evident in cells exposed to 200 μM PFOA. The results of this study suggested that PFOA exposure increased ROS generation, which led to activation of caspase-9 and induction of the apoptotic pathway in HepG2 cells.

Wielsøe et al. (2015) observed a significant increase in ROS production in HepG2 cells exposed to 2.0E-7, 2.0E-6, and 2.0E-5M PFOA for 24 hours, along with a dose-dependent increase in DNA damage. Total antioxidant concentration was significantly decreased after 24 hours of exposure to all PFOA concentrations tested. This study demonstrated that genotoxic effects in vitro are the result of oxidative DNA damage following excess ROS production.

3.5.4.2.4.6 Conclusions

PFOA exposure is associated with several mechanisms that can contribute to carcinogenicity. There is robust evidence that PFOA activates PPARα and initiates downstream events that lead to hepatic tumorigenesis, including key events and modulating factors of the PPARα activator-induced MOA for rodent hepatocarcinogenesis (Corton et al., 2018; Corton et al., 2014; Klaunig et al., 2003).

Additionally, PFOA exposure is associated with several mechanisms that can contribute to carcinogenicity, including epigenetic changes and oxidative stress, which may occur in conjunction with or independently of PPARα activation. It is plausible that these mechanisms may occur independently of PPARα-dependent mechanisms. These observations are consistent with literature reviews recently published by state health agencies which concluded that the hepatotoxic effects of PFOA may not entirely depend on PPARα activation (CalEPA, 2021; Gleason et al., 2017). For example, CalEPA concluded that PFOA "can induce biological activity and hepatotoxicity that is independent of PPARα activation. This indicates that the toxicity observed in rodent studies may not act entirely through the PPARα activation pathway. As such, OEHHA cannot conclude that all hepatotoxic endpoints of PFOA and PFOS in rodents are the result of PPARα activation" (CalEPA, 2021). Similarly, NJDWQI agreed that "effects of PFOA clearly occur through both PPAR-alpha independent and PPAR-alpha dependent processes" (Gleason et al., 2017). The existence of multiple MOAs in addition to PPARα activation suggest that PFOA-induced liver cancer in rats may be more relevant to humans than previously thought. Additional research is warranted to better characterize the MOAs for PFOA-induced hepatic tumorigenesis.

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 PFOA-induced hepatic carcinogenesis, there is an absence of definitive information supporting a single, scientifically justified MOA; in fact, there is evidence supporting the potential for

multiple plausible MOAs. Therefore, EPA takes the health-protective approach and concludes that the hepatic tumors observed by Biegel et al. (2001) and NTP (2020) can be relevant to human health.

3.5.4.3 Conclusions

The available mechanistic data continue to suggest that multiple MOAs could play role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in human populations as well as animal models. The few available mechanistic studies focusing on PFOA-induced renal toxicity highlight several potential underlying mechanisms of PFOA exposure-induced renal tumorigenesis, including altered cell proliferation and apoptosis, epigenetic alterations, and oxidative stress. However, due to data limitations, it is difficult to distinguish which mechanism(s) are operative for PFOA-induced kidney cancer. Similarly for testicular cancer, the available literature highlights several potential MOAs by which PFOA exposure may result in increased incidence of LCTs in animals, though it is unclear whether these MOAs are relevant to testicular cancers associated with PFOA exposure in humans. Combined, the epidemiological and animal toxicological literature indicate that the testes are a common site of PFOA-induced tumorigenesis. Overall, the EPA concluded that the available mechanistic data suggest that multiple MOAs could play role in the renal, testicular, pancreatic, and hepatic tumorigenesis associated with PFOA exposure in studies of human populations and animal models. IARC (2016) and Zahm (2023), CalEPA (CalEPA, 2021) and NJDWQI (Gleason et al., 2017) similarly concluded that there is evidence for many potential mechanisms for PFOA-induced carcinogenicity. For example, IARC concluded there is strong mechanistic evidence of carcinogenicity in exposed humans and that PFOA is immunosuppressive, induces epigenetic alterations, induces oxidative stress, modulates receptor-mediated effects (via (PPAR) α, constitutive androstane receptor/pregnane X receptor [CAR/PXR], and PPARγ), and alters cell proliferation, cell death, and nutrient and energy supply (Zahm et al., 2023).

3.5.5 Cancer Classification

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the EPA reviewed the weight of the evidence and determined that PFOA is *Likely to Be Carcinogenic to Humans*, as "the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor *Carcinogenic to Humans.*" This determination is based on the evidence of kidney and testicular cancer in humans and LCTs, PACTs, and hepatocellular adenomas and carcinomas in rats.

The *Guidelines* (U.S. EPA, 2005a) provide examples of data that may support the *Likely to Be Carcinogenic to Humans* descriptor; the available PFOA data are consistent with the following factors:

- "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";
- "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";

- "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 PFOA has carcinogenic potential in humans and at least one animal model. A plausible, though not definitively causal, association exists between human exposure to PFOA and kidney and testicular cancers in the general population and highly exposed populations. As stated in the *Guidelines for Carcinogen Risk Assessment,* "an inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies." Two *medium* confidence independent studies provide evidence of an association between kidney cancer and elevated PFOA serum concentrations (Shearer et al., 2021; Vieira et al., 2013), while two studies in the same cohort provide evidence of an association between testicular cancer and elevated PFOA serum concentrations (Barry et al., 2013; Vieira et al., 2013). The PFOA cancer database would benefit from additional large *high* confidence cohort studies in independent populations.

The evidence of carcinogenicity in animals is based on three studies that used the same strain of rat. Taken together, these results provide evidence of increased incidence of three different tumor types (LCTs, PACTs, and hepatocellular tumors) in males administered diets contaminated with PFOA. Additionally, pancreatic acinar cell adenocarcinomas are a rare tumor type (NTP, 2020), and their occurrence in PFOA-treated animals in this study increases the confidence that this incidence is treatment-related since these tumors are unlikely to be observed in the absence of a carcinogenic agent (U.S. EPA, 2005a). The historical control incidence for pancreatic acinar cell adenocarcinomas in the female rats is 0/340 and in the male rats is 2/340, highlighting the rarity of this particular tumor type (NTP, 2020). 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 testicular cancer and animal studies reporting increased incidence of LCTs, evidence of carcinogenicity of PFOA from other cancer sites where concordance between humans and animals is not present is still relevant to the carcinogenicity determination for PFOA. See Table 3-35 below for specific rationale on how PFOA aligns with examples supporting the *Likely to Be Carcinogenic to Humans* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Table 3-35. Comparison of the PFOA Carcinogenicity Database with the *Likely* Cancer Descriptor as Described 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).	**PFOA data are consistent with this description.** Epidemiological evidence supports a plausible association between exposure and cancer, though there are uncertainties regarding the MOAs for tumor types observed in humans. There is supporting experimental evidence, including carcinogenicity data from animal experiments.

Likely to Be Carcinogenic to Humans	
"An agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans" (U.S. EPA, 2005a).	**PFOA data are consistent with this description.** PFOA has tested positive in one species (rat), both sexes, and multiple sites (liver, pancreas, testes, uterus). There is also evidence of carcinogenicity in humans.
"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 PFOA.** The report by NTP (2020) does not indicate that perinatal exposure exacerbates the carcinogenic potential of PFOA.
"A rare animal tumor response in a single experiment that is assumed to be relevant to humans" (U.S. EPA, 2005a).	**PFOA data are consistent with this description.** The pancreatic adenocarcinomas observed in multiple male dose groups are a rare tumor type in this strain (NTP, 2020).
"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).	**PFOA data are consistent with this description.** Multiple positive tumor studies in the same strain of rat are supported by plausible associations between human exposure and kidney and testicular cancer.

Notes: DNA = deoxyribonucleic acid; MOA = mode of action.

EPA recognizes that other state and international health agencies have recently classified PFOA as carcinogenic to humans (IARC as reported in Zahm et al., 2023; 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's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)" and, similarly, IARC's classification criteria are not identical to the EPA's guidelines (IARC, 2019). Rationale for why PFOA does not meet the Carcinogenic to Humans descriptor according to the 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 perfluorooctanoic acid (PFOA) exposure can result in adverse health effects across a range of health outcomes. In response to recommendations made by the SAB and the conclusions from EPA's systematic review of the available health effects evidence, presented in the EPA's preliminary analysis, the 2021 SAB review draft *Proposed Approaches to the Derivation of a Draft Maximum Contaminant Level Goal for Perfluorooctanoic Acid (PFOA) in Drinking Water* (U.S. EPA, 2021c), 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 outcomes with evidence *demonstrates* or evidence *indicates* integration judgments, based on the synthesis of the available human, animal, and mechanistic evidence (Section 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 PFOA, 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. Health outcomes for which the results were *suggestive* are discussed in the evidence profile tables provided in Appendix C (U.S. EPA, 2024a), as well as Section 5.5.

In the previous section describing the hazard judgment decisions (Section 3.4 and 3.5), EPA qualitatively considered *high, medium,* and sometimes *low* confidence studies of PFOA 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 PFOA. 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, etc.) for POD derivation, analyzed the outcome of interest in the general population or susceptible population, and demonstrated a larger number of the study attributes outlined above were preferred. If available, *high* and *medium* confidence studies with exposures levels within or near the range of typical environmental human exposures, especially exposure levels comparable to human exposure levels in the general United States population, 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 PFOA 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 PFOA HESD (U.S. EPA, 2016c).

4.1.1.1 Hepatic Effects

As reviewed in Section 3.4.1.4, *evidence indicates* that elevated exposures to PFOA are associated with hepatic effects in humans. As described in Table 3-4, the majority of epidemiological studies assessed endpoints related to serum biomarkers of hepatic injury (18 *medium* confidence studies), while fewer studies reported on liver disease or injury (5 *medium* confidence studies) and other serum markers of liver function (4 *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 of general population adults from the updated literature searches reported

positive associations between PFOA serum concentrations and ALT, three of which reported statistically significant responses (Jain, 2019; Nian et al., 2019; Salihovic et al., 2018; Darrow et al., 2016; Gleason et al., 2015). These more recently published studies provided additional evidence for increased ALT in adults beyond the three *medium* confidence studies reporting positive associations for ALT from the 2016 PFOA 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 and 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 toxicological 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 study findings 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 status without stratifying by exposure level, which were not amenable to modeling (Jain, 2019).

Exclusions of these studies resulted in the consideration of four *medium* confidence studies for POD derivation (Nian et al., 2019; Darrow et al., 2016; 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, the two largest studies of PFOA and ALT are Gallo et al. (2012) and Darrow et al. (2016), both 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 of increased ALT across deciles of PFOA exposure and Darrow et al. (2016) provided an exposure-response gradient for PFOA. 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 (1,605) 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 PFOA. 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 PFOA and these associations remained after accounting for other PFAS in the regression models. However, several methodological limitations precluded its use for POD derivation. Limitations include lack of clarity about the base of logarithmic transformation applied to PFOA 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, three *medium* confidence epidemiological studies were prioritized for POD derivation(Nian et al., 2019; Darrow et al., 2016; Gallo et al., 2012) (Table 4-1).

Liver toxicity results reported in animal toxicological studies after PFOA exposure are concordant with the observed increased ALT indicative of hepatic damage observed in epidemiological studies. Specifically, studies in rodents found that oral PFOA treatment resulted in increased relative liver weight (17/20 *high* and *medium* confidence studies), biologically significant alterations in levels of at least one serum biomarker of liver injury (i.e., ALT, AST, and ALP) (6/9 *high* and *medium* confidence studies), and evidence of histopathological alterations including hepatocyte degenerative or necrotic changes (12/12 *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 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 PFOA-induced hepatic effects are relevant to humans.

For animal toxicological hepatic endpoints, EPA preferred studies reporting quantitative biologically or statistically significant 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 seven studies that quantitatively reported incidences of hepatic cell or tissue death or necrosis, five were excluded; two studies were excluded because they did not report statistically or biologically significant responses (Butenhoff et al., 2012; Perkins et al., 2004) and three were excluded because they had relatively small sample sizes (i.e., $n \leq 10$) (Cope et al., 2021; Blake et al., 2020; NTP, 2019). After these exclusions, EPA identified two studies reporting adverse liver effects in male rodents due to PFOA exposure, NTP (2020), a chronic dietary study in Sprague-Dawley rats (see study design details in Section 3.4.4.2.1.2), and Loveless et al. (2008), a 29-day gavage dosing study in CD-1 mice, for POD derivation (Table 4-1). NTP (2020) conducted histopathological examinations of liver tissue in male rats and reported dose-dependent increases in the incidence of hepatocellular single cell death and hepatocellular necrosis. As this is one of the few available chronic PFOA toxicity studies that presented a large sample size ($n = 50$), numerous and relatively low dose levels, and assessment of a suite of hepatic endpoints, both the single cell death and necrosis endpoints in males from the 107-week time point were considered for derivation of PODs. Similar to the NTP study (2020), Loveless et al. (2008) reported a number of hepatotoxic effects, a low dose range, relatively large sample sizes ($n = 19–20$), and dose-dependent increases in histopathological outcomes indicating adverse effects in male mice gavaged with PFOA for 29 days. In addition, Loveless et al., (2008, 988599) was the only study in mice to report quantitative histopathological examinations of liver tissue in non-pregnant adults and had the longest exposure duration of the available mouse studies. Therefore, the incidences of two endpoints, focal cell necrosis and individual cell necrosis, in male mice from Loveless et al. (2008) were also considered for the derivation of PODs.

4.1.1.2 Immunological Effects

As reviewed in Section 3.4.2.4, *evidence indicates* that elevated exposures to PFOA are associated with immunological effects in humans. As described in Table 3-9, the majority of epidemiological studies assessed endpoints related to immunosuppression (1 *high* and 20 *medium* confidence studies) and immune hypersensitivity (1 *high* and 20 *medium* confidence studies), while 2 *medium* confidence studies 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 PFOA exposure and altered immune function.

Evidence of immunosuppression in children associated with exposure to PFOA reported by epidemiological studies was consistent across studies and endpoints. Specifically, epidemiological studies reported associations between PFOA exposure and reduced humoral immune response to routine childhood immunizations, including lower levels of tetanus and diphtheria (Timmermann et al., 2021; Abraham et al., 2020; Budtz-Jørgensen and Grandjean, 2018; Grandjean et al., 2012), HiB (Abraham et al., 2020), 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 PFOA 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 PFOA.

As noted by Dewitt et al. (2019; 2017; 2016a) 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. Only one *low* confidence study reported findings on antibody response to Hib (Abraham et al., 2020), which was excluded from POD derivation because of the limited evidence and the *low* confidence rating. Three studies (Zhang et al., 2023; Pilkerton et al., 2018; Stein et al., 2016b) reported on antibody response to rubella in adolescents and one study reported on antibody response in young children (Granum et al., 2013). From the adolescent studies, one study observed decreased rubella antibody response in a specific subpopulation of only seropositive adolescents (Stein et al., 2016b) and the other two studies did not report statistically significant associations between PFOA and rubella antibody response in the overall population (Zhang et al., 2023; Pilkerton et al., 2018). Granum et al. (2013) reported a statistically significant association between PFOA exposure and rubella antibody response in young children. Because studies reporting rubella antibody response were mixed (2/4 demonstrating associations), rubella studies were not further considered for POD derivation. Overall, EPA prioritized studies reporting responses to tetanus and diphtheria because the responses were consistently observed across a large number of studies (*medium* and *low* confidence) in children from multiple populations for these two vaccine types.

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 and PFOA exposure in the same cohort (i.e., 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. One *medium* confidence cross-sectional study (Timmermann et al., 2021) reported on tetanus and diphtheria antibody response and PFOA exposure in Greenlandic children. This study was also prioritized for POD derivation. The results from the Faroe Island and Greenlandic populations are qualitatively

supported by a *low* confidence cross-sectional study of associations between diphtheria and tetanus antibody responses and PFOA in German children (Abraham et al., 2020).

In total, two *medium* confidence epidemiologic studies that reported decreased antibody responses in children exposed to PFOA (Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018) were considered for POD derivation (Table 4-1). These two epidemiological studies report data characterizing antibody responses to vaccinations in children using a variety of PFOA exposure measures across various populations and vaccinations. Budtz-Jørgensen and Grandjean (2018) investigated anti-tetanus and anti-diphtheria responses in Faroese children aged 5–7 and PFOA exposure measured at age 5 or prenatally; Timmerman et al. (2021) investigated anti-tetanus and anti-diphtheria responses and PFOA exposure in Greenlandic children aged 7–12. Both studies examined antibody responses associated with PFOA exposure in well-characterized cohorts, and in the case of Budtz-Jørgensen and Grandjean (2018), multiple prior publications supported the finding of an inverse relationship between PFOA exposure concentrations and antibody response in the same study cohorts.

Immunotoxicity results reported in animal toxicological studies in adult rodents are concordant with the immunosuppression observed in epidemiological studies. Specifically, studies in rodents found that oral PFOA treatment resulted in reduced immune response (i.e., reduced globulin and immunoglobulin levels upon immune challenges) (four *medium* confidence studies) and altered immune cell populations (e.g., altered white blood cell counts, altered splenic and thymic cellularity) (one *high* and four *medium* confidence studies). Immunosuppression evidenced by functional assessments of the immune responses, such as analyses of globulin and immunoglobulin levels after challenges, are comparable and thus, supportive of the immunosuppression reported as decreased antibody responses seen in human populations and were therefore prioritized for quantitative assessment. Additionally, EPA identified immunosuppressive effects in multiple species and both sexes of animal toxicological studies, further supporting the selection of these endpoints for dose-response analyses. Animal toxicological studies assessing alterations in immune cell populations were not considered further as there were a limited number of studies assessing specific endpoints of interest. Although the other reported immune effects, such as altered organ weights and histopathology, are consistent with evidence indicating alterations in immune function and response from animal toxicological studies, they were not considered for POD derivation as these effects may be confounded by changes in body weight, effects were not consistent across studies, and/or a limited number of studies assessed specific outcomes. Of the four *medium* confidence studies reporting impaired IgM response in mice, EPA selected Dewitt et al. (2008), a 15-day drinking water exposure study in female mice, and Loveless et al. (2008), a 29-day study in male mice, for POD derivation as these two studies presented data for a larger number of dose groups spanning a broader dose range than either Dewitt et al. (2016b) or De Guise et al. (2021).

4.1.1.3 Cardiovascular Effects

As reviewed in Section 3.4.3.4, *evidence indicates* that exposure to PFOA are associated with cardiovascular effects in humans. As described in Table 3-12, the majority of epidemiological studies assessed endpoints related to serum lipids (2 *high*, 27 *medium*, and 19 *mixed*[18] confidence

[18] *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.

studies) and blood pressure and hypertension (2 *high* and 18 *medium* confidence studies), while some studies also reported on cardiovascular disease (1 *high* and 6 *medium* confidence studies) and atherosclerosis (1 *high* and 3 *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 consistent evidence of associations between PFOA exposure and continuous measures of blood pressure and risk of hypertension in adults from the general population, including adults living in high-exposure communities located near PFAS manufacturing plants, which qualitatively support an association between PFOA and cardiovascular effects in humans.

The majority of studies in adults in the general population, including high-exposure communities, reported positive associations between PFOA serum concentrations and serum lipids. Studies in adults were prioritized based on reported age-dependent fluctuations in serum lipids as a result of puberty (Daniels et al., 2008), which may impact the consistency of results from studies in children. Specifically, *medium* confidence epidemiological studies in adults reported positive associations between PFOA exposure and total cholesterol (TC) (15/18 studies) and low-density lipoprotein (LDL) (12/17 studies). Of these two endpoints, EPA selected TC for quantitative assessments because the association was the most consistently observed in adults and the 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 PFOA 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 PFOA exposure disrupts lipid metabolism, suggests that toxic disruptions of lipid metabolism by PFOA 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 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).

A total of 15 *medium* confidence studies (Canova et al., 2020; Fan et al., 2020; Lin et al., 2020e; Dong et al., 2019; Jain and Ducatman, 2019b; Lin et al., 2019; Liu et al., 2018d; Winquist and Steenland, 2014; Eriksen et al., 2013; Fitz-Simon et al., 2013; Nelson et al., 2010; Costa et al., 2009; Steenland et al., 2009; Sakr et al., 2007a; Olsen et al., 2003) reported positive associations between exposure to PFOA and total cholesterol in adults from the general population. One study (Winquist and Steenland, 2014) was excluded from POD derivation because the study estimated the risk of levels above clinical thresholds for TC and these data were not amenable to modeling continuous changes in TC. Three studies were excluded from POD derivation because they were in occupationally exposed adult populations only and would not represent typical exposure scenarios for human environmental exposure (Costa et al., 2009; Sakr et al., 2007a; Olsen et al., 2003). Three studies (Canova et al., 2020; Lin et al., 2020e; 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, 40–60 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 from POD derivation because the study reported findings stratified by BMI status without stratification by exposure.

Although the positive associations between PFOA and TC were supported by the findings of 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 PFOA 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 PFOA 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). 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 PFOA 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 describe a variety of PFOA exposure measures across various adult 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), Steenland et al. (2009) investigated effects in a high-exposure community (the C8 Health Project study population), and Lin et al. (2019) collected data from prediabetic adults from the DPP and DPPOS study (1996–1999).

Though results reported in animal toxicological studies support the alterations in lipid metabolism associated with PFOA exposure observed in epidemiological studies, there are species differences in the direction of effect with increasing 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 provide an increased understanding of the observed non-monotonicity of serum lipid levels and decreased serum lipid levels at higher dose levels in rodents (Section 3.4.3.2). 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 PFOA are associated with developmental effects in humans. As described in Table 3-15, the majority of epidemiological studies assessed endpoints related to fetal growth restriction (26 *high* and 25 *medium* confidence studies) and gestational duration (13 *high* and 13 *medium* confidence studies), while fewer studies reported on endpoints related to fetal loss (2 *high* and 6 *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 (2/4), EPA did not prioritize this endpoint for POD derivation as there were a relatively limited number of studies compared with endpoints related to gestational duration and fetal growth restriction and results from the *high* confidence studies were mixed. The impacts observed on fetal loss are supportive of an association between PFOA exposure and adverse developmental effects.

Approximately half of the available studies reporting metrics of gestational duration observed increased risk associated with PFOA exposure, including among *high* confidence studies. Six of the 14 *medium* or *high* confidence studies reported inverse associations for gestational age at birth and 5 of the 11 *medium* or *high* confidence studies reported an increased risk of preterm birth. Gestational age was not prioritized for quantitative analyses because the majority of studies did not report inverse associations and this endpoint is more prone to measurement error (see Section 3.4.4.1.2). There were generally more consistent findings showing positive associations between PFOA exposure and preterm birth, particularly from the *high* confidence studies. However, there were some concerns with sample timing and potential influence of pregnancy hemodynamics on the observed outcomes, as the majority of studies reporting increased odds of preterm birth sampled PFOA concentrations later in pregnancy. While overall there appears to be some associations between PFOA exposure and gestational duration, the inconsistencies in the database and lack of studies sampling in the first trimester of pregnancy resulted in this effect not being considered for POD derivation. Additionally, the database for fetal growth restriction was

both larger and consisted of more *medium* and *high* confidence studies. Therefore, studies demonstrating fetal growth restriction were prioritized for POD derivation.

The majority of *high* and *medium* confidence epidemiological studies (17/25) reported associations between PFOA and decreased mean birth weight in infants. Studies on changes in standardized birth weight measures (i.e., z-scores) also reported some inverse associations in *high* and *medium* confidence studies. Endpoints characterizing fetal growth restriction were included for POD derivation because 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 irreversible adverse health outcomes at various later life stages. 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 as reported in Yang 2022, 10176603; Osmond and Barker, 2000). 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 that evaluated 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 PFOA and mean birth weight (Gardener et al., 2021; Luo et al., 2021; Yao et al., 2021; Chu 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; Govarts et al., 2016; Whitworth et al., 2012) were considered for POD derivation. Three studies (Gardener et al., 2021; 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 because they used non-preferred exposure measures such as infant whole blood samples from a heel stick and postpartum maternal exposure samples, which are prone to exposure misclassification. Four studies (Lauritzen et al., 2017; Lind et al., 2017a; Manzano-Salgado et al., 2017a; Valvi et al., 2017) were not considered for POD derivation because of inconsistencies in associations by sex or study location with no clear biological explanation for the inconsistency.

As a result of these exclusions, the 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; Govarts et al., 2016) were considered for POD derivation (Table 4-1). The candidate epidemiological studies described a variety of PFOA exposure measures across both fetal and neonatal developmental windows. 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. Yao et al. (2021) was not further considered because the PFOA exposure concentrations in this cohort were considerably higher than typical human environmental exposure levels and the exposure median in this study was at least 10 times higher than the other studies considered. Two of the six studies examined PFOA levels primarily during trimester one (Sagiv et al., 2018 Wikström, 2020, 6311677) and one during trimesters two and three (Starling et al., 2017). Two studies examined PFOA collected within days of birth (Chu et al., 2020; Govarts et al., 2016). Wikström et al. (2020) reported associations between PFOA levels and decreased birth weight in 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 on first trimester PFOA samples

collected between 1999–2002 in a Project Viva birth cohort in the U.S. Chu et al. (2020) reported inverse associations between maternal PFOA collected within three days of delivery and birth weight in the Chinese Guangzhou Birth Cohort Study (2013). Starling et al. (2017) reported associations between PFOA collected in later pregnancy (range: 20 to 34 weeks gestational age) and decreased birth weight in the Healthy Start prospective cohort in Colorado (2009–2014). Govarts et al. (2016) reported a modest inverse association between PFOA in cord blood and birth weight in the Flemish Human Environment Health Survey II (FLEHS II) cohort (2008–2009).

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 PFOA exposure resulted in reduced offspring weight (8/11 studies; 2 *high* and 6 *medium* confidence), decreased offspring survival (6/9 studies; 1 *high* and 5 *medium* confidence), developmental delays (2/2 studies; both *medium* confidence), physical abnormalities (2/2 studies; both *medium* confidence) and altered placental parameters (2/2 studies; both *medium* confidence). Some of the 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 toxicological studies with effects in offspring, as opposed to placental or maternal effects, because these effects provide concordance with the approximate timing of decreased birth weight observed in human infants. Though several studies measured pregnant dam weight or dam weight at birth, there were inconsistencies in results across the database, with some studies reporting decreased maternal weight, some reporting no effect, and some reporting increased maternal weight as a result of PFOA exposure. These inconsistencies may stem from the potential confounding effect of reduced offspring weight observed in those same studies. 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 detect developmental effects. Multiple animal toxicological studies observed effects at low dose levels and demonstrated a dose-related response in decreased offspring weight, decreased pup survival, and developmental delays. Therefore, these endpoints were prioritized for dose-response analysis.

Numerous studies in both rats and mice reported decreased offspring body weight after gestational PFOA exposure. Reduced fetal body weight was consistently observed, with 5/5 studies in mice reporting this effect (Blake et al., 2020; Li et al., 2018a; Suh et al., 2011; Yahia et al., 2010; Lau et al., 2006). Reduced pup body weight was also consistently observed; the majority of the available studies (10/13) reported this effect, two of which were *high* confidence studies in rats (NTP, 2020; Butenhoff et al., 2004a), indicating consistency across species. EPA selected both reduced pup and fetal weights because the timing is concordant with the endpoint of decreased infant birth weight prioritized for POD derivation from the human epidemiological

studies and also represents two different developmental stages (i.e., fetus and pup) across the sensitive perinatal period of development.

Of the five studies reporting decreased fetal body weight in mice, results from Li et al. (2018a) were selected for POD derivation because the exposure duration encompassed the majority of gestation, the study used a relatively large number of dose groups, and the effect was observed in multiple dose groups. The two *high* confidence rat studies reporting reduced pup weight were not selected for POD derivation due to study design limitations, including the use of relatively high dose levels, and non-monotonic responses, although they provide qualitative support for this effect in mice. Of the eight studies reporting reduced pup body weight in mice (Song et al., 2018; Hu et al., 2012; White et al., 2011; Hu et al., 2010; Yahia et al., 2010; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006), decreased pup weight relative to litter at PND 22 as reported by Lau et al. (2006) was ultimately selected for POD derivation because this study reported results as pup weight averaged by litter rather than individual pups, used an exposure duration that spanned the majority of gestation, used a larger number of dose groups than the other studies, and reported the effect in multiple dose groups.

In addition to effects on offspring weight, six studies in mice (Song et al., 2018; White et al., 2011; Yahia et al., 2010; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006) reported alterations in pup survival after gestational exposure to PFOA. Pup survival was selected over fetal survival because the metrics used to determine fetal mortality varied (e.g., reported as prenatal loss, litter loss, resorption, reduced fetal survival) and difficult to directly compare, Additionally, pup survival provides concordance with the timing of the effect of decreased infant birth weight in humans. Among the six available studies examining pup survival, Abbott et al. (2007) was determined to be *low* confidence for this endpoint and was therefore excluded for quantitative assessment. EPA selected results from Song et al. (2018) (PND 21) and Lau et al. (2006) (PND 0 and 23) for POD derivation because this study presented data for a larger number of treatment groups spanning broader or lower dose ranges as compared with Wolf et al. (2007), White et al. (2011), and Yahia et al. (2010).

Three studies in mice (Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006) reported developmental delays, specifically delayed eye opening, as a result of gestational PFOA exposure. Abbott et al. (2007) was not further considered for POD derivation due to the extensive litter loss in dose groups greater than 1 mg/kg/day and the effect was only observed in that dose group, limiting the available dose-response range as compared to Lau et al. (2006) and Wolf et al. (2007). EPA selected results from Lau et al. (2006) over Wolf et al. (2007) for POD derivation because Lau et al. (2006) presented data for a larger number of dose groups spanning a greater dose range. Additionally, Lau et al. (2006) reported the effect in multiple dose groups.

Table 4-1 summarizes the studies and endpoints considered for POD derivation.

Table 4-1. Summary of Observed Endpoints in Humans and Rodent Studies Considered for Dose-Response Modeling and Derivation of Points of Departure

Endpoint	Reference, Confidence	Strain/Species/ Sex	POD Derived?	Justification
Immune Effects				
Reduced Antibody Concentrations for Diphtheria and Tetanus	Budtz-Jørgensen and Grandjean (2018)[a] *Medium* Timmerman et al. (2021) *Medium*	Human (male and female children)	Yes	Decreases in antibody responses to pathogens such as diphtheria and tetanus were observed at multiple ages during childhood, associated with both prenatal and childhood PFOA exposure levels. Effect was large in magnitude and generally coherent with epidemiological evidence for other antibody effects. Effects were observed in multiple populations and are supported by studies of other vaccine types (e.g., rubella (Granum et al., 2013)).
Reduced immunoglobulin M (IgM) Response	Loveless et al. (2008) *Medium* DeWitt et al. (2008) *Medium*	C57BL/6N mice (adult females), Crl:CD-1(ICR)BR mice (adult males)	Yes	Functional assessment indicative of immunosuppression. Immune effects were consistently observed across multiple studies including reduced spleen and thymus weights, altered immune cell populations, and decreased splenic and thymic cellularity. Reduced IgM response is coherent with epidemiological evidence of reduced immune response to vaccinations.
Developmental Effects				
Decreased Birth Weight	Chu et al. (2020) *High* Govarts et al. (2016) *High* Sagiv et al. (2018) *High* Starling et al. (2017) *High* Wikström et al. (2020) *High*	Human (male and female infants)	Yes	Evidence for developmental effects is based on consistent inverse effects for FGR including birth weight 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.
Decreased Birth Weight	Yao et al. (2021) *High*	Human (male and female infants)	No	Effect was supportive of epidemiological evidence for this effect, but the exposure median in this study was at least 10 times higher than the other studies considered (see Appendix D, (U.S. EPA, 2024a)).
Decreased Pup Survival	Song et al. (2018) *Medium* Lau et al. (2006) *Medium*	Kunming mice (F_1 males and females, PND 21)	Yes	Effects on pre- and postnatal offspring survival were consistently observed across multiple studies and species. Decreased pup survival was reported in six studies and three strains of mice (Song et al., 2018; White et al., 2011; Yahia et al., 2010; Abbott et al., 2007; Wolf et al., 2007; Lau et al., 2006) and is

Endpoint	Reference, Confidence	Strain/Species/ Sex	POD Derived?	Justification
		CD-1 mice (F_1 males and females, PND 0 and PND 23)		coherent timing of the critical effect selected in humans (i.e., decreased birth weight in infants). This critical effect is supported by observations of prenatal loss, litter loss/resorption, reduced fetal survival, and increased postweaning mortality observed in mice and rats. Song et al. (2018) and Lau et al. (2006) were selected for POD derivation because they reported data for a larger number of dose groups and tested lower or broader dose ranges than the other four studies reporting this effect.
Decreased Fetal Body Weight	Li et al. (2018a) *Medium*	Kunming mice (F_1 males and females, GD 18)	Yes	Effects on pre- and postnatal offspring weight were consistently observed across multiple studies and species. Decreased fetal weight was observed in 5/5 studies in mice and is supported by reduced pup weight observed in studies of mice and rats. Li et al. (2018a) was selected for POD derivation because the study tested a relatively large number of dose groups and had decreased variability compared with the other four studies. Note that decreases in maternal body weight were also considered for POD derivation but was not a selected endpoint because the decreased fetal body weight could be a potential confounder and was found to be a more sensitive effect.
Decreased Pup Body Weight (relative to litter)	Lau et al. (2006) *Medium*	CD-1 mice (F_1 males and females, PND 22)	Yes	Effects on pre- and postnatal offspring weight were consistently observed across multiple studies and species. Decreased pup weight was observed in nine studies across two species and is supported by reduced fetal weight reported by five studies in mice. Reduced pup weight at PND 22 reported by Lau et al. (2006) was selected for POD derivation because the study reported pup weight relative to litter, tested a relatively large number of dose groups compared with the other six studies in mice, and reported the effect in multiple dose groups.
Delayed Time to Eye Opening	Lau et al. (2006) *Medium*	CD-1 mice (F_1 males and females, PND 14 – PND 18)	Yes	Effect also observed in Wolf et al. (2007) and Abbott et al. (2007). Lau et al. (2006) was selected for dose-response because this study reported dose response information across a larger number of dose groups (5) and a relatively low dose range (1, 3, 5, 10 and 20 mg/kg/day).
		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 PFOA and blood pressure.

Endpoint	Reference, Confidence	Strain/Species/ Sex	POD Derived?	Justification
Hepatic Effects				
Increased ALT	Gallo et al. (2012) *Medium* Darrow et al. (2016)[b] *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 including the C8 Health Project (Darrow et al., 2016; Gallo et al., 2012) and Isomers of C8 Health Project in China (Nian et al., 2019).
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 PFOA 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.
Necrosis (focal, individual cell, both) in the Liver	Loveless et al. (2008) *Medium* NTP (2020) *High*	Crl:CD-1(ICR)BR mice (adult males), Sprague-Dawley rats (adult males)	Yes	Effect was accompanied in both studies by other liver lesions including cytoplasmic alteration and apoptosis. Necrotic liver cells were also observed in male mice in Crebelli et al. (2019) and pregnant dams in Blake et al. (2020). Effect is further supported by changes in serum ALT levels in animals and humans. Data from females were not considered for POD derivation as they appear to be less sensitive, potentially due to toxicokinetic differences between the sexes in rats.

Notes: ALT = alanine transaminase; BMD = benchmark dose; F1 = first generation; NHANES = National Health and Nutrition Examination Survey; POD = point of departure.
[a] Supported by Grandjean et al. (2012), Grandjean et al. (2017a), and 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 (PODs) 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 PFOA-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 (e.g., mortality, infertility). 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 4.2 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 PFOA 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 U.S. EPA (2012a)) to estimate the probability of an individual with an adverse serum ALT level as a function of PFOA 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% extra risk BMR for comparison to the selected 5% BMR in Appendix E (U.S. EPA, 2024a), as recommended by agency guidance (U.S. EPA, 2012a).

For the adverse effects of single cell and focal liver necrosis observed in adult rats following PFOA 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 and tetanus antibody response in children associated with PFOA 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) and Timmerman et al. (2021) measured antibody concentrations in childhood and PFOA 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. As such, EPA determined that there is no clear definition of an adverse effect threshold for the endpoints of reduced tetanus or diphtheria antibody concentrations in children.

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 $\log_2$-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 effect of reduced IgM response observed in animal toxicological studies, there is currently inadequate available biological or toxicological information to permit determination of a minimal biologically significant response level. In accordance with recommendations in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) for continuous data in adult animal models with no known biologically significant response level, a BMR of 1 SD change from the control mean was employed (see Table 4-2).

4.1.2.3 Cardiovascular Effects

For the cardiovascular endpoint of increased serum TC in adults associated with PFOA 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 U.S. EPA (2012a)) to estimate the probability of an individual with an adverse TC level as a function of PFOA 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 PFOA 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 PFOA 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 delayed time to eye opening and decreased pup survival observed in animal studies, a BMR of 0.5 SD from the control was employed (see Table 4-2). For decreased fetal and pup weights observed in animal studies, a BMR of 5% relative deviation was employed. These BMR selections 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 (i.e., fetal and pup 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 or pup body weight, a BMR of 0.1 SD for the frank effects of decreased fetal or pup survival, and a BMR of 1 SD for delayed time to eye opening for comparison purposes, based on severity of the endpoints, as recommended by agency guidance (U.S. EPA, 2012a) (see Appendix E, (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 and tetanus in children	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

Endpoint	BMR	Rationale
		cases where there is no accepted definition of an adverse level of change or clinical cutoff for the health outcome (U.S. EPA, 2012a)
Reduced immunoglobulin M (IgM) response	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)
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)
Delayed Time to Eye Opening	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

Endpoint	BMR	Rationale
	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, 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
Single Cell and Focal Liver 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 PFOA 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 F, (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 PFOA serum concentrations following single dose exposure to animals. Training set data demonstrated an MSLE of 0.40 for PFOA. For test set data, the MSLE was 1.4 for PFOA. As evidenced in the supplementary code, the discrepancy in model predictions for test set data is driven by higher animal PFOA doses that were outside the scope of the original model calibration. 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 PFOA.

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 PFOA 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 in the subsections 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 PFOA

Parameter	Units	CD1 Mouse (F)[a]	C57BL/6 Mouse (F)[a]	Sprague-Dawley Rat (F)[a]	Sprague-Dawley Rat (M)[a]	Cynomolgus Monkey (M/F)[a]
Body Weight[b] (BW)	kg	0.02	0.02	0.20 (0.16–0.23)	0.24 (0.21–0.28)	7 (M), 4.5 (F)
Cardiac Output[c] (Q_{cc})	L/h/kg$^{0.74}$	8.68	8.68	12.39	12.39	19.8
Absorption Rate (k_a)	1/h	290 (0.6–73,000)	340 (0.53–69,000)	1.7 (1.1–3.1)	1.1 (0.83–1.3)	230 (0.27–73,000)
Central Compartment Volume (V_{cc})	L/kg	0.18 (0.16–2.0)	0.17 (0.13–2.3)	0.14 (0.11–0.17)	0.15 (0.13–0.16)	0.4 (0.29–0.55)
Intercompartment Transfer Rate (k_{12})	1/h	0.012 ($3.1 \times e^{-10}$ – 38,000)	0.35 (0.058–52)	0.098 (0.039–0.27)	0.028 (0.0096–0.08)	0.0011 ($2.4 \times e^{-10}$ – 35,000)
Intercompartment Ratio ($R_{V2:V21}$)	Unitless	1.07 (0.26–5.84)	53 (11–97)	9.2 (3.4–28)	8.4 (3.1–23)	0.98 (0.25–3.8)
Maximum Resorption Rate (T_{maxc})	µmol/h	4.91 (1.75–2.96)	2.7 (0.95–22)	1.1 (0.25–9.6)	190 (5.5–50,000)	3.9 (0.65–9,700)
Renal Resorption Affinity (K_T)	µmol	0.037 (0.0057–0.17)	0.12 (0.033–0.24)	1.1 (0.27–4.5)	0.092 ($3.4 \times e^{-4}$ – 1.6)	0.043 ($4.3 \times e^{-5}$ – 0.29)
Free Fraction	Unitless	0.011 (0.0026–0.051)	0.034 (0.014–0.17)	0.086 (0.031–0.23)	0.08 (0.03–0.22)	0.01 (0.0026–0.038)
Filtrate Flow Rate (Q_{filc})	Unitless	0.077 (0.015–0.58)	0.017 (0.01–0.081)	0.039 (0.014–0.13)	0.22 (0.011–58)	0.15 (0.02–24)
Filtrate Volume (V_{filc})	L/kg	0.00097 ($3.34 \times e^{-9}$ – 7.21)	$7.6 \times e^{-5}$ ($2.7 \times e^{-10}$ – 6.4)	$2.6 \times e^{-5}$ ($2.9 \times e^{-10}$ – 28)	0.0082 ($1.3 \times e^{-8}$ – 7.6)	0.0021 ($3.3 \times e^{-9}$ – 6.9)

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 CD1 mouse were from Lou et al. (2009), for the C57BL/6 mouse were from DeWitt et al. (2008), for the rat were from Kemper (2003), and for the monkey from Butenhoff et al. (2004b).

[b] Estimated average body weight for species used except with Kemper (2003) where individual rat weights were available and assumed to be constant.

[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 PFOA concentration-time data in the species of interest, EPA compared model fits to in vivo datasets either not considered in or published after the 2016 PFOA HESD (Table 4-4). For rats, this included Kudo et al. (2002), Kim et al. (2016), and Dzierlenga et al. (2019a). Model simulations demonstrated good agreement with available data for adult time-course PFOA PK predictions in the rat. For mice however, only one adult PFOA study was available for comparison (Fujii et al., 2015) and that study only tracked PFOA concentrations through 24 hours. As mentioned in Section 3.3.2.1, a 24 hour observation window is insufficient to accurately estimate the terminal excretion half-life of PFOA. Therefore, only the original study used for parameter determination, Lou et al. (2009), 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.

Because male mouse parameters are not available for PFOA, only female parameters are used for all PFOA modeling in mice. This assumption is addressed in Wambaugh et al. (2013) and is based on a lack of evidence for sex-dependent PK differences in the mouse.

Table 4-4. Model Predicted and Literature PK Parameter Comparisons for PFOA

	Male					Female				
	t1/2,α (days)	**t1/2,β (days)**	**Vd,α (L/kg)**	**Vd,β (L/kg)**	**CL (L/d/kg)**	**t1/2,α (days)**	**t1/2,β (days)**	**Vd,α (L/kg)**	**Vd,β (L/kg)**	**CL (L/d/kg)**
					Rat					
Model	5.8	16.5	0.12	0.35	0.0147	0.16	2.84	0.16	2.81	0.686
Literature	1.64[a], 2.8[b]	10.25[b]	0.11[a,c],	0.15[b,c]	0.047[a], 0.013[b]	0.19[a], 0.028[b]	0.22[b]	0.17[a,c],	0.12[b,c]	0.613[a], 0.81[b]
					Mouse					
Model	–	–	–	–	–	17.8	18.9	0.18	0.19	0.007
Literature	–	–	–	–	–	–	–	–	–	–

Notes: CL = clearance; PK = pharmacokinetic; $t_{1/2,\alpha}$ = initial-phase elimination half-life; $t_{1/2,\beta}$ = terminal-phase elimination half-life; $V_{d,\alpha}$ = volume of distribution during the initial phase; $V_{d,\beta}$ = volume of distribution during the terminal phase.

[a] Information obtained from Kim et al. (2016).

[b] Information obtained from Dzierlenga et al. (2019a).

[c] Literature volumes of distribution represent central compartment volumes from a one-compartment or two-compartment model.

4.1.3.1.3 Life-Course Modeling

The Wambaugh et al. (2013) model was modified to account for gestation, lactation, and postweaning phases (Figure 4-1). Using the original model structure and published parameters, simulations assumed that dams were dosed prior to conception and up to the date of parturition. Following parturition, a lactational phase involved PFOA transfer from the breastmilk to the suckling pup where the pup was modeled with a simple one-compartment PK model. Finally, a postweaning 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.

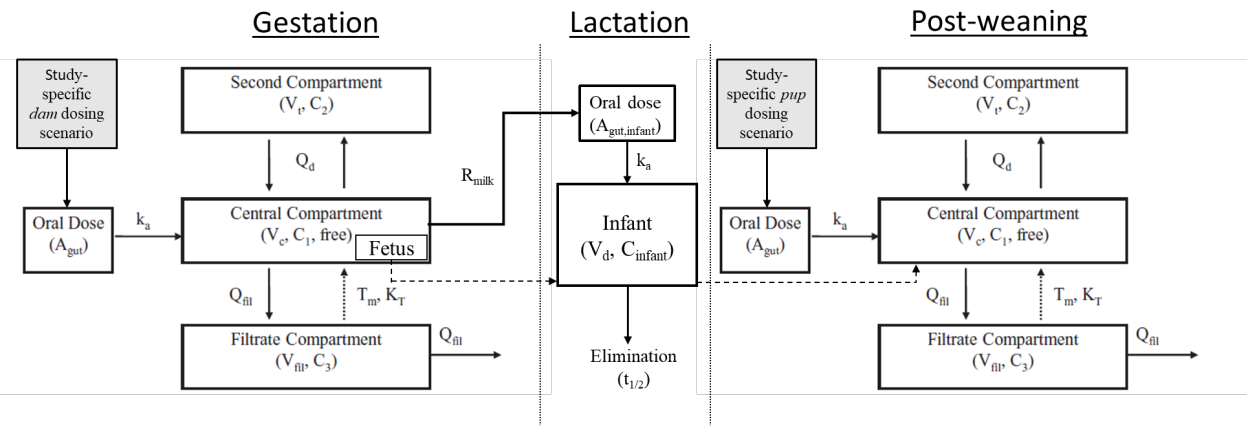


Figure 4-1. Model Structure for Lifestage Modeling

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 PFOA and milk PFOA 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 postweaning exposure and infant growth.

A simple one-compartment model for infant lactational exposure was chosen because of differences between PFOA Vd reported in the literature and Wambaugh et al. (2013) model-predicted Vd following extrapolation to a relatively low infant body weight. Because Vd is assumed to be extracellular water in human, 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 (Dzierlenga et al., 2019a; Lou et al., 2009) for the one-compartment model to describe infant toxicokinetics. Finally, the Wambaugh

et al. (2013) model was not parameterized on a postpartum infant, and it was not possible to evaluate the mechanistic assumptions for renal elimination with postnatal toxicokinetic data. While there is one study that doses PFOA in young, postweaning, juvenile animals (Hinderliter et al., 2006b), concentrations at only two time points are reported for each age group making it not possible to estimate infant/juvenile pharmacokinetic parameters such as half-life. 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 PFOA.

Table 4-5. Additional PK Parameters for Gestation/Lactation for PFOA

Parameter	Units	Rat	Mouse
Maternal Milk:Blood Partition Coefficient (P_{milk})	Unitless	0.11[a,b]	0.32[e]
Fetus:Mother Concentration Ratio (R_{fm})	Unitless	0.42[b]	0.25[f]
Elimination Half-Life ($t_{1/2}$)	Days	2.23[c]	18.5 g
Volume of Distribution (V_d)	L/kg	0.18[d]	0.2 g
Starting Milk Consumption Rate (r^0_{milk})	kg_{milk}/day	0.001[h]	0.0001[i]
Week 1 Milk Consumption Rate (r^1_{milk})	kg_{milk}/day	0.003[h]	0.0003[i]
Week 2 Milk Consumption Rate (r^2_{milk})	kg_{milk}/day	0.0054[h]	0.00054[i]
Week 3 Milk Consumption Rate (r^3_{milk})	kg_{milk}/day	0.0059[h]	0.00059[i]

Notes: PK = pharmacokinetic.
[a] Information obtained from Loccisano et al. (2013) (derived from Hinderliter et al. (2005)).
[b] Information obtained from Hinderliter et al. (2005).
[c] Average of male/female half-lives reported in Dzierlenga et al. (2019a), Kim et al. (2016), and Kemper et al. (2003).
[d] Information obtained from Kim et al. (2016) and Dzierlenga et al. (2019a).
[e] Information obtained from Fujii et al. (2020).
[f] Information obtained from Blake et al. (2020).
[g] Information obtained from Lou et al. (2009).
[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 PFOA 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 PFOA, 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 simulated two studies for PFOA exposure (one in mice and one in rats) to ensure the model predicted the time-course concentration curves for both

the dam and the pup. For all gestation/lactation studies, time zero represents conception followed by a gestational window (21 days for the rat, 17 days for the mouse). Dosing prior to day zero represents pre-mating exposure to PFOA.

Figure 4-2 demonstrates the model's ability to predict gestation and lactation study design in rat dams exposed to 30 mg/kg/day PFOA from GD 1-LD 22 that gave birth to pups who are exposed through gestation and lactation until weaning (Hinderliter et al., 2005). Comparatively, Figure 4-3 demonstrates model fits for PFOA exposure in mice from a cross-fostering study (White et al., 2009). In each case, the original Wambaugh et al. (2013) model with increasing maternal weight predicts dam concentrations in female rats and mice while the one-compartmental lactational transfer model predicts infant concentrations for pups exposed either *in utero* or during lactation only.

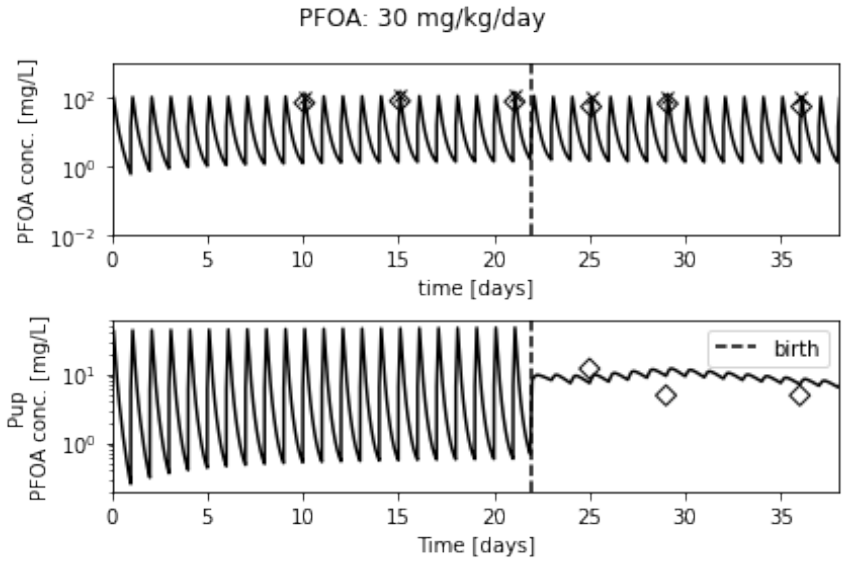


Figure 4-2. Gestation and Lactation Predictions of PFOA in the Rat

Top panel represents time-course model predicted dam concentrations (solid line) where open diamonds (◊) represent the in vivo dam concentrations reported in Hinderliter et al. (2005) and x's represent the model-predicted value at the reported time. Bottom panel demonstrates the model predicted pup concentrations (solid line) where open diamonds (◊) represent the reported pup concentrations in Hinderliter et al. (2005) with PFOA exposure is from the breast milk. Vertical dashed line represents birth.

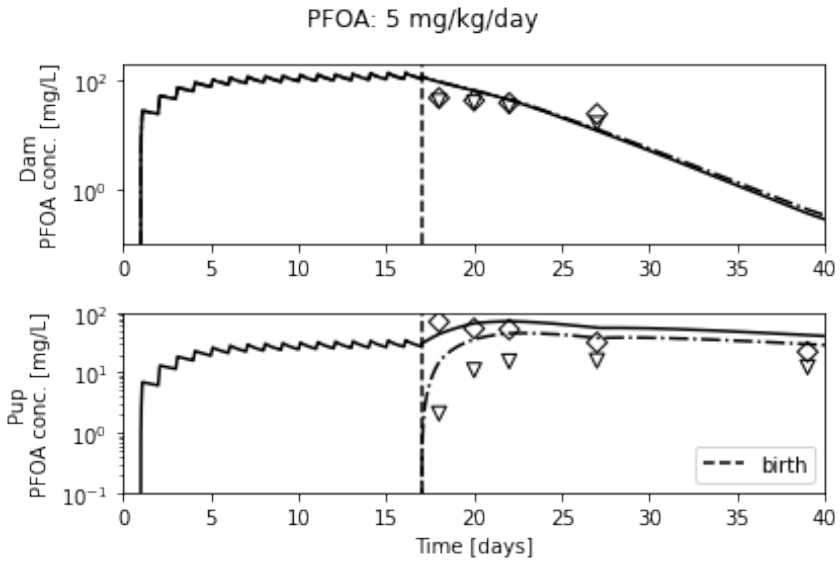


Figure 4-3. Gestation and Lactation Predictions of PFOA in the Mouse in a Cross-Fostering Study

Top panel represents predicted dam concentrations while bottom panel represents the predicted pup concentrations from White et al. (2009). Solid lines (–) represent a 5 mg/kg/day maternal dose paired with nursing pups that were exposed to PFOA in utero and open diamonds (◊) represent the reported dam and infant concentrations for this exposure scenario. Comparatively, dot-dashed lines (• –) represent the simulations from the cross-fostering study where dams were exposed to 5 mg/kg/day PFOA and pups born to the control dam were exposed through lactation. Open triangles (▽) represent the reported dam and infant concentrations for this cross-foster study.

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 points of departure 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 PFOA in blood were considered for all the dose metrics. For studies in adult animals the dose-metric options were generally a maximum blood concentration (C_{max}, mg/L) and a time averaged blood concentration i.e., the area under the curve over the duration of the study (AUC, mg * day/L) or the blood concentration over the last 7 days (C_{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 C_{max} and averaged 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 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 nondevelopmental 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 mechanism of action (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.

Finally, for NTP (2020), an additional dose metric was derived which averages out the concentration in the pup from conception to the end of the 2 years ($C_{avg\_pup\_total}$). Specifically, it adds the area under the curve in gestation/lactation to the area under the curve from diet (postweaning) and then divides by 2 years.

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 suggest 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)), 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 PFOA is available in Sections 5.6.2 and 5.7.

Several adjustments were undertaken to facilitate the application of the model for this use. First, the model was converted from acslX language to an R/MCSim framework. This allows the code to be more accessible to others by updating it to a contemporary modeling language, 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, the modeling language conversion body weight curves for nonpregnant adults were revised based on 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, the volume of distribution (V_d), the ratio of PFOA concentration in cord blood to maternal serum, and the ratio of PFOA 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 PFOA serum concentrations are approximately 70% of the original values during pregnancy, and the child's serum concentration is approximately 60% 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 PFOA (U.S. EPA, 2016c). The 2016 PFOA 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 PFOA 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 PFOA in Humans

Parameter	Updated Value	Original Value[a]
Volume of Distribution (mL/kg)	170[b]	170
Half-life (yr)	2.7[c]	3.8
Clearance (mL/kg/d)	0.120[d]	0.085
Cord Serum:Maternal Serum Ratio	0.83[e]	0.79
Milk:Serum Partition Coefficient	0.049[f]	0.058

Notes:
[a] Verner et al. (2016).
[b] Thompson et al. (2010a).
[c] Li et al. (2017d).
[d] Calculated from half-life and volume of distribution. $Cl = Vd * \ln(2)/t_{1/2}$.
[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 2.7 years was determined for PFOA as reported in Li et al. (2018c; 2017d). This value comes from a large population (n = 455) who originally had contaminated drinking water for which the study documents the decrease in exposure levels after the installation of filtration with an average final serum sample taken 3.9 years after the beginning of water filtration. Li et al. (2018c) also reported a similar half-life of 2.7 years for PFOA in a separate community with a similar study design. In that study, serial blood samples were collected from participants after the beginning of drinking water filtration after a long period of exposure to drinking water contaminated with PFOA. The second study involved 106 participants with a median number of six samples per person but with only a 2-year observation period Li et al. (2017d). The good agreement between the second study and the previous, larger study in diverse populations support the use of this value as a good estimate of the PFOA elimination half-life.

A summary of PFOA half-life values is presented in 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 of PFOA, 170 mL/kg, was sourced from Thompson et al. (2010a) who used a one-compartment PK model. This calculation involves several assumptions: that the participants' serum concentrations are at steady-state, their exposure can be estimated from the drinking water concentration in their community, there is 91% bioavailability for exposure from drinking water, and the half-life of PFAS is 2.3 years, which comes from the report of Bartell et al. (2010). EPA considered updating this parameter to 200 mL/kg, which is the value that would be calculated using the EPA chosen half-life value of 2.7 years. However, the value of 2.3 years was calculated under very similar conditions as the other data in the Thompson et al. (2010a) population and 2.3 years may better reflect the clearance rate in that specific population at that time. This calculation was performed in a population with PFOA contamination. V_d is a parameter that is relatively easily obtained from an analysis of PK data from controlled experimental studies, as it is related to the peak concentration observed after dosing and is expected to be similar between human and nonhuman primates (Mordenti et al., 1991). For comparison, the optimized V_d for PFOA from oral dosing in monkeys was 140 mL/kg (Andersen et al., 2006).

Another group has approached the calculation of V_d by taking the average of reported animal and human values and reported values of 200 mL/kg for PFOA (Gomis et al., 2017). This calculation included the V_d value from Thompson et al. (2010a) and did not include additional values derived from human data. The resulting average value shows that the value from Thompson et al. (2010a) is reasonable; EPA selected the Thompson et al. (2010a) result based on the fact that it is the only value derived from human data that EPA considers to be reliable for risk estimation in the general population.

A summary of PFOA 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 PFOA concentration in cord blood to maternal serum, and the ratio of PFOA 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.

Table 4-7. Summary of Studies Reporting the Ratio of PFOA 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	0.038	No
Seung-Kyu Kim et al. (2011b)	2919258	0.025	No
Liu et al. (2011)	2919240	0.11	No
Cariou et al. (2015)[a]	3859840	0.034	Yes
Sunmi Kim et al. (2011a)[b]	1424975	0.04	Yes
Verner et al. (2016)	3299692	0.058[c]	–

Source	HERO ID	Milk:Maternal Plasma Ratio	Included in Verner et al. (2016) Analysis
Additional Studies	–	0.049[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 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 4.1.1.2) 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/U.S. EPA/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 PFOA Toxicological Endpoints and Dosimetric Interpretation of Epidemiological Endpoints

Different approaches were taken to estimate POD_{HED}s depending on the species (i.e., human versus 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 the 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 nonlinear 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 ($C_{last7,avg}$), 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.

This approach for interspecies extrapolation follows EPA's guidance to prefer the use of a PK or PBPK model over the use of a data-derived extrapolation factor (DDEF) (U.S. EPA, 2014). A PK model allows for predictions of dosimetry for specific exposure scenarios in animals and humans and can incorporate PK details such as maternal accumulation and subsequent gestation/lactational transfer to a fetus/infant. Using a hierarchical decision-making framework, a DDEF approach is only considered when a validated PK or PBPK model is not available. Furthermore, EPA considers DDEF values based on the ratio of maximum blood concentration from acute, high-dose exposures to likely not be protective for typical exposure scenarios to humans, chronic low-dose exposure or lactational exposure to a nursing infant (Dourson et al., 2019). While a repeat dose DDEF has been presented (Dourson et al., 2019), this factor relied on maximum concentrations from Elcombe et al. (2013), for which the results are not considered relevant to the general population as discussed in Section 4.1.3.2.

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; PFOA concentrations at age 5 and anti-tetanus antibody serum concentrations at age 7	$BMDL_{0.5SD}$	3.47 ng/mL	3.05×10^{-7}	BMR of 0.5 SD provided reasonably good estimate of 5% extra risk; single- and multi-PFAS models resulted in same BMDL; selected BMDL was based on significant regression parameter
	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOA concentrations in the mother[c] and anti-tetanus antibody serum concentrations at age 5	$BMDL_{0.5\ SD}$	3.31 ng/mL	5.21×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; single- and multi-PFAS models resulted in similar BMDLs; selected BMDL was based on significant regression parameter
	Timmerman et al. (2021) *Medium*	Human, male and female; PFOA concentrations and anti-tetanus antibody concentrations at ages 7–12	$BMDL_{0.5SD}$	2.26 ng/mL	3.34×10^{-7}	BMR of 0.5 SD may not be a reasonably good estimate of 5% extra risk; BMDL was based on nonsignificant regression parameter; no multi-PFAS modeling was conducted
Decreased serum anti-diphtheria antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female; PFOA concentrations at age five and anti-diphtheria antibody serum concentrations at age 7	$BMDL_{0.5SD}$	3.32 ng/mL	2.92×10^{-7}	Single- and multi-PFAS models resulted in comparable BMDLs though there was a 30% change in the effect size when controlling for PFOS; selected BMDL was based on significant regression parameter
	Budtz-Jørgensen and Grandjean (2018)[b]	Human, male and female; PFOA concentrations in the mother[c] and anti-	$BMDL_{0.5SD}$	1.24 ng/mL	1.95×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; single- 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
	Medium	diphtheria antibody serum concentrations at age 5				multi-PFAS models resulted in similar BMDLs though there was a 30% change in the effect size when controlling for PFOS
	Timmerman et al. (2021) *Medium*	Human, male and female; PFOA concentrations and anti-diphtheria antibody concentrations at ages 7–12	$BMDL_{0.5SD}$	1.49 ng/mL	2.20×10^{-7}	BMR of 0.5 SD may not be a reasonably good estimate of 5% extra risk; BMDL was based on nonsignificant regression parameter
Decreased IgM response to SRBC	Dewitt et al. (2008) *Medium*	C57BL/6N Mice, females, adults, Study 1	$BMDL_{1SD}$, Polynomial Degree 4	18.2 mg/L $C_{last7,avg}$	2.18×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
	Dewitt et al. (2008) *Medium*	C57BL/6N Mice, females, adults, Study 2	NOAEL[d] (1.88 mg/kg/day)	45.3 mg/L $C_{last7,avg}$	5.43×10^{-3}	Test for constant variance and test for nonconstant variance failed therefore a NOAEL approach was taken
	Loveless et al. (2008) *Medium*	Crl:CD-1(ICR)BR Mice, males, adults	$BMDL_{1SD}$, Exponential 3	57.6 mg/L $C_{last7,avg}$	6.91×10^{-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; PFOA serum concentrations in third trimester	$BMDL_{5RD}$, Hybrid	2.0 ng/mL	3.15×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; selected BMDL was based on significant regression parameter
	Govarts et al. (2016) *High*	Human, male and female; PFOA concentrations in umbilical cord	$BMDL_{5RD}$, Hybrid	1.2 ng/mL	2.28×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; selected BMDL was based on nonsignificant 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
	Sagiv et al. (2018) *High*	Human, male and female; PFOA serum concentrations in first and second trimesters	$BMDL_{5RD}$, Hybrid	9.1 ng/mL	1.21×10^{-6}	Selected BMDL was based on nonsignificant regression parameter
	Starling et al. (2017) *High*	Human, male and female; PFOA serum concentrations in second and third trimesters	$BMDL_{5RD}$, Hybrid	1.8 ng/mL	2.65×10^{-7}	PFOA concentrations may be influenced by pregnancy hemodynamics; selected BMDL was based on significant regression parameter
	Wikström et al. (2020) *High*	Human, male and female; PFOA serum concentrations in first and second trimesters	$BMDL_{5RD}$, Hybrid	2.2 ng/mL	2.92×10^{-7}	Selected BMDL was based on significant regression parameter
Decreased Pup Survival	Song et al. (2018) *Medium*	Kunming Mice, F_1 males and females (PND 21)	$BMDL_{0.5SD}$, Polynomial Degree 3	12.3 mg/L $C_{avg\_pup\_gest\_lact}$	6.40×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 0)	NOAEL[d] (3 mg/kg/day)	19.1 mg/L $C_{avg\_pup\_gest}$	3.23×10^{-3}	No models had adequate fit. Test for constant variance failed, and test for nonconstant variance failed. NOAEL approach taken
	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 23)	NOAEL[d] (3 mg/kg/day)	26.6 mg/L $C_{avg\_pup\_gest\_lact}$	1.38×10^{-3}	Test for constant variance failed. For nonconstant variance models, goodness of fit for nonconstant models was poor. NOAEL approach taken
Decreased Fetal Body Weight	Li et al. (2018a) *Medium*	Kunming Mice, F_1 males and females (GD 18)	NOAEL[d] (1 mg/kg/day)	8.5 mg/L $C_{avg\_pup\_gest}$	1.44×10^{-3}	No models had adequate fit. Test for constant variance failed, and test for nonconstant variance failed. NOAEL approach taken
Decreased Pup Body Weight	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 23)	NOAEL[d] (1 mg/kg/day)	15.8 mg/L $C_{avg\_pup\_gest\_lact}$	8.2×10^{-4}	No models had adequate fit. Test for constant variance failed. For nonconstant

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
						variance models, goodness of fit for nonconstant models was poor. NOAEL approach taken
Delayed Time to Eye Opening	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 14 – PND 18)	$BMDL_{0.5SD}$, Polynomial Degree 2	8.0 mg/L $C_{avg\_pup\_gest\_lact}$	4.17×10^{-4}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Cardiovascular Effects (Serum Lipids)						
Increased Total Cholesterol	Dong et al. (2019) *Medium*	Human, male and female, age 20-80	$BMDL_{5RD}$, Hybrid	2.29 ng/mL	2.75×10^{-7}	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	4.25 ng/mL	5.10×10^{-7}	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	$BMDL_{0.5SD}$, Linear	5.28 ng/mL	6.34×10^{-7}	Analyses include individuals prescribed cholesterol medication and significant regression parameter
Hepatic Effects						
Increased ALT	Gallo et al. (2012) *Medium*	Human, female, age 18 and older	$BMDL_{5RD}$, Hybrid	17.9 ng/mL	2.15×10^{-6}	BMDL based on significant regression parameter
	Darrow et al. (2016) *Medium*	Human, female, age 18 and older	$BMDL_{5RD}$, Hybrid	66.0 ng/mL	7.92×10^{-6}	BMDL based on modeled serum PFOA concentrations and significant regression parameter
	Nian et al. (2019) *Medium*	Human, female, age 22 and older	$BMDL_{5RD}$, Hybrid	3.76 ng/mL	4.51×10^{-7}	BMDL based on 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
Increased Focal Necrosis	Loveless et al. (2008) *Medium*	Crl:CD-1(ICR)BR Mice, adult male	$BMDL_{10RD}$, Dichotomous Hill	10.0 mg/L $C_{last7,avg}$	1.20×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and presented most protective BMDL in consideration of the adversity of effect
Increased Individual Cell Necrosis	Loveless et al. (2008) *Medium*	Crl:CD-1(ICR)BR Mice, adult male	$BMDL_{10RD}$, Probit	36.0 mg/L $C_{last7,avg}$	4.32×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Increased Hepatocyte Single Cell Death	NTP (2020) *High*	Sprague-Dawley Rats, males; perinatal and postweaning	$BMDL_{10RD}$, Gamma	100 mg/L $C_{avg\_pup\_total}$	1.20×10^{-2}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC
Increased Necrosis	NTP (2020) *High*	Sprague-Dawley Rats, males; perinatal and postweaning	$BMDL_{10RD}$, Multistage Degree 1	26.9 mg/L $C_{avg\_pup\_total}$	3.23×10^{-3}	Selected model showed adequate fit ($p > 0.1$) and lowest AIC

Notes: AIC = Akaike information criterion; ALT = alanine aminotransferase; AUC = area under the curve; $BMDL_{0.5SD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a change in the mean response equal to 0.5 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 for a 10% change in response; $C_{avg\_pup\_gest}$ = average blood concentration normalized per day during gestation; $C_{avg\_pup\_total}$ = average blood concentration in pup; $C_{last7,avg}$ = average blood concentration over the last 7 days; F_1 = first generation; IgM = immunoglobulin M; NOAEL = no-observed-adverse-effect level; NTP = National Toxicology Program; POD_{HED} = point-of-departure human equivalence 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), and 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] No models provided adequate fit; therefore, a NOAEL/LOAEL approach was selected.

4.1.4.1 Hepatic Effects

Increased ALT in individuals aged 18 and older (Darrow et al., 2016; 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 (Nian et al., 2019; Gallo et al., 2012) or modeled (Darrow et al., 2016) PFOA serum concentrations collected from adults aged 18 years and older, 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 (see 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 (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Focal Necrosis, Crl:CD-1(ICR)BR mice, male, $C_{last7,avg}$ (Loveless et al., 2008)

Increased incidence of focal necrosis of the liver was observed in male ICR mice. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The $C_{last7,avg}$ was selected for all non-developmental studies (i.e., studies with exposure during adulthood only) 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 human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Individual Cell Necrosis, Crl:CD-1(ICR)BR mice, male, $C_{last7,avg}$ (Loveless et al., 2008)

Increased incidence of individual cell necrosis of the liver was observed in male ICR mice. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). The $C_{last7,avg}$ was selected for all non-developmental studies (i.e., studies with exposure during adulthood only) 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 human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Necrosis, Sprague-Dawley rats, males, perinatal and postweaning, $C_{avg\_pup\_total}$ (NTP, 2020)

Increased incidence of necrosis of the liver was observed in adult male Sprague-Dawley rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). For

endpoints derived from NTP (2020), an additional dose metric was developed which averages the concentration in the offspring from conception to the end of the 2-year postnatal exposure period ($C_{avg\_pup\_total}$; see 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 human clearance value (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$)).

Hepatocyte Single Cell Death, Sprague-Dawley rats, males, perinatal and postweaning, $C_{avg\_pup\_total}$ (NTP, 2020)

Increased incidence of single cell death of the liver was observed in adult male Sprague-Dawley rats. Dichotomous models were used to fit dose-response data. A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) (Section 4.1.2). For endpoints derived from NTP (2020), an additional dose metric was developed which averages the concentration in the offspring from conception to the end of the 2-year postnatal exposure period ($C_{avg\_pup\_total}$; see 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 human clearance value (0.120 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 PFOA 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 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 1-year breastfeeding period, where the exposure to the child was only through lactation, which was much greater than the exposure to the mother. After 1 year, the exposure to the child, relative to body weight, was set to the same value as the mother. The model provided predictions for a child aged 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 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. 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 PFOA 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 selected 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 was 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 PFOA 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 IgM response to SRBC, C57BL/6N mice, Female, Studies 1 and 2, $C_{last7,avg}$ (Dewitt et al., 2008)

Decreased mean response of SRBC-specific IgM antibody titers was observed in female C57BL/6N mice (Studies 1 and 2). Using the Wambaugh et al. (2013) model, daily exposure to PFOA in the drinking water was simulated for 15 days using female C57BL/6 mice parameters (Section 4.1.3.1). Continuous models were used to fit dose-response data. A 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 $C_{last7,avg}$ was selected for all non-developmental studies (i.e., studies with exposure during adulthood only) 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). For Study 1, the BMDS produced a BMDL in mg/L. For Study 2, the tests for constant and nonconstant variance failed therefore a NOAEL approach was taken. 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 (0.120 mL/kg/day; calculated from half-life and volume of distribution; $Cl = V_d * \ln(2)/t_{1/2}$).

Decreased IgM response to SRBC, Crl:CD-1(ICR)BR mice, Male, $C_{last7,avg}$ (Loveless et al., 2008)

Decreased mean response of IgM serum titer was observed in male Crl:CD-1(ICR)BR mice. Using the Wambaugh et al. (2013) model, daily oral gavage exposure to PFOA was simulated for 29 days using male CD1 mice parameters. Continuous models were used to fit dose-response data. A 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 $C_{last7,avg}$ was selected for all non-developmental studies (i.e., studies with exposure during adulthood only) 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 human clearance value (0.120 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 adults 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 PFOA 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 (0.120 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 PFOA 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 (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 (0.120 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 PFOA 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 was selected 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 (0.120 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 PFOA 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 PFOA 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 was independent of the sex of the child in the model.

Decreased birthweight using the serum PFOA concentrations collected from umbilical cord samples (Govarts et al., 2016)

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 PFOA serum concentrations collected from an umbilical cord sample, 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 delivery and reverse dosimetry was performed to calculate the POD_{HED} that resulted in the POD serum level in cord serum. This metric was independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOA 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 PFOA serum concentrations collected from the mother primarily in the first trimester (median gestational age: 9 weeks; range: 5–19 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 was independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOA 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 PFOA 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 competed 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 was independent of the sex of the child in the model.

Decreased birthweight using the mother's serum PFOA 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 PFOA 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 Pup Survival, Kunming Mice, F_1 males and females (PND 21), $C_{avg\_pup\_gest\_lact}$ (Song et al., 2018)

Decreased mean response of number of offspring survival at weaning on PND 21 was observed in F1 male and female Kunming mice. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.5 standard deviations from the control mean was selected for POD derivation 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.2, (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 1-year breastfeeding period. 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 Survival, CD-1 Mice, F_1 males and females (PND 0), $C_{avg\_pup\_gest}$ (Lau et al., 2006)

Decreased mean response of pup survival was observed in F1 male and female CD-1 mice at PND 0. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.5 standard deviations from the control mean was chosen 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.2, (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 tests for constant and nonconstant variance failed therefore 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 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. This metric was independent of the sex of the child in the model.

Decreased Pup Survival, CD-1 Mice, F_1 males and females (PND 23), $C_{avg\_pup\_gest\ lact}$ (Lau et al., 2006)

Decreased mean response of pup survival was observed in F1 male and female CD-1 mice at PND 23. Continuous models were used to fit dose-response data. A BMR of a change in the mean equal to 0.5 standard deviations from the control mean was chosen 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.2, (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 tests for constant and nonconstant variance failed therefore 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 1-year breastfeeding period. 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 Fetal Body Weight, Kunming Mice, F_1 males and females (GD 18), $C_{avg\_pup\_gest}$ (Li et al., 2018a)

Decreased mean response of fetal body weight was observed in F_1 male and female Kunming mice. Continuous models were used to fit dose-response data. A BMR of 5% extra risk 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.2, (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 tests for constant and nonconstant variance failed therefore 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 4.1.2). 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. This metric was independent of the sex of the child in the model.

Decreased Pup Body Weight (relative to litter), CD-1 Mice, F_1 males and females (PND 23), $C_{avg\_pup\_gest\_lact}$ (Lau et al., 2006)

Decreased mean response of pup body weight was observed in F_1 male and female CD-1 mice at PND 23. Continuous models were used to fit dose-response data. A BMR of 5% extra risk 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.2, (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 bodyweight. 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 1-year breastfeeding period. 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.

Delayed Time to Eye Opening, CD-1 Mice, F_1 males and females (PND 14 – PND 18), $C_{avg\_pup\_gest\_lact}$ (Lau et al., 2006)

Decreased mean response of time to eye opening was observed in F1 male and female CD-1 mice. Continuous models were used to fit dose-response data. BMR of a change in the mean equal to 0.5 standard deviations from the control mean 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 1 standard deviations from the control mean was provided for comparison purposes (see Appendix E.2, (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 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 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 because the lactational duration in humans that equates to time to eye opening 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 different growth curves specific to male and female children were used in the model, the model predicted slightly (less than 5%) different serum concentrations for each sex. The lower HED was selected to be more health protective.

4.1.5 Derivation of Candidate Chronic Oral Noncancer Reference Doses (RfDs)

Though multiple candidate 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 Sections 4 and 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

Three *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Nian et al., 2019; Darrow et al., 2016; Gallo et al., 2012). EPA considered all three studies as they represented the low-dose range of effects across hepatic endpoints and provided data from relatively large populations, including U.S. populations. 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 three studies reported analyses examining different forms of confounding factors and consideration of cumulative PFOA exposure (Darrow et al., 2016), sensitivity analyses excluding participants with lifestyle characteristics (e.g., excluding smokers, drinkers, medicine takers) impacting outcome assessment (Nian et al., 2019), and nonlinear exposure-response relationships (Gallo et al., 2012). All three of these studies provided the necessary data for modeling.

One *high* confidence animal toxicological study was carried forward for candidate RfD determination (NTP, 2020). NTP (2020) was prioritized for candidate RfD development because it was determined to be a *high* confidence study and it used a chronic exposure duration that encompassed sensitive periods of development, whereas Loveless et al. (2008) was a *medium* confidence study that used a short-term (28-day) exposure duration and predated current criteria for hepatic histopathological assessment of cell death (Elmore et al., 2016). Increased liver necrosis from NTP (2020) was selected for candidate RfD derivation over the effect of increased

hepatocyte single cell death due to the increased biological severity of the former endpoint. Increased liver necrosis additionally resulted in a more protective POD_{HED}.

4.1.5.2 Immune Effects

Two *medium* confidence epidemiological studies were carried forward for candidate RfD determination (Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018). EPA considered both studies as they both represented the low-dose range of effects across immunological endpoints and provided data regarding sensitive populations (i.e., children). Although EPA derived POD_{HED}s for two time points reported by Budtz-Jørgensen and Grandjean (2018) (i.e., PFOA serum concentrations at age 5 and antibody concentrations at age 7; PFOA 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 PFOA concentrations measured in the mother for candidate RfD derivation because of concerns surrounding potential 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. In total, four immunological POD_{HED}s from two epidemiological studies were carried forward for candidate RfD derivation.

One *medium* confidence animal toxicological study was carried forward for candidate RfD determination (Dewitt et al., 2008). The POD_{HED} from Study 1 was selected over Study 2 because the former was amenable to benchmark dose modeling and had a POD_{HED} based on a BMDL, the preferred POD for animal toxicological studies (U.S. EPA, 2022d, 2012a). Study quality evaluations and further consideration did not identify notable characteristics distinguishing the two candidate studies (Dewitt et al., 2008; Loveless et al., 2008), but because the POD_{HED}s of reduced IgM response in rodents represented effects at the highest dose range of responses and because the observed effects were from *medium* confidence less-than-chronic studies, EPA selected the most health protective POD_{HED} based on Dewitt et al. (2008) for candidate RfD derivation. The candidate RfD derived from Dewitt et al. (2008) is expected to be protective of the immune effects observed in Loveless et al. (2008).

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

Two *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). Of the five epidemiological studies for which POD_{HED}s were derived, Sagiv et al. (2018) and Wikström et al. (2020) assessed maternal PFOA serum concentrations 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 two 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, both studies were considered further for candidate RfD derivation. The other three studies assessed PFOA 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 (Chu et al., 2020; Starling et al., 2017; Govarts et al., 2016).

Two *medium* confidence animal toxicological studies representing two endpoints, decreased pup survival and delayed time to eye opening, were carried forward for candidate RfD determination (Song et al., 2018; Lau et al., 2006). These two datasets were amenable to benchmark dose modeling and had POD_{HED}s based on BMDLs, the preferred POD for animal toxicological studies (U.S. EPA, 2022d, 2012a). In contrast, the endpoints of decreased fetal body weight derived from data published by Li et al. (2018a) and decreased pup survival and decreased pup weight derived from data published by Lau et al. (2006) were not amenable to BMD modeling and had NOAELs as the basis of the POD_{HED}s. Therefore, these POD_{HED}s were excluded from further consideration for candidate RfD derivation. As the delayed time to eye opening and decreased pup survival endpoints reported by Lau et al. (2006) and Song et al. (2018), respectively, encompassed sensitive populations (i.e., fetuses and pups) and different effects in two different strains of mice, these two POD_{HED}s were considered further for candidate RfD derivation. These two endpoints appear to be more sensitive (i.e., have lower POD_{HED}s) than the effects reported by Li (2018a) and Lau (2006).

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

UF	Value	Justification
		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: BMDL = benchmark dose level; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; 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 UF.

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. This was the case for all epidemiological endpoints and studies advanced for candidate RfD derivation.

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 PFOA 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 PFOA. In animals, comprehensive oral short-term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and developmental toxicity studies including assessment of immune effects following developmental exposure. Moreover, there is a 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 that was applied to candidate RfDs derived from all of the epidemiological studies 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_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: BMDL = benchmark dose level; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; 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 = total 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 database UF (UF_D) of 1 was applied to account for deficiencies in the database for PFOA. In animals, comprehensive oral short-term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and developmental toxicity studies including assessment of immune effects following developmental exposure. Moreover, there is a 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	Study, Confidence	Strain/Species/ Sex/Age	POD_{HED} (mg/kg/day)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate RfD[a] (mg/kg/day)
Immune Effects										
Decreased serum anti-tetanus antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female, PFOA concentrations at age 5 and antibody concentrations at age 7	3.05×10^{-7}	1	10	1	1	1	10	$3.05 \times 10^{-8} = 3 \times 10^{-8}$
	Timmerman et al. (2021) *Medium*	Human, male and female, PFOA and antibody concentrations at ages 7–12	3.34×10^{-7}	1	10	1	1	1	10	$3.34 \times 10^{-8} = 3 \times 10^{-8}$
Decreased serum anti-diphtheria antibody concentration in children	Budtz-Jørgensen and Grandjean (2018)[b] *Medium*	Human, male and female, PFOA concentrations at age 5 and antibody concentrations at age 7	2.92×10^{-7}	1	10	1	1	1	10	$2.92 \times 10^{-8} = 3 \times 10^{-8}$
	Timmerman et al. (2021) *Medium*	Human, male and female, PFOA and antibody concentrations at ages 7–12	2.20×10^{-7}	1	10	1	1	1	10	$2.20 \times 10^{-8} = 2 \times 10^{-8}$
Decreased IgM response to SRBC	Dewitt et al. (2008) *Medium*	Mouse, female, adults, study 1	2.18×10^{-3}	3	10	10	1	1	300	$7.27 \times 10^{-6} = 7 \times 10^{-6}$
Developmental Effects										
Decreased Birth Weight	Sagiv et al. (2018) *High*	Human, male and female, PFOA concentrations in first and second trimesters	1.21×10^{-6}	1	10	1	1	1	10	$1.21 \times 10^{-7} = 1 \times 10^{-7}$
	Wikström et al. (2020) *High*	Human, male and female, PFOA concentrations in first and second trimesters	2.92×10^{-7}	1	10	1	1	1	10	$2.92 \times 10^{-8} = 3 \times 10^{-8}$
Decreased Offspring Survival	Song et al. (2018) *Medium*	Kunming Mice, F_1 males and females	6.40×10^{-4}	3	10	1	1	1	30	$2.13 \times 10^{-5} = 2 \times 10^{-5}$

Endpoint	Study, Confidence	Strain/Species/ Sex/Age	POD_{HED} (mg/kg/day)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate RfD[a] (mg/kg/day)
Delayed Time to Eye Opening	Lau et al. (2006) *Medium*	CD-1 Mice, F_1 males and females (PND 14 – PND 18)	4.17×10^{-4}	3	10	1	1	1	30	$1.39 \times 10^{-5} = 1 \times 10^{-5}$
Cardiovascular Effects										
Increased Serum Total Cholesterol	Dong et al. (2019) *Medium*	Human, male and female, age 20-80	2.75×10^{-7}	1	10	1	1	1	10	$2.75 \times 10^{-8} = 3 \times 10^{-8}$
	Steenland et al. (2009) *Medium*	Human, male and female, age 18 and older	5.10×10^{-7}	1	10	1	1	1	10	$5.10 \times 10^{-8} = 5 \times 10^{-8}$
Hepatic Effects										
Increased Serum ALT	Gallo et al. (2012) *Medium*	Human, female, age 18 and older	2.15×10^{-6}	1	10	1	1	1	10	$2.15 \times 10^{-7} = 2 \times 10^{-7}$
	Darrow et al. (2016) *Medium*	Human, female, age 18 and older	7.92×10^{-6}	1	10	1	1	1	10	$7.92 \times 10^{-7} = 8 \times 10^{-7}$
	Nian et al. (2019) *Medium*	Human, female, age 22 and older	4.51×10^{-7}	1	10	1	1	1	10	$4.51 \times 10^{-8} = 5 \times 10^{-8}$
Necrosis	NTP (2020) *High*	Sprague-Dawley rats, perinatal and postweaning (2-year), male	3.23×10^{-3}	3	10	1	1	1	30	$1.08 \times 10^{-4} = 1 \times 10^{-4}$

Notes: ALT = alanine aminotransferase; NTP = National Toxicology Program; POD_{HED} = point-of-departure human equivalence dose; RfD = reference dose; SRBC = sheep red blood cells; UF_A = interspecies uncertainty factor; UF_H = intraspecies uncertainty factor; UF_S = subchronic-to-chronic extrapolation uncertainty factor; UF_L = extrapolation from a LOAEL-to-NOAEL uncertainty factor; UF_D = database uncertainty factor; UF_C = composite uncertainty factor.

[a] RfDs were rounded to one significant figure.

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

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 4-4, the candidate RfDs derived from epidemiological studies were all within 1 order of magnitude of each other (10^{-7} to 10^{-8} 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 either 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 4-4, 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 PFOA 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 PFOA. 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.

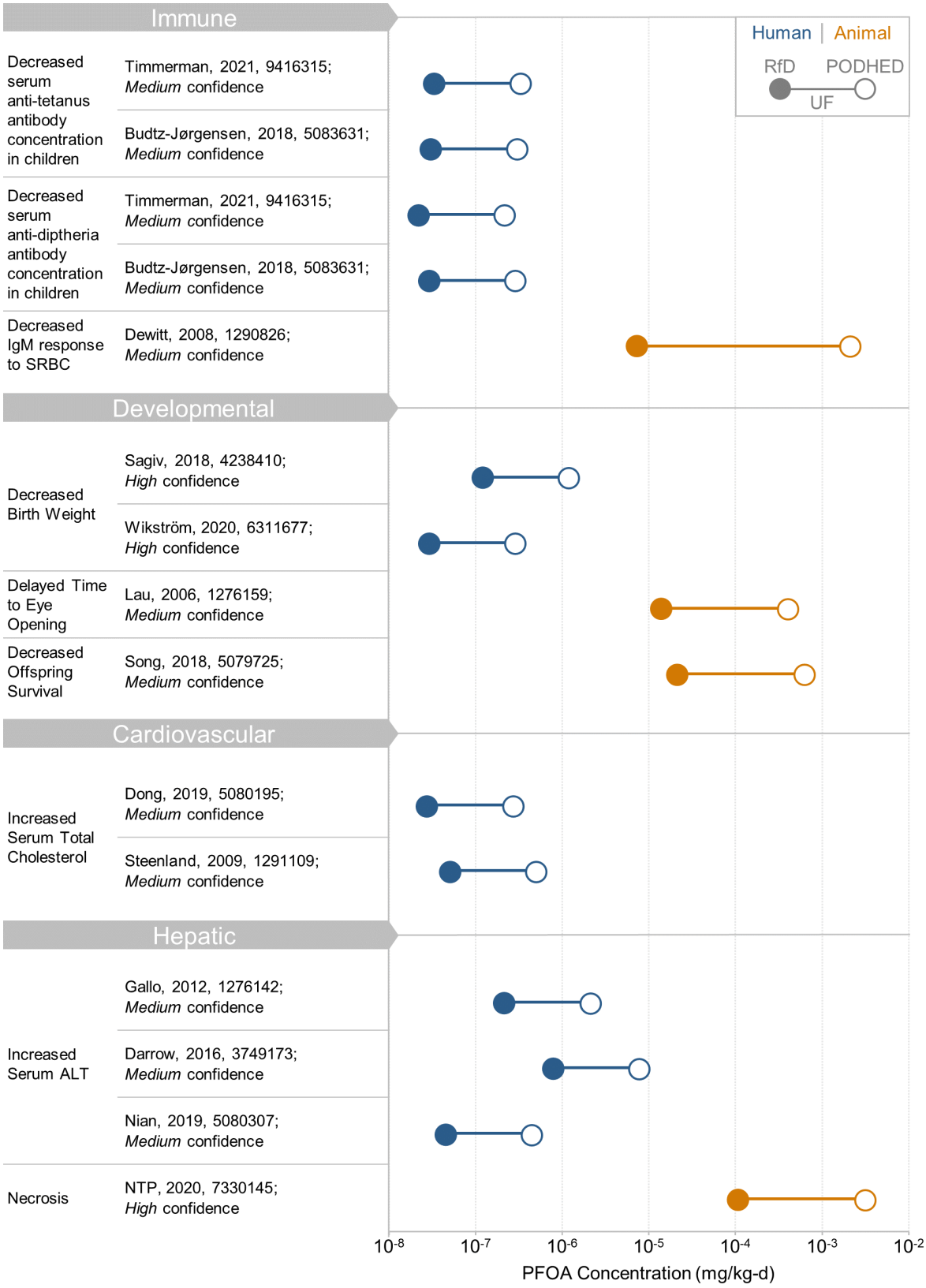


Figure 4-4. Comparison of Candidate RfDs Resulting from the Application of Uncertainty Factors to POD_{HED}s Derived from Epidemiological and Animal Toxicological Studies

As described in the subsections below, EPA selected amongst the candidate RfDs to identify an RfD representative of each of the four priority health outcomes (i.e., health outcome-specific RfDs), as well as an overall RfD that is protective of the effects of PFOA on all health outcomes and endpoints (Figure 4-5).

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

Three *medium* confidence epidemiological studies were selected for candidate RfD derivation for the endpoint of increased ALT (Nian et al., 2019; Darrow et al., 2016; Gallo et al., 2012). The two largest studies of PFOA and ALT in adults, Gallo et al. (2012) and Darrow et al. (2016), were both conducted in over 30,000 adults from the C8 Study. Gallo et al. (2012) reported measured serum ALT levels, unlike Darrow et al. (2016) which reported a modeled regression coefficient as the response variable. Another difference between the two studies is reflected in exposure assessment: Gallo et al. (2012) includes measured PFOA serum concentrations, while Darrow et al. (2016) based PFOA exposure on modeled PFOA serum levels. Due to these factors, the candidate RfD derived from Darrow et al. (2016) was excluded from further consideration as the health outcome-specific RfD for hepatic effects.

The third study by 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 PFOA, as well significant increases in ORs of elevated ALT. Both Nian et al. (2019) and Gallo et al. (2012) provided measured PFOA serum concentrations and a measure of serum ALT levels. However, the Gallo et al. (2012) study was conducted in a community exposed predominately to PFOA, whereas Nian et al. (2019) was conducted in a community exposed predominately to PFOS. The candidate RfD derived from Gallo et al. (2012) was ultimately selected as the health outcome-specific RfD due to reduced risk of bias related to potential confounding from other PFAS in this population. The resulting health outcome-specific RfD is 2×10^{-7} mg/kg/day (Figure 4-5).

4.1.6.1.2 Immune Effects

Candidate RfDs were derived from two *medium* confidence epidemiological studies for the endpoint of decreased antibody production in response to various vaccinations in children (Timmermann et al., 2021; Budtz-Jørgensen and Grandjean, 2018). Candidate RfDs were derived from Timmerman et al. (2021) were considered lower confidence candidate RfDs than those derived from Budtz-Jørgensen and Grandjean (2018). PODHEDs 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 PODHEDs: 1) the analyses considered co-exposures of other PFAS (i.e.,

PFOS); and 2) the response reported by this study was more precise in that it reached statistical significance. Therefore, the candidate RfDs from Timmerman et al. (2021) were not considered for selection as the health outcome-specific RfD.

The RfDs for anti-tetanus response in 7-year-old Faroese children and anti-diphtheria response in 7-year-old Faroese children, both from Budtz-Jørgensen and Grandjean (2018) were ultimately selected for the immune outcome as they are the same value and have no distinguishing qualitative (e.g., strength of evidence) or quantitative (e.g., model fit) characteristics that would facilitate selection of one over the other. The resulting health outcome-specific RfD is 3×10^{-8} mg/kg/day (Figure 4-5). 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 TC (Dong et al., 2019; Steenland et al., 2009). These candidate studies offer a variety of PFOA 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 PFOA and TC as categorical and continuous variables. The results using the natural log transformed TC and the natural log transformed PFOA were stated to fit the data slightly better than the ones using untransformed PFOA. However, the dramatically different changes in regression slopes between the two analyses by Steenland et al. (2009) resulting in extremely different PODs raise concerns about the appropriateness of using this data. Therefore, the resulting health outcome-specific RfD based on results from Dong et al. (2019) is 3×10^{-8} mg/kg/day (Figure 4-5). Note that both candidate RfDs for the cardiovascular outcome were within one order of magnitude of the selected health outcome-specific RfD.

4.1.6.1.4 Developmental Effects

Two *high* confidence epidemiological studies were selected for candidate RfD derivation for the endpoint of decreased birth weight (Wikström et al., 2020; Sagiv et al., 2018). These candidate studies assessed maternal PFOA serum concentrations primarily in the first trimester, minimizing concerns surrounding bias due to pregnancy-related hemodynamic effects. Both were *high* confidence prospective cohort studies with many study strengths including sufficient study sensitivity and sound methodological approaches, analysis, and design, as well as no evidence of bias. Between these two studies, PFOA exposure concentrations observed in Wikström et al. (2020) are more comparable to current exposure levels in the U.S. general population and therefore may be more relevant to the general population than the candidate RfD derived from Sagiv et al. (2018). 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 health outcome-specific RfD for developmental effects. The resulting health outcome-specific RfD is 3×10^{-8} mg/kg/day (Figure 4-5). Note that both candidate RfDs based on epidemiological studies for the

developmental outcome were within one order of magnitude of the selected health outcome-specific RfD.

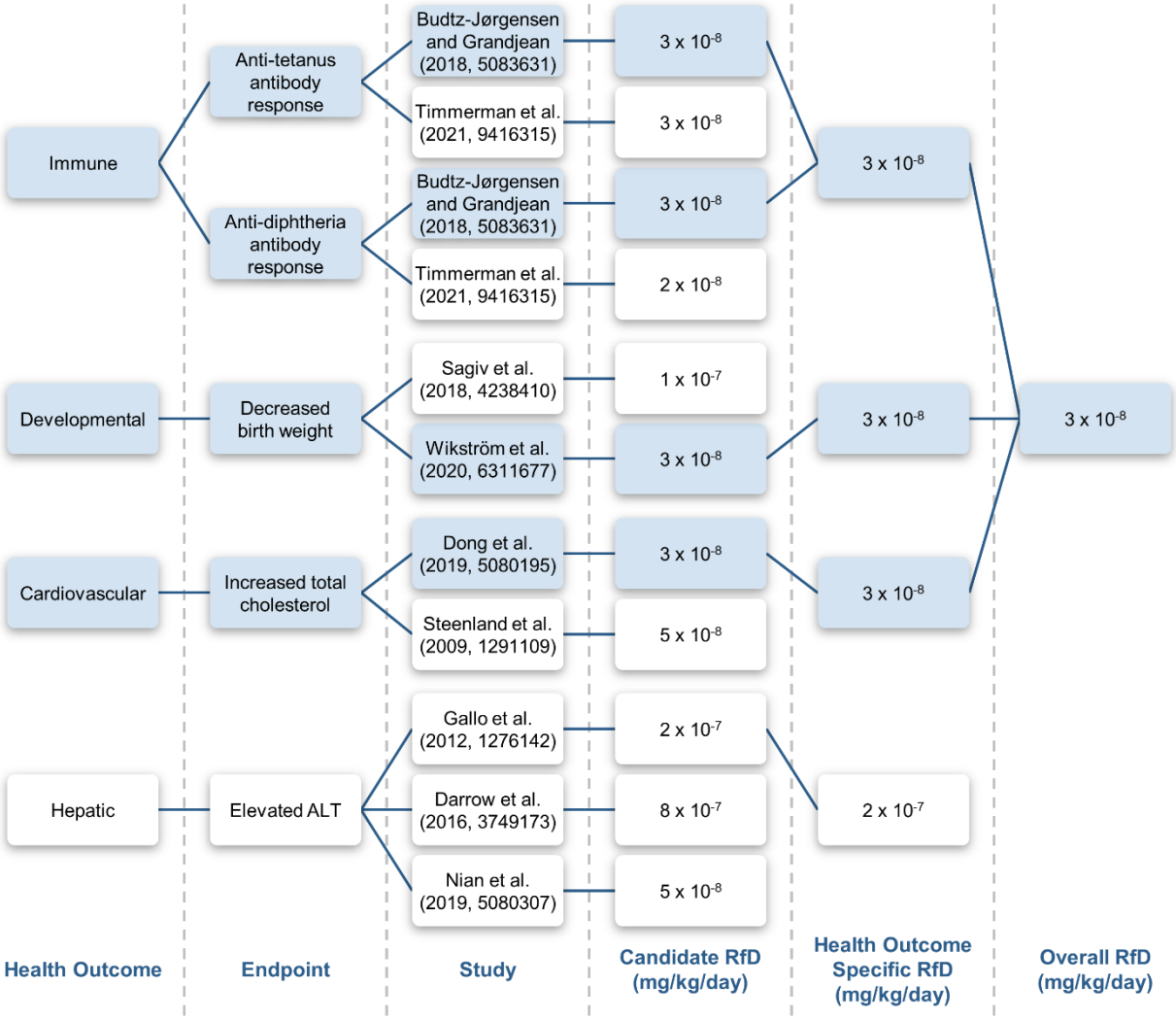


Figure 4-5. Schematic Depicting Selection of the Overall RfD for PFOA

RfD = reference dose.
Blue highlighted boxes indicate outcomes, endpoints, studies, candidate RfDs, and health outcome-specific RfDs that were selected as the basis of the overall RfD.

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 PFOA exposure. In fact, candidate RfDs within the immune, developmental, and cardiovascular outcomes are the same value (i.e., 3×10^{-8} mg/kg/day). Therefore, EPA has selected an overall RfD for PFOA of 3×10^{-8} mg/kg/day. The immune, developmental, and cardiovascular RfDs based on endpoints of decreased anti-tetanus and anti-diphtheria antibody concentrations in children, 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 (e.g., infants, children; see Section 5.8), as well as hepatic effects in adults that may result from PFOA exposure. As two of the co-critical effects identified for PFOA are developmental endpoints and can potentially result from a short-term exposure during critical periods of development, EPA concludes that the overall RfD for PFOA 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 PFOA 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 4, there is evidence from both epidemiological and animal toxicological studies that oral PFOA exposure may result in adverse health effects across many health outcomes, including cancer (Section 3.5). In Section 3.5.5, EPA concluded that PFOA 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 (e.g., selection of Vieira et al. (2013) over other C8 Health Project studies (see Section 4.2.1.1)).

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 (see Section 4.2.4). 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 evidence indicates that there is an increase in risk for kidney or Renal cell carcinoma (RCC) and testicular cancers with PFOA exposure (Bartell and Vieira, 2021; Shearer et al., 2021; Chang et al., 2014; Raleigh et al., 2014; Barry et al., 2013; Vieira et al., 2013; Steenland and Woskie, 2012). Results are most consistent for kidney cancer in adults based on a nested case-control study (Shearer et al., 2021), two C8 Health Project studies (Barry et al., 2013; Vieira et al., 2013), two occupational mortality studies (Raleigh et al., 2014; Steenland and Woskie, 2012), and a meta-analysis of epidemiological literature that concluded that there was an increased risk of kidney tumors correlated with increased PFOA serum concentrations (Bartell and Vieira, 2021). Therefore, the endpoint of kidney cancer was selected for CSF derivation.

Testicular cancer was identified as supporting evidence for carcinogenicity in humans in the 2016 PFOA HESD (U.S. EPA, 2016c). However, additional epidemiological studies examining risk of testicular cancer were not identified in the updated literature search and only two studies in the same high-exposure community (C8 Health Project) reported this association (Barry et al., 2013; Vieira et al., 2013). Therefore, the endpoint of testicular cancer in humans was not selected for dose-response modeling. Evidence was mixed or limited for other cancer sites (e.g., breast, liver cancers), which were not considered further.

Two studies reporting associations between kidney cancer and PFOA serum concentrations, Shearer et al. (2021) and Vieira et al. (2013), were selected for dose-response modeling. Shearer et al. (2021) was selected because it is a well-conducted, U.S.-based multicenter case-control study in the general population reporting a relatively large number of cases (N = 326). Median PFOA levels in controls was 5.0 ng/mL, comparable with 4.8 ng/mL in adults 60 and over from NHANES 1999–2000. Additionally, the analyses accounted for numerous confounders including BMI, smoking, history of hypertension, eGFR, previous freeze-thaw cycle, calendar and study year of blood draw, sex, race and ethnicity, study center. There was also a statistically significant increase in odds of RRC per doubling of PFOA (OR = 1.71, 95% CI: 1.23, 2.37) and in the highest versus lowest quartile (OR = 2.63, 95% CI: 1.33, 5.2) and a statistically significant increasing trend with increasing PFOA exposure across quartiles (p-trend = 0.007). Statistically significant increased odds of RCC were observed in participants ages 55–59 years, and in both men and women, separately.

EPA also selected the C8 Health Project study (Vieira et al., 2013) for dose-response modeling. The Vieira et al. (2013) study was a cancer registry-based case-control conducted in 13 counties in Ohio and West Virginia that surround the DuPont Washington Works PFOA facility (C8 study area). Analyses were adjusted for several factors including age, sex, diagnosis year, smoking status (current, past, unknown, or never), and insurance provider (government-insured Medicaid, uninsured, unknown, or privately insured). There was a statistically significant increase in the odds of kidney cancer when comparing both the high (OR = 2.0; 95% CI: 1.3, 3.2) and the very high (OR = 2.0; 95% CI: 1.0, 3.9) exposure categories to the unexposed reference population. Vieira et al. (2013) was selected for modeling over Barry et al. (2013), the populations of which likely overlapped, because Barry et al. (2013) did not report the necessary exposure measurements for CSF calculation. Specifically, exposure levels were reported separately for the community participants and workers, but not for the overall study population and therefore, CSF calculations were not feasible. Vieira (2013, 2919154) included the most complete and up-to-date data from this population, including all information needed for CSF derivation.

The high-exposure occupational study by Steenland and Woskie (2012) was not selected for dose-response analysis because it was limited by the small number of observed cancer cases (six kidney cancer deaths) and the exposure levels reported in the study population (average annual serum concentration of 350 ng/mL) are less comparable to the U.S. general population than the levels reported by Shearer et al. (2021) and Vieira et al. (2013). The study by Raleigh et al. (2014) was also not selected prioritized because of the concerns of exposure assessment methods (i.e., estimated air PFOA concentrations rather than biomonitoring data) and study quality (i.e., relatively small numbers of cases and lack of information regarding adjustment of risk factors for kidney cancer such as smoking status and BMI).

4.2.1.2 Animal Toxicological Studies

Three chronic studies are available that investigate the relationship between dietary PFOA exposure and carcinogenicity in male and female rats (NTP, 2020; Butenhoff et al., 2012; Biegel et al., 2001). Combined, at least two of the three studies report increased incidences each of hepatic, testicular, and pancreatic neoplastic lesions. Increased incidences of neoplastic lesions were primarily observed in male rats, though results in females, particularly the reports of rare tumor types (i.e., pancreatic acinar cell adenomas and adenocarcinomas), are supportive of potential carcinogenicity of PFOA. Additionally, NTP (2020) observed marginally increased incidences of uterine adenocarcinomas in female Sprague-Dawley rats during the extended evaluation (i.e., uterine tissue which included cervical, vaginal, and uterine tissue remnants). Uterine adenocarcinomas were not selected for CSF derivation because "the strength of the response was marginal and there was a low concurrent control incidence that lowered confidence in the response" (NTP, 2020). Butenhoff et al. (2012) identified mammary fibroadenomas and ovarian tubular adenomas in female rats, though there were no statistical differences in incidence rates between PFOA-treated groups and controls. These tumor types were also not selected for CSF derivation because the incidences were not observed by NTP (2020). As these results are inconclusive and there was increased magnitude of hepatic and pancreatic tumor incidences in males, likely due to the increased sensitivity of male rats resulting from toxicokinetic differences between the sexes (see Section 3.3.1), quantitative analyses were focused on males rather than females.

Butenhoff et al. (2012) and Biegel et al. (2001) reported dose-dependent increases in testicular LCTs. Additionally, LCT incidence at similar dose levels was comparable between the two studies (11 and 14%, respectively). PACTs were observed in both the NTP (2020) and Biegel et al. (2001) studies. NTP (2020) reported increased incidences of pancreatic acinar cell adenomas and adenocarcinomas in males in all treatment groups compared with their respective controls. These rare tumor types were also observed in female rats in the highest dose group, though the increased incidence did not reach statistical significance. Biegel et al. (2001) reported increases in the incidence of PACTs in male rats treated with PFOA, with zero incidences observed in control animals. In addition, both NTP (2020) and Biegel et al. (2001) reported dose-dependent increases in the incidence of liver adenomas in male rats. NTP (2020) also reported several male rats with hepatocellular carcinomas in the highest dose group (300/80 ppm). Butenhoff et al. (2012) additionally reported incidences of hepatocellular carcinomas in male rats from every treatment group, including controls, and female rats in the highest dose group. Given the consistency across the three available studies, the observation of malignant pancreatic and

hepatic tumors, and the site concordance between the testicular tumors in rats and humans, tumors from all three sites (i.e., liver, pancreas, testes) were selected for CSF derivation.

In further evaluation of the studies, Biegel et al. (2001) was not considered for dose-response modeling because it is a single-dose study. Therefore, NTP (2020) was selected for candidate CSF derivation for the PACTs and hepatocellular tumors and Butenhoff et al. (2012) was selected for candidate CSF derivation for LCTs.

4.2.2 Candidate CSF Derivation

4.2.2.1 Epidemiological Studies

EPA calculated CSFs for RCC from Shearer et al. (2021) and for kidney cancer from Vieira et al. (2013) based on the method used in CalEPA (2021) and for its *Public Health Goals for Arsenic in Drinking Water* (OEHHA, 2004). Details are provided in the Appendix (U.S. EPA, 2024a). The underlying model involves a linear regression between PFOA exposure and cancer relative risk used to estimate the dose-response between PFOA and RCC or kidney cancer risk. This was calculated using a weighted linear regression of the quartile specific RRs, with the weights defined as the inverse of the variance of each RR. Since the incidence of kidney cancer is relatively low and because the cases and controls were matched on age (or models were adjusted for age in Vieira et al. (2013)), the ORs represent a good approximation of the underlying RRs. The CSF is then calculated as the excess cancer risk associated with each ng/mL increase in serum PFOA (internal CSF). The internal CSF was calculated by first converting the linear regression model discussed above from the RR scale to the absolute risk scale. This was done assuming a baseline risk (R_0) of RCC or kidney cancer in an unexposed or lower exposure reference group. Since this is not available in a case-control study, the lifetime risk of RCC in U.S. males is used. For Shearer et al. (2021), the lifetime RCC risk was estimated by multiplying the lifetime risk of kidney cancer in U.S. males (American Cancer Society, 2020) by the percentage of all kidney cancers that are the RCC subtype (90%). This gives an R_0 of $0.0202 \times 90\% = 0.0182$. For Vieira et al. (2013), the lifetime kidney cancer of R_0 of 0.0202 was used, and the model fit was better when the highest exposure level was excluded. The internal CSF was then calculated as either the product of the upper 95% CI or the central tendency of the dose-response slope and R_0 and represents the excess cancer risk associated with each ng/mL increase in serum PFOA. The internal serum CSF was converted to an external dose CSF, which describes the increase in cancer risk per 1 ng/(kg-day) increase in dose. This was done by dividing the internal serum CSF by the selected clearance value, which is equivalent to dividing by the change in external exposure that results in a 1 ng/mL increase in serum concentration at steady-state. The clearance value used was the same as that used in the updated Verner model for endpoints related to developmental exposure (Table 4-6).

The results of the modeling and the candidate CSFs derived are presented in Table 4-12.

Table 4-12. Candidate Cancer Slope Factors Based on Epidemiological Data

Tumor Type	Reference, Confidence	Strain/ Species/Sex/ Age	POD Type, Model	Internal CSF – Increase in Cancer Risk per 1 ng/mL Serum Increase	CSF – Increase in Cancer Risk per 1 ng/(kg*d) Increase in Dose
Renal cell carcinoma (RCC)	Shearer et al. (2021) *Medium*	Human, male and female 55–74 yr	CSF serum in adults (per ng/mL of serum PFOA); upper limit of the 95% CI	3.52×10^{-3} $(ng/mL)^{-1}$ (see Appendix (U.S. EPA, 2024a)for additional detail)	0.0293 $(ng/kg/d)^{-1}$
Kidney cancer	Vieira et al. (2013) *Medium*	Human, male and female, median age 67 years	CSF serum in adults (per ng/mL of serum PFOA); upper limit of the 95% CI, highest exposure group excluded	4.81×10^{-4} $(ng/mL)^{-1}$ (see Appendix (U.S. EPA, 2024a)for additional detail)	0.00401 $(ng/kg/d)^{-1}$

Notes: CI = Confidence Interval; CSF = cancer slope factor; POD = point of departure.

EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a) notes that approaches for combining datasets in dose-response modeling may be used when datasets are statistically and biologically compatible. This type of approach was utilized in the CalEPA analysis of kidney cancer (CalEPA, 2021). EPA therefore considered this approach for candidate CSF derivation and performed a sensitivity analysis to derive a CSF_{serum} based on the pooled data from Shearer et al. (2021) and Vieira et al. (2013). These analyses are presented in Appendix E (U.S. EPA, 2024a). However, EPA identified several considerable differences between the two studies, including the outcome measured (RCC versus any kidney cancer) and the exposure metric (measured vs. modeled serum PFOA), among others. Additionally, the slope of the dose-response relationship was very different between the two studies (0.0981, 95% CI: 0.0025, 0.1937 *vs.* 0.0122, 95% CI: 0.0006, 0.0238 from Shearer et al. (2021) and Vieira et al. (2013), respectively). Given these differences, EPA determined that these two studies are not statistically or biologically comparable and therefore, they were not pooled for dose-response modeling (U.S. EPA, 2012a).

4.2.2.2 Animal Toxicological Studies

In the 2016 PFOA HESD (U.S. EPA, 2016c), EPA derived a CSF based on LCTs reported by Butenhoff et al. (2012). At that time, the dose-response relationship for the LCTs observed by Butenhoff et al. (2012) was modeled using EPA's Benchmark Dose Software (BMDS) Version 2.3.1. The multistage cancer model predicted the dose at which a 4% increase in tumor incidence would occur. The 4% increase was chosen as the low end of the observed response range within the Butenhoff et al. (2012) results. EPA has reanalyzed the LCTs reported by Butenhoff et al. (2012) in the current effort using the updated animal and human PK models described in Section 4.1.3 and an updated version of BMDS (Version 3.2). These modeling results are described in Appendix E (U.S. EPA, 2024a). A BMR of 10% was modeled because it is the recommended standard level for comparison across chemicals (U.S. EPA, 2012a). However, for this dataset, a

BMR of 10% resulted in a BMDL value higher than the lowest dose tested (see Appendix E (U.S. EPA, 2024a)). Therefore, a BMR of 4% was ultimately selected because it was representative of the low end of the observed response range within the study results (U.S. EPA, 2012a).

EPA also derived candidate CSFs for the tumor types observed in the NTP study that provide further evidence of carcinogenic activity of PFOA in male Hsd:Sprague-Dawley rats: hepatocellular neoplasms (hepatocellular adenomas and carcinomas) and acinar cell neoplasms (adenomas and adenocarcinomas) of the pancreas (NTP, 2020) (Table 4-13). A BMR of 10% was selected for these tumor types, consistent with the BMD Technical Guidance (U.S. EPA, 2012a). For all tumor types, dichotomous models were used to fit dose-response data.

For LCTs reported by Butenhoff et al. (2012), 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. For tumor types reported by NTP (2020), the $C_{avg\_pup\_total}$ was selected for this model to account for the perinatal window of exposure. As discussed previously in Section 4.1.3.1.3, the $C_{avg\_pup\_total}$ metric averages out the concentration in the pup from conception to the end of the 2 years by adding the area under the curve in gestation/lactation to the area under the curve from diet (postweaning) and dividing by 2 years. The BMDS produced BMDLs in mg/L for all tumor types. The animal PODs were converted to POD_{HED}s by multiplying the POD by the human clearance value (Table 4-6). This POD_{HED} is equivalent to the constant exposure, per body weight, which would result in serum concentration equal to the POD at steady state. The candidate CSF is then calculated by dividing the BMR by the POD_{HED}. These modeling results are described further in the Appendix (U.S. EPA, 2024a).

Table 4-13. Candidate Cancer Slope Factors Based on Animal Toxicological Data from 2-year Cancer Bioassays

Tumor Type	Reference, Confidence	Strain/ Species/Sex	POD Type, Model	POD Internal Dose/Internal Dose Metric[a]	POD_{HED}	CSF (BMR/POD_{HED})	Notes on Model Selection
Leydig Cell Adenomas in the Testes	Butenhoff et al. (2012) *Medium*	Male Sprague-Dawley Rats	$BMDL_{4RD}$, Multistage Degree 1	27,089.3 AUC_{avg} (mg × d/L)	4.75×10^{-3} mg/kg/day	8.42 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.
Hepatocellular Adenomas or Carcinoma	NTP (2020) *High*	F_1 Male Sprague-Dawley Rats, Perinatal and Postweaning Exposure	$BMDL_{10RD}$, Multistage Degree 2	88.7 ($C_{avg\_pup\_total}$ in mg/L)	1.06×10^{-2} mg/kg/day	9.4 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.
Hepatocellular Adenomas	NTP (2020) *High*	F_1 Male Sprague-Dawley Rats, Perinatal and Postweaning Exposure	$BMDL_{10RD}$, Multistage Degree 2	93.0 ($C_{avg\_pup\_total}$ in mg/L)	1.12×10^{-2} mg/kg/day	9.0 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.
Pancreatic Acinar Cell Adenoma or Adenocarcinoma	NTP (2020) *High*	F_1 Male Sprague-Dawley Rats, Perinatal and Postweaning Exposure	$BMDL_{10RD}$, Multistage Degree 3	15.2 ($C_{avg\_pup\_total}$ in mg/L)	1.83×10^{-3}	54.7 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.
Pancreatic Acinar Cell Adenoma	NTP (2020) *High*	F_1 Male Sprague-Dawley Rats, Perinatal and Postweaning Exposure	$BMDL_{10RD}$, Multistage Degree 1	15.7 ($C_{avg\_pup\_total}$ in mg/L)	1.88×10^{-3}	53.2 $(mg/kg/day)^{-1}$	Model selected based on lowest AIC as all models had adequate fit and BMDLs were within sufficiently close.

Notes: AUC = area under the curve; $BMDL_{4RD}$ = benchmark dose level corresponding to the 95% lower confidence limit of a 4% change; $BMDL_{10RD}$ = lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% change; BMR = benchmark response; CSF = cancer slope factor; NTP = National Toxicology Program.

[a] See Appendix (U.S. EPA, 2024a) for additional details on benchmark dose modeling.

4.2.3 Overall CSF Selection

Overall, recently published studies and the candidate CSFs indicate that PFOA is a more potent carcinogen than previously understood and described in the 2016 PFOA HESD (U.S. EPA, 2016c). To select an overall CSF, EPA focused on the CSFs derived from the epidemiological data consistent with the IRIS Handbook which states "when both laboratory animal data and human data with sufficient information to perform exposure-response modeling are available, human data are generally preferred for the derivation of toxicity values" (U.S. EPA, 2022d). As with data underlying noncancer RfDs, the use of human data eliminates the uncertainties associated with interspecies extrapolation and the toxicokinetic differences between species which are major uncertainties associated with the PFOA animal toxicological studies due to the half-life differences and sex-specific toxicokinetic differences in rodent species. The use of human data also ensures that the values are based on human-relevant exposure conditions and human-relevant tumor types/sites.

Therefore, EPA selected the critical effect of renal cell carcinomas in human males reported by Shearer et al. (2021) as the basis of the overall CSF for PFOA. Shearer et al. (2021) is a well-conducted, multicenter case-control epidemiological study nested within NCI's PLCO with median PFOA levels relevant to the general U.S. population. The CSF derived from Shearer et al. (2021) was selected as the overall CSF over the CSF derived from Vieira et al. (2013) due to multiple study design considerations. Specifically, Shearer et al. (2021) exhibited several preferred study attributes compared with the Vieira et al. (2013) include specificity in the health outcome considered (RCC vs. any kidney cancer), the type of exposure assessment (serum biomarker vs. modeled exposure), the source population (multicenter vs. Ohio and West Virginia regions), and study size (324 cases and 324 matched controls vs. 59 cases and 7,585 registry-based controls).

The resulting overall CSF for PFOA based on RCC reported by Shearer et al. (2021) is 0.0293 $(ng/kg/day)^{-1}$ (29,300 $(mg/kg/day)^{-1}$).

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 the potential for increased risk for cancer due to early lifestage susceptibility to chemical exposure (U.S. EPA, 2005a, b). Per EPA guidelines, 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, most of the studies assessing mutagenicity following PFOA exposure were negative and therefore, PFOA 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 PFOA (U.S. EPA, 2011a).

EPA additionally evaluated whether there are chemical-specific data for early-life exposure to PFOA and determined that there is insufficient information available from epidemiological and animal toxicological studies to adequately determine whether 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 PFOA exposure. However, there are two studies that assessed cancer risk after PFOA exposure during various developmental stages.

An NTP 2-year cancer bioassay in rats chronically exposed to PFOA both perinatally and postweaning did not report an increased cancer risk compared with chronic postweaning-only exposure (see further study design details in Section 3.4.4.2.1.2 and study results in Section 3.5.2), which suggests no increased cancer risk as a result of lifetime exposure compared with postweaning-only exposure. The NTP cancer bioassay does not include dose groups that were only exposed during early-lifestages (i.e., only during development) and therefore, the findings of the NTP cancer bioassay do not provide a basis for quantitatively estimating the difference in susceptibility between early-life and adult exposures. The other study, by Filgo et al. (2015), reported equivocal evidence of hepatic tumors in three strains of F_1 female mice perinatally treated with PFOA from GD 1–17, with potential residual exposure through lactation, and necropsy at 18 months of age. This study is also limited in that there was no adult-only exposure comparison group, the authors only assessed female mice, and the authors only histopathologically examined the liver (Filgo et al., 2015). In summary, the available studies do not provide information on whether early-life PFOA exposures result in increased cancer incidence compared with adult-only exposure. Due to the lack of evidence supporting postnatal early-life susceptibility to PFOA exposure, EPA did not adjust the risk value using chemical-specific data.

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 Perfluorooctanoic Acid (PFOA*) and Drinking Water Health Advisory (U.S. EPA, 2016a, c), EPA qualitatively considered epidemiological studies as a supporting line of evidence but did not quantitatively consider them for POD derivation, citing the following as reasons to exclude the epidemiological data that were available at that time from quantitative analyses:

- Unexplained inconsistencies in the epidemiological database,
- The use of mean serum PFOA concentrations rather than estimates of exposure,
- Declining serum PFOA 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 PFOA, 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 PFOA (see Sections 3.1.1, 3.4, and 3.5). Further, new tools that have facilitated the use of study quality evaluation as part of systematic review have enabled EPA to systematically assess 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 PFOA 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 five health outcomes and five epidemiological endpoints within these outcomes (i.e., decreased antibody response to vaccination in children, decreased birthweight, increased total cholesterol, increased ALT, and increased risk of kidney cancer) 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 PFOA 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 4.1.3, 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 PFOA exposure concentrations from the serum PFOA 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 PFOA toxicity due to differential toxicokinetics.

An additional source of uncertainty in using epidemiological data for POD derivation for chronic, non-developmental effects, is the documented decline in human serum PFOA levels over time, which raises concerns about whether one-time serum PFOA measurements are a good representation of lifetime peak exposure. Because of PFOA's long half-life in serum, however, one-time measurements likely reflect several years of exposure (Lorber and Egeghy, 2011). Importantly, EPA considered multiple time periods when estimating PFOA exposure, ranging from the longest period with available data on PFOA 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 PFOA exposure estimates based on the serum PFOA data for 1999–2018, which is likely to capture the peak PFOA exposures in the United States, which occurred in the 1990's (Dong et al., 2019). The modeling results show that the 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 PFOA 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 PFOA 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 PFOA 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 PFOA exposure could be explained in part by confounding from other PFAS exposures, including the metabolism of precursor compounds to PFOA 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 PFOA 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 the *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 PFOA serum concentrations, especially as biotransformation efficiency appears to vary depending on the precursor of interest (Mcdonough et al., 2022; D'eon and Mabury, 2011; Lorber and Egeghy, 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 aqueous film-forming foam use may have different precursor PFOA 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 PFOA serum concentrations and endpoints of interest in populations with varying exposure histories, including the general population and high-exposure communities. EPA observed associations for several endpoints in populations known to have been predominantly exposed to PFOA (e.g., 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 PFOA 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, many of which reported on a national scale (e.g., using NHANES data). The identified studies (most from the early-mid 2000's) reported that serum concentrations of PFOA were lower among people of color on average nationwide (Buekers et al., 2018; Kato et al., 2014; Nelson et al., 2012; Calafat et al., 2007). However, certain races/ethnicities may have relatively higher serum concentrations than others depending on the geographic location and the specific PFAS sampled (Park et al., 2019c). 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. The Appendix (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 PFOA 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 PFOA, such confounding may occur if there are other PFAS that are moderately or highly correlated with PFOA, associated with the outcome of interest, and not on the causal pathway between PFOA and the outcome. If the association between co-occurring PFAS and the outcome is in the same direction as the association between PFOA 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 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 PFOA and the outcome of interest, adjusted for other covariates and the co-pollutants. Another approach is to screen large groups of exposures to identify which ones are most strongly related to the outcome, using principal components analysis, elastic net regression, and Bayesian kernel machine regression (BKMR). Each of these approaches has strengths and limitations. For example, when PFOA and the co-pollutants 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 co-pollutants, 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 PFOA 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 co-pollutants, in order to assesses the extent to which there may be confounding by other PFAS in studies reporting the associations between PFOA and birth weight.

5.1.1.2 Multipollutant Models of PFOA and Birth Weight

When assessing the associations between PFOA 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 PFOA and another PFAS and when the magnitude of the association between the co-exposure and the health effect is large.

In order to identify the most co-occurring PFAS, Table 5-1 shows correlations between PFOA and other PFAS exposures in the nine studies on PFOA and birth weight that included mutually adjusted models. Four of these studies are *medium* confidence studies (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 (around 0.6) between PFOA and PFOS were consistently observed in six of the seven studies that reported such information. Correlations between PFOA with other commonly examined PFAS, including PFNA (four studies), PFDA (four studies), and PFHxS (five studies), were less consistent but generally weaker than correlations with PFOS, suggesting that other PFAS may not consistently co-occur with PFOA.

Table 5-1. Correlation Coefficients Between PFOA and Other PFAS in Mutually Adjusted Studies

Reference	Study Setting	Correlations with PFOA			
		PFOS	PFNA	PFDA	PFHxS
Ashley-Martin et al. (2017)[a] *High*	Canada (10 cities)	0.59	–[b]	–	0.47
Luo et al. (2021)[a] *High*	Guangzhou, China	0.11	0.28	0.19	0.02
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	0.76	0.56	0.61
Lenters et al. (2016)[e] *Medium*	Greenland; Kharkiv, Ukraine; Warsaw, Poland	0.61	0.30	0.50	0.34
Meng et al. (2018)[d] *Medium*	Denmark	0.66	0.47	0.28	0.33
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 log_{10}-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 type not specified.

[g] Correlations not reported numerically. Heat map of correlation coefficients (Figure S2, in Woods et al. (Woods et al., 2017)) shows positive correlations between PFOA and PFOS, 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 PFOA and birth weight is evaluated by comparing the magnitude and direction of the effects from the single-PFOA 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 studies included PFOS but varied with respect to other PFAS considered (Table 5-2). Lenters et al. (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 or more precise results following statistical adjustment for other PFAS.

Starling et al. (Starling et al., 2017) observed a statistically significant association between PFOA and birth weight reductions in the single pollutant model. This association increased in magnitude but precision was decreased in the multipollutant OLS model with four other PFAS. PFOA was also retained in the elastic net regression model, showing an inverse relationship with birth weight, but the association was attenuated. Lenters et al. (Lenters et al., 2016) reported associations between PFOA and reduced birth weight in single pollutant OLS and in elastic net regression models with PFOA retained but the association attenuated. Luo et al. (Luo et al., 2021) observed non-significant inverse associations between PFOA and birth weight in single pollutant and in BKMR models. Manzano-Salgado et al. (Manzano-Salgado et al., 2017a) and Shoaff et al. (Shoaff et al., 2018) reported null results in single and in multi-PFAS regression models. Meng et al. (Meng et al., 2018) observed an association between PFOA and reduced birth weight in the single pollutant model; this association was attenuated in a multipollutant model with PFOS.

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 impact of co-pollutants difficult. Ashley-Martin et al. (Ashley-Martin et al., 2017) and Robledo et al. (Robledo et al., 2015) reported non-significant inverse associations between PFOA and birth weight in girls in multipollutant models. Woods et al. (Woods et al., 2017) reported on an overlapping population from the same HOME cohort as Shoaff et al. (Shoaff et al., 2018) and observed non-significant inverse associations from a multipollutant Bayesian hierarchical linear model. PFOA was not selected for inclusion in an elastic net regression model 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 often attenuated 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 PFOA birth weight associations (Ashley-Martin et al., 2017; Woods et al., 2017; Robledo et al., 2015). Considering all nine studies together, it is challenging to draw definitive conclusions about the extent of confounding by co-occurring PFAS, particularly given differences in modeling approaches, PFAS adjustment sets, and exposure contrasts used across studies. Additionally, these studies represented only a small fraction of the total number of studies examining associations between PFOA 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 PFOA and birth weight deficits are fully attributable to confounding by co-occurring PFAS.

Similar conclusions can be drawn for other health outcomes. Budtz-Jorgensen (Budtz-Jørgensen and Grandjean, 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 PFOA exposure, and there was no notable attenuation of the observed effects after estimates were adjusted for PFOS (see Section 3.4.2.1.2.1) (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. (Lin et al., 2010) performed multipollutant modeling for the effects on serum ALT and observed that when PFOS, PFHxS, and PFNA were simultaneously added in the fully adjusted regression models, the significant positive association between PFOA exposure and ALT remained and was slightly larger. Fan et al. (Fan et al., 2020) examined cross-sectional associations between exposure to PFOA and increased TC in single- and multipollutant models in a sample of adults from NHANES (2012–2014). Exposure to PFOA was associated with statistically significantly elevated TC in the single-pollutant model, but the association was no longer significant in multipollutant analyses. A statistically significant 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 PFOA and the four priority noncancer health outcomes are confounded by 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 PFOA 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 Other PFAS Adjustment on PFOA Birth Weight Results	PFAS Adjustments
Ashley-Martin et al. (Ashley-Martin et al., 2017) *High*	NR	Girls: −89.51 (−263.40, 84.38) Boys: −35.51 (−198.99, 127.97)	–[c]	log_{10}-unit (ng/mL) increase	–	PFOS, PFHxS
Luo et al. (Luo et al., 2021) *High*	−62.37 (−149.08, 24.35)	−24 (−84, 36)[d]	–	Single PFAS model: ln-unit (ng/mL) increase Multi-PFAS model: 75th vs. 25th percentile	Attenuated	PFOS, PFBA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS, 6:2 Cl-PFESA, 8:2 Cl-PFESA
Manzano-Salgado et al. (Manzano-Salgado et al., 2017a) *High*	−9.33 (−38.81, 20.16)	1.02 (−42.73, 44.77)	–	log_2-unit (ng/mL) increase	Slightly attenuated	PFOS, PFNA, PFHxS
Shoaff et al. (Shoaff et al., 2018) *High*	−0.03 (−0.17, 0.10)[e]	0.00 (−0.16, 0.18)[e]	–	log_2-unit (ng/mL) increase	Slightly attenuated	PFOS, PFNA, PFHxS
Starling et al. (Starling et al., 2017) *High*	−51.4 (−97.2, −5.7)	−69.66 (−148.19, 8.87)	−14.47	ln-unit (ng/mL) increase	Attenuated	PFOS, PFNA, PFDA, PFHxS
Lenters et al. (Lenters et al., 2016) *Medium*	−78.52 (−137.01, −20.03)	–	−38.82	2 SD ln-unit (ng/mL) increase	Attenuated	PFOS, PFNA, PFDA, PFHxS, PFHpA, PFUnDA, PFDoDA
Meng et al. (Meng et al., 2018)[f] *Medium*	−35.6 (−66.3, −5.0)	−9.94 (−52.63, 32.75)	–	log_2-unit (ng/mL) increase	Attenuated	PFOS

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 Other PFAS Adjustment on PFOA Birth Weight Results	PFAS Adjustments
Robledo et al. (Robledo et al., 2015)[g] *Medium*	NR	Girls: −61.64 (−159.15, 35.87) Boys: 4.78 (−85.44, 95.01)	–	1 SD ln-unit (ng/mL) increase	–	PFOS, PFDA, PFNA, PFOSA, Et-PFOSA-AcOH, Me-PFOSA-AcOH
Woods et al. (Woods et al., 2017) *Medium*	NR	−13 (−54, 27)[h]	N/S	log_{10}-unit (ng/mL) increase	–	PFOS, PFNA, PFDA, PFHxS

Notes: N/S = PFOA not selected in elastic net regression model; SD = standard deviation.
[a] From ordinary least squares regression models unless otherwise specified.
[b] Outcome variable unit is grams unless otherwise specified.
[c] Not applicable.
[d] Results estimated from Luo et al. (Luo et al., 2021) Figure 3 using WebPlotDigitizer. Results are from a Bayesian kernel machine regression model comparing the PFOA at its 75th vs. 25th percentile, holding other PFAS constant at their 50th percentiles.
[e] Outcome variable unit in Shoaff et al. (Shoaff et al., 2018) models is birth weight *z*-score.
[f] Meng et al. (Meng et al., 2018) estimates associations between serum PFOA and birth weight in three samples of the Danish National Birth Cohort, two of which were analyzed by the same laboratory for PFOA, PFOS, PFDA, PFNA, PFHxS, and PFHpS and one of which was analyzed by a separate laboratory for PFOA and PFOS only.
[g] Robledo et al. (Robledo et al., 2015) estimated associations using both maternal and paternal PFAS concentrations; results shown here are from maternal PFAS models, also adjusted for "the individual and partner sum of remaining chemical concentrations in each chemical's respective class."
[h] Effect estimates and posterior 95% credible intervals based on a Bayesian hierarchical linear model. Results estimated from Woods et al. (Woods et al., 2017) Figure 1 using WebPlotDigitizer.

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-, 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, PFOA 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. For PFOA, sex-specific differences in the toxicokinetics of rats is an additional source of uncertainty. To address toxicokinetic differences between species and sexes, 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 that 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 PFOA exposure on serum lipid levels (i.e., potential non-monotonic dose-response relationships that are not easily assessed in animal studies due to low dose levels needed to elicit the same response observed in humans).

Third, there may be differences in mechanistic responses between humans and animal models. One example of this is the PPARα response. It is unclear to what extent PPARα influences the responses to PFOA exposure observed in humans, though it has been shown that the rodent PPARα response is greater than that 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 PFOA 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 PFOA HESD (U.S. EPA, 2016c) 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 PFOA 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 PFOA HESD, EPA did not use BMD modeling to derive PODs, and instead relied on the NOAEL/LOAEL approach for all candidate studies and endpoints (U.S. EPA, 2016c). 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 PFOA HESD, EPA derived a candidate RfD based on the endpoints of decreased parental body weight and increased parental absolute and relative kidney weight reported by Butenhoff et al. (Butenhoff et al., 2004a), all of which shared a common POD (LOAEL). 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 entire 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 together 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 2016 from the Butenhoff et al. (Butenhoff et al., 2004a) study, systemic effects such as body weight and renal effects such as kidney weight were reevaluated and determined to have *evidence suggestive* of an association with PFOA exposure. As described in Section 4.1.1 of this assessment, EPA derived PODs only for endpoints from health outcomes with *evidence indicating* or *evidence demonstrating* an association with PFOA exposure.

Additionally, for the current assessment, EPA preferentially selected endpoints for which there were a greater number of studies supporting the observed effect. For example, for the 2016 PFOA HESD, EPA derived a candidate RfD based on the co-critical effect of accelerated male puberty reported by Lau et al. (Lau et al., 2006). Results of the current assessment's literature search showed that no *high* or *medium* confidence studies supporting that observed effect have been published since 2016. As Lau et al. (Lau et al., 2006) was also the only study identified in 2016 that reported an acceleration of male puberty (a second study reported a delay in male puberty (Butenhoff et al., 2004a) and there were several other developmental endpoints (e.g., reduced offspring weight and survival, delayed eye opening) that were supported by multiple studies, EPA did not further consider this endpoint from Lau et al. (Lau et al., 2006) for POD derivation in the present assessment. Similarly, upon further evaluation during the current assessment of the co-critical effects of reduced forelimb and hindlimb ossification in pups reported by Lau et al. (Lau et al., 2006), it was determined that an unexplained non-linear dose-response trend adds uncertainty to selection of the LOAEL as the POD. As reduced ossification was only observed at the highest dose tested (10 mg/kg/day) by the one other study (Yahia et al., 2010) that tested dose levels close to the LOAEL from Lau et al. (Lau et al., 2006) (1 mg/kg/day) and because no studies identified during literature searches for the current assessment reported this effect, EPA relied on other endpoints from Lau et al. (Lau et al., 2006) that were amenable to BMD modeling, exhibited dose-dependent response trends, and were supported by at least one other study in the available literature.

For some health effects that served as the basis for candidate RfDs in the 2016 PFOA HESD, new studies published since 2016 provide more information about these same endpoints. For example, in 2016, EPA derived a candidate RfD based on increased liver weight and necrosis in rats reported by Perkins et al. (Perkins et al., 2004). Since that time, NTP (NTP, 2020) published an animal bioassay that has additional or improved study attributes compared to the older study. Specifically, the NTP (NTP, 2020) study was identified as a *high* confidence study (rather than *medium* confidence) that used a chronic (rather than 14-week) exposure duration, larger sample sizes (n = 50 rather than n = 15), and a dose range that was more sensitive to the histopathological effects in both male and female rats. Therefore, EPA considered liver necrosis data as reported by NTP (NTP, 2020) for POD derivation rather than data from the *medium* confidence study by Perkins et al. (Perkins et al., 2004).

For transparency, EPA has provided a comparison of studies and endpoints used to derive candidate RfDs for both the 2016 PFOA HESD and the present assessment (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	
Dewitt et al. (Dewitt et al., 2008), *medium* confidence – reduced immunoglobulin M (IgM) response	Dewitt et al. (Dewitt et al., 2008), *medium* confidence – reduced IgM response
Developmental	

Studies and Effects Used in 2016 for Candidate RfD Derivation[b]	Studies and Effects Used in 2024 for Candidate RfD Derivation
Lau et al. (Lau et al., 2006), *medium* confidence – reduced pup ossification (forelimb and hindlimb) and accelerated male puberty (preputial separation)	Lau et al. (Lau et al., 2006), *medium* confidence – delayed time to eye opening
Wolf et al. (Wolf et al., 2007), *medium* confidence – decreased pup body weight	Song et al. (Song et al., 2018), *medium* confidence – decreased pup survival
Hepatic	
Perkins et al. (Perkins et al., 2004), *medium* confidence – increased liver weight and necrosis	NTP (NTP, 2020), *high* confidence – liver necrosis

Notes: RfD = reference dose; IgM = immunoglobulin M; NTP = National Toxicology Program.
[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 PFOA HESD or the current assessment.
[b] Candidate RfDs from the 2016 PFOA HESD that correspond to non-priority health outcomes (e.g., renal) are not presented here.

5.4 Consideration of Alternative Conclusions Regarding the Weight of Evidence of PFOA Carcinogenicity

While reviewing the weight of evidence for PFOA, EPA also evaluated consistencies of the carcinogenicity database with other cancer descriptors according to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). In the 2016 PFOA HESD, EPA determined that the available carcinogenicity database for PFOA at that time was consistent with the descriptions for *Suggestive Evidence of Carcinogenic Potential* (U.S. EPA, 2016c). Upon reevaluation for this assessment, the agency identified several new studies reporting on cancer outcomes that strengthened the evidence. As a result of conducting a weight of evidence evaluation of the available carcinogenicity database, EPA determined that PFOA is consistent with the descriptions for *Likely to Be Carcinogenic to Humans* according to the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), as described above. More specifically, the available data for PFOA surpass many of the descriptions for *Suggestive Evidence of Carcinogenic Potential* provided in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). The examples for which the PFOA database exceeds the *Suggestive* descriptions (outlined below) 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.' The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system (see discussions of conflicting evidence and differing results, below);
- 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;
- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend." (U.S. EPA, 2005a).

There are multiple *medium* or *high* confidence human and animal toxicological studies that provide evidence of multiple tumor types resulting from exposure to PFOA. The observed tumor types are generally consistent across human subpopulations (i.e., kidney (Shearer et al., 2021; Vieira et al., 2013) and testicular (Barry et al., 2013; Vieira et al., 2013)) and studies of equal confidence did not provide conflicting evidence for these cancer types. Studies within the same species of rat consistently report multisite tumorigenesis (i.e., testicular, pancreatic, and hepatic (NTP, 2020; Butenhoff et al., 2012; Biegel et al., 2001)) and there is no indication that a high background incidence or other intrinsic factors related to these tumor types are driving the observed responses. The SAB PFAS Review Panel agreed that: "a) the evidence for potential carcinogenicity of PFOA has been strengthened since the 2016 PFOA HESD; b) the results of human and animal studies of PFOA are consistent with the examples provided above and support a designation of 'likely to be carcinogenic to humans'; and c) the data exceed the descriptors for the three designations lower than 'likely to be carcinogenic'" (U.S. EPA, 2022e). See Table 5-4 below for specific details on how PFOA exceeds the examples supporting the *Suggestive Evidence of Carcinogenic Potential* cancer descriptor in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

While the SAB panel agreed that the data for PFOA exceed a *Suggestive* cancer descriptor, the final report also recommends "explicit description of how the available data for PFOA do not meet the criteria for the higher designation as 'carcinogenic'" (U.S. EPA, 2022e). After reviewing the descriptions of the descriptor *Carcinogenic to Humans,* EPA has determined that at this time, the evidence supporting the carcinogenicity of PFOA 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 all of 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 PFOA and cancer is currently lacking. The SAB similarly concluded that "the available epidemiologic data do not provide convincing evidence of a causal association but rather provide evidence of a plausible association, and thus do not support a higher designation of 'carcinogenic to humans'" (U.S. EPA, 2022e).

Additionally, though the available evidence indicates that there are positive associations between PFOA and multiple cancer types, there is uncertainty regarding the identification of carcinogenic MOA(s) for PFOA, particularly for renal cell carcinomas and testicular cancer in humans. The evidence of carcinogenicity in animals is limited to a single strain of rat, although PFOA tested positive for multisite tumorigenesis. The animal and mechanistic databases do not provide clarity to discern the MOA(s) of PFOA in humans, though there is some animal toxicological study

evidence supporting hormone-mediated MOAs for testicular tumors and oxidative stress-mediated MOAs for pancreatic tumors. The full mode of action 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. See Table 5-4 below for specific details on how PFOA 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 PFOA Carcinogenicity Database with Cancer Descriptors as Described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)

Comparison of Evidence for *Carcinogenic* and *Suggestive* Cancer Descriptors	
Carcinogenic to Humans	
"This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer" (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** There is evidence of a plausible association between PFOA exposure and cancer in humans, however, the database is limited to only two independent populations, 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.
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 events of the agent's mode of action but not enough for a causal association," (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** There is evidence of an association between human exposure and cancer, however, there is limited mechanistic information that could contribute to the determination of a causal relationship.
"There is extensive evidence of carcinogenicity in animals," (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** While there are three chronic cancer bioassays available, each testing positive in at least one tumor type, they were all conducted in the same strain of rat. The database would benefit from additional *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 and" (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** A definitive MOA has not been identified for each of the PFOA-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" (U.S. EPA, 2005a).	**PFOA data are not consistent with this description.** The animal database does not provide significant clarity on the MOA(s) of PFOA in humans, though there is some evidence supporting hormone-mediated MOAs for testicular tumors and oxidative stress-mediated MOAs for pancreatic tumors.
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).	**PFOA data exceed this description.** Statistically significant increases in tumor incidence of multiple tumor types were observed across several human and animal toxicological studies.

Comparison of Evidence for *Carcinogenic* and *Suggestive* Cancer Descriptors	
"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 PFOA 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).	**PFOA data exceed this description.** The studies from which carcinogenicity data are available were determined to be *high* or *medium* 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).	**PFOA data exceed this description.** Increases in kidney cancer in humans were statistically significant in two exposure groups in one study (Vieira et al., 2013), and there was a statistically significant increased odds for the highest exposure quartile and an increasing trend across exposure quartiles in a second study (Shearer et al., 2021). Statistically significant increases in hepatic and pancreatic tumors in male rats were observed in multiple dose groups with a statistically significant trend overall (NTP, 2020).

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 PFOA 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 PFOA 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 PFOA, two health outcomes that had characteristics of both *evidence suggests* and *evidence indicates* were the reproductive and endocrine 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 Perfluorooctanoic Acid (PFOA) (CASRN 335-67-1) in Drinking Water* (U.S. EPA, 2021c). 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 PFOA exposure may cause adverse reproductive or endocrine effects.

Epidemiological studies published since the 2016 PFOA HESD considered for evidence integration for adverse endocrine effects included many *high* and *medium* confidence studies. There was *slight evidence* to suggest human endocrine toxicity, including associations between PFOA exposure and changes in serum thyroxine (T4) in children, though there was considerable uncertainty in the results due to inconsistencies across sexes and age groups and a limited number of studies. Animal toxicological studies considered for evidence integration included eight *high* or *medium* confidence studies. Collectively, the animal evidence for an association between PFOA exposure and effects on the endocrine system was considered *moderate*, based on observed alterations in thyroid and adrenocortical hormone levels, increased thyroid gland weight, and increased thyroid follicular cell hypertrophy. Overall, the available evidence was *suggestive* but not *indicative* of adverse endocrine effects due to PFOA exposure. Therefore, EPA did not prioritize this health outcome for dose-response modeling. See Appendix C (U.S. EPA, 2024a) for a detailed description of endocrine evidence synthesis and integration.

Epidemiological studies of reproductive effects in males published since the 2016 PFOA HESD that were considered for evidence integration included three *medium* confidence studies (Cui et al., 2020; Petersen et al., 2018; Lopez-Espinosa et al., 2016) and one *low* confidence study (Di Nisio et al., 2019). Although there was *slight evidence* to suggest human male reproductive toxicity, including for effects on testosterone levels and sperm parameters, the associations were inconsistent across studies and populations, and it was difficult to assess the impacts of the alterations. Animal toxicological studies considered for evidence integration included three *high* confidence studies (NTP, 2020, 2019; Biegel et al., 2001) and five *medium* confidence studies (Song et al., 2018; Lu et al., 2016; Zhang et al., 2014b; Butenhoff et al., 2012; Li et al., 2011). The available animal data provided *slight evidence* that exposure to PFOA results in adverse effects to the male reproductive system, including changes to the testes and epididymis. However, the evidence from animal studies was inconsistent. Therefore, this health outcome was not prioritized for dose-response modeling. See Appendix C (U.S. EPA, 2024a) for a detailed description of male reproductive evidence synthesis and integration.

Female reproductive epidemiological studies published since the 2016 PFOA HESD that were considered for evidence integration included 1 *high* confidence study (Ding et al., 2020) and 10 *medium* confidence studies (Kim et al., 2020; Donley et al., 2019; Ernst et al., 2019; Wang et al., 2019; Crawford et al., 2017; Lum et al., 2017; Timmermann et al., 2017b; Wang et al., 2017; Lopez-Espinosa et al., 2016; Romano et al., 2016). Although there was *slight evidence* to suggest human female reproductive toxicity, including preeclampsia and gestational hypertension, there was conflicting evidence on altered puberty onset and limited data suggesting reduced fertility and fecundity. The associations were inconsistent across reproductive hormone parameters, and it was difficult to assess the adversity of these alterations. Animal toxicological studies considered for evidence integration included one *high* confidence study (NTP, 2019) and three *medium* confidence studies (Zhang et al., 2020b; Chen et al., 2017c; Butenhoff et al., 2012). The available animal data provided *slight evidence* that exposure to PFOA can result in alterations in ovarian physiology and hormonal parameters in adult female rodents following exposure to doses as low as 1 mg/kg/day. However, as with the available epidemiological studies, the evidence from animal studies was inconsistent. Therefore, this health outcome was not prioritized for dose-response modeling. See Appendix C (U.S. EPA, 2024a) for a detailed description of female reproductive evidence synthesis and integration.

Similar adverse reproductive and endocrine effects have been observed among the family of PFAS. For example, the developing fetus and thyroid were identified as targets following oral exposure to PFBS (U.S. EPA, 2021f), though the observed reproductive effects were considered equivocal. Additionally, EPA's 2021 assessment of GenX chemicals identified the reproductive system as a potential toxicological target (U.S. EPA, 2021e) and the final IRIS Toxicological Reviews for both PFBA (U.S. EPA, 2022c) and PFHxA (U.S. EPA, 2023b) 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 PFOA and reproductive and endocrine effects.

As the databases for endocrine and reproductive outcomes were *suggestive* of human health effects resulting from PFOA 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 suggest the potential for endocrine and reproductive effects after PFOA exposure. Studies on endocrine and reproductive 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. (Wambaugh et al., 2013) animal internal dosimetry model because it had availability of model parameters across 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 confidence 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 F, (U.S. EPA, 2024a)). However, these uncertain model parameters did not impact the median prediction used for BMD modeling and simply demonstrate that the available data are unable to identify all parameters across every species over the range of doses used for model calibration. Finally, the model is only parameterized using adult, single dose, PFOA study designs. 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. (Wambaugh et al., 2013) model fit the selected PFOA developmental study data well, though there are additional 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, PFOA concentrations in breast milk are assumed to partition passively from the maternal blood. This assumption does not account for the presence of active transport in the mammary gland or time-course changes for PFOA uptake to the milk. Despite these limitations, the incorporation of model parameters related to developmental lifestages is a significant improvement over the model used in the 2016 PFOA HESD, which did not implement life-course modeling (U.S. EPA, 2016c).

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 PFOA, 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.

In the Verner et al. (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 PFOA 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 PFOA 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 is well known for pharmaceuticals, but PFOA 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 might impact the distribution of PFOA.

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 PFOA in humans (see Appendix B, (U.S. EPA, 2024a)). The slow excretion of PFOA 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 PFOA 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 PFOA 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 depend on the ratio of the prior and ongoing exposures. 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. One probable example of this is the elimination half-life of approximately 120 or 200 days reported by Dourson and Gadagbui (Dourson and Gadagbui, 2021), who analyzed a clinical trial with exposures to PFOA of between 50 and 1,200 mg weekly for a period of 6 weeks. In this study, the average plasma level after 6 weeks ranged from 34 µg/mL at 0.1 mg/kg/day to 492.7 µg/mL at 2.3 mg/kg/day (Dourson et al., 2019). This is orders of magnitude greater than the blood levels seen in the general population (the 95th percentile serum PFOA concentration in NHANES 2007–08 was 9.7 ng/mL (Kato et al., 2011)) and is in the range of the maximum values seen at the peak of PFOA manufacturing (Post et al., 2012). The high exposure and short follow-up time may be the source of the short half-life observed in this population. In addition, this study was also carried out in patients with advanced cancer, which may have an effect on the rate of PFOA excretion.

A recent review publication (Campbell et al., 2022) addressing the variation in reported half-life values for PFOA promoted a half-life value of 1.3 years, based on the authors' analysis of half-life values estimated from paired blood and urine samples (Zhang et al., 2013c). The rationale for this was the exclusion of studies that may be biased upward by ongoing exposure, and studies that did not analyze linear and branched isomers of PFOA separately. A commentary in response to the review disputed this conclusion and the approach used to make it (Post et al., 2022). The authors pointed out two citations that explore the effect of explicitly correcting for background exposure: Russell et al. (Russell et al., 2015) and Bartell (Bartell, 2012). Both estimated half-lives >2 years after accounting for ongoing exposure. They go on to list several high-quality studies that estimated half-lives much longer than the value calculated from Zhang et al. (Zhang et al., 2013c). They also pointed out methodological limitations of Zhang et al. (Zhang et al., 2013c) and noted that another estimate of renal clearance using the same approach resulted in a considerably different value (Gao et al., 2015b). EPA is aware of two other studies estimating renal clearance of PFOA from measurements in urine, and both estimated longer half-lives than the value calculated by Zhang et al. (Zhang et al., 2013c). Fu et al. (Fu et al., 2016) estimated a half-life of 4.1 years and Fujii et al. (Fujii et al., 2015) estimated a renal clearance value of 0.044 mL/kg/day, equivalent to a half-life of 7.3 years. These additional measurements of PFOA half-life using a similar study design show that Campbell et al. (Campbell et al., 2022) selected an outlier study, both from other urinary clearance studies and from direct-observation studies.

Another factor EPA considered when evaluating Zhang et al. (Zhang et al., 2013c) was that the estimated value for the half-life of PFOS, geometric mean of 5.8 years for young females and 18 years for males and older females, is higher than is typically estimated. This result for PFOS illustrates that there are uncertainties in any single estimate. Campbell et al. (Campbell et al., 2022) selected an outlier study for the half-life of PFOA, both from other urinary clearance studies and from direct-observation studies. The range of results from among various studies represents a range of uncertainty and EPA has chosen a half-life based on study quality (i.e., representative population, environmentally relevant exposure, and multiple sampling of each individual) that results in a value intermediate among the published estimates.

There are few reported V_d values for humans because this parameter requires knowledge of the total dose or exposure, and V_d values are difficult to determine from environmental exposures. In addition to the V_d reported by Thompson et al. (Thompson et al., 2010b), which was selected by EPA for model parameterization, Dourson and Gadagbui (Dourson and Gadagbui, 2021) reported a human V_d of 91 mL/kg from a clinical trial on PFOA. This value is much lower than other reported values across mammalian species and may reflect an earlier initial distribution step rather than the distribution observed after chronic exposure. Chronic exposure may result in a greater distribution to tissues relative to the plasma, and this process may be slowed by extensive binding of PFOA to plasma proteins. Additionally, the exposure levels used in the clinical trial are much higher than typically seen in the general population, which could result in a different distribution profile.

Lastly, the description of breastfeeding in the updated Verner et al. (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 PFOA concentrations, and that weaning was an immediate process with the infant transitioning from a breastmilk-only 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 1-year breastfeeding duration 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 PFOA exposure via the introduction to solid foods. However, since lactational exposure is much greater than exposure after weaning, a breastfeeding scenario that does not account for potential PFOA 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 PFOA 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 PFOA 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. (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). For PFOA, the analyses did not generate viable models and therefore the comparison could not be made. These analyses are presented in detail in Appendix E (U.S. EPA, 2024a).

For PFOS, however, $BMDL_5$ 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 PFOA 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.

Given the previous issues that EPA encountered for other PFAS when implementing PBPK models and the known issue with the Loccisano model and the models based upon it, EPA selected a one-compartment model because it was the most robust available approach for this effort. Following the consideration and analysis of different models, EPA concluded that a one-compartment model is sufficient to predict blood (or serum/plasma) concentrations. Serum/plasma is a good biomarker for exposure, because a major proportion of the PFOA 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 describing the dosimetry of PFOA.

The two one-compartment approaches identified in the literature for PFOA was the model of Verner et al. (Verner et al., 2016) and the model developed by the Minnesota Department of Health (MDH model) (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 PFOA.

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 PFOA 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 PFOA during childhood growth without the need for an explicit term in the equations. Not accounting for growth will result in the overprediction of serum concentrations in individuals exposed during growth. Despite this, PFOA 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 PFOA. 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, PFOA is amphiphilic, not simply hydrophilic, and its distribution is driven by interactions with binding proteins and

transporters, not by passive diffusion with body water. While it is plausible that V_d is larger in children, it is unknown to what degree.

Since increased V_d in children is plausible but neither supported nor contradicted by direct evidence, EPA evaluated the effect of variable V_d by implementing this change 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. Since 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 for 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 exposure interval used in the critical study and/or the target population relevant to the 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, the 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 PFOA. Specifically, EPA concluded that the determination that assuming V_d in children equal to the adult values and calculating an 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 PFOA. These potentially susceptible populations include populations exhibiting a more severe response than others despite similar PFOA exposure due to increased biological sensitivity, as well as populations exhibiting a more severe response due to higher PFOA exposure and/or exposure to other chemicals or nonchemical stressors. Populations with greater biological sensitivity may include pregnant women and their developing fetuses, lactating women, the elderly, children, adolescents, and people with certain underlying medical conditions (see Section 5.8.1). Additionally, some available data indicates that there may be sex-specific differences in sensitivity to potential effects of PFOA (see Section 5.8.2). Populations that could exhibit a greater response to PFOA exposure due to higher exposures to PFOA or other chemicals include communities overburdened by chemical exposures or nonchemical stressors such as communities with environmental justice concerns (see Section 5.8.3).

The potential health effects after PFOA 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 PFOA across populations to assess whether racial/ethnic or socioeconomic differences are associated with greater PFOA exposure. However, the available research on PFOA's potential impacts on sensitive populations is limited and more research is needed. Health effects differences in sensitivity to PFOA exposure have not allowed for the identification or characterization of all potentially sensitive subpopulations. This lack of knowledge about susceptibility to PFOA represents a potential source of uncertainty in the assessment of PFOA.

5.8.1 Fetuses, Infants, Children

One of the more well-studied sensitive populations to PFOA exposure is developing fetuses, infants, and children. Both animal toxicological and epidemiological data suggest that the

developing fetus is particularly sensitive to PFOA-induced toxicity. As described in Sections 3.4.4.1 and 3.4.2.1, results of some epidemiological studies indicate an association between PFOA exposure during pregnancy and/or early childhood and adverse outcomes such as decreased birth weight and decreased antibody response to vaccination. 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 PFOA during gestation). Additionally, PFOA exposure to humans during certain lifestages or exposure windows (e.g., prenatal or early postnatal exposure windows) may be more consequential than others. 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 PFOA 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 that are considered eradicated (such as diphtheria or tetanus) could return to the United States (Hotez, 2019). As noted by Dietert et al. (Dietert et al., 2010), the risks of developing infectious diseases may increase if immunosuppression occurs in the developing immune system.

5.8.2 Sex Differences

In humans, potential sex differences in the disposition of PFOA in the body, as well as in the potential for adverse health effects in response to PFOA exposure, have not been fully elucidated. With respect to sex differences in the development of adverse health effects in response to PFOA exposure, the available epidemiological data are insufficient to draw conclusions, although some studies reported sex differences (e.g., an association between PFOA exposure and serum ALT in girls but not boys (Attanasio, 2019; Mora et al., 2018)). In some studies in rats, males appeared to be more sensitive to some effects than females, even when females received much higher PFOA doses (NTP, 2020; Butenhoff et al., 2004a).

With respect to ADME, research in humans indicates that PFOA half-lives in males are generally longer than those in females (Li et al., 2018c; Gomis et al., 2017; Fu et al., 2016). Some animal studies (in rats in particular) show the same sex difference, but additional research is needed to determine whether the underlying mechanisms identified in rats are relevant to humans. Female rats have been shown to absorb PFOA faster than male rats (Kim et al., 2016), and PFOA may distribute to some compartments (i.e., liver cytosol) to a greater extent in female rats compared with males (Han et al., 2005). Several studies have demonstrated that female rats and rabbits eliminate PFOA from the body faster than males (Dzierlenga et al., 2019a; NTP, 2019; Hinderliter et al., 2006b; Hundley et al., 2006). These studies and others are further described in Section 3.3.1 and Appendix B (U.S. EPA, 2024a).

Several studies have been conducted to elucidate the cause of the sex difference in the elimination of PFOA by rats (Cheng et al., 2006; Hinderliter et al., 2006b; Kudo et al., 2002). Many of the studies have focused on the role of transporters in the kidney tubules, especially the OATs and OATPs located in the proximal portion of the descending tubule (Yang et al., 2010;

Nakagawa et al., 2009; Yang et al., 2009b; Nakagawa et al., 2008). Generally, both *in vivo* and *in vitro* studies reported differences in renal transporters that are regulated by sex hormones and show consistent results indicating increased resorption of PFOA in male rats (see Section 3.3.1 and Appendix B, (U.S. EPA, 2024a)). Hinderliter et al. (Hinderliter et al., 2006b) found that a developmental change in renal transport occurs in rats between 3 and 5 weeks of age that allows for expedited excretion of PFOA in females and an inverse development in males. When considered together, the studies of the transporters suggest that female rats are efficient in transporting PFOA across the basolateral and apical membranes of the proximal kidney tubules into the glomerular filtrate, but male rats are not.

Although sex differences in rats have been relatively well studied, sex differences observed in mice were less pronounced (Lou et al., 2009; Lau et al., 2006) and were actually reversed in cynomolgus monkeys and hamsters (Hundley et al., 2006; Butenhoff et al., 2004b), indicating species-specific factors impacting elimination across sexes.

Although there is some evidence to suggest sex differences in humans exposed to PFOA, the mechanisms for these potential differences have not been fully explored. For example, postmenopausal females and adult males have longer PFOA elimination half-lives than premenopausal adult females (Zhang et al., 2013c). Partitioning to the placenta, amniotic fluid, fetus, menstruation, and breast milk represent important routes of elimination in humans and may account for some of the sex differences observed for blood and urinary levels of PFOA by sex and age. It is unclear whether hormone-dependent renal transporters play an additional role in the observed sex differences in PFOA half-life in humans. Additional research is needed to further elucidate these sex differences and their implications, and to ascertain whether the sex differences observed in some animal species are relevant to humans. This data gap represents a source of uncertainty in the elucidation of the risks of PFOA to humans.

5.8.3 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 PFOA exposure because of either greater biological sensitivity or higher exposure to PFOA 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 PFOA exposure are limited. To fully address equity and environmental justice concerns about PFOA, these data gaps regarding differential exposure and health effects after PFOA 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 PFAS National Primary Drinking Water Regulation* (U.S. EPA, 2024b)). EPA acknowledges that exposure to PFOA, 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 PFOA manufacturing and/or contamination) and that studies of these communities are high priority research needs.

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