Public Health Goals

FIRST PUBLIC REVIEW DRAFT

Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water

July 2021

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Proposed Public Health Goals for Perfluorooctanoic Acid and Perfluorooctane Sulfonic Acid in Drinking Water

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PREFACE

Public Health Goal (PHG) technical support documents provide information on health effects from contaminants in California drinking water. PHGs are developed for chemical contaminants based on the best available data in the scientific literature and using the most current principles, practices, and methods used by public health professionals. These documents and the analyses contained therein provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

Under the California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365), the Office of Environmental Health Hazard Assessment (OEHHA) develops PHGs for drinking water contaminants in California based exclusively on public health considerations. OEHHA periodically reviews PHGs and revises them as necessary based on the occurrence of the respective chemical in California drinking water supplies and the availability of new scientific data. This document presents proposed PHGs for perfluorooctanoic acid and perfluorooctane sulfonic acid.

OEHHA is releasing this draft assessment for public comment and independent, external peer review. OEHHA will address the comments in a second draft and will release it for further public comment before publishing the final assessment.

PHGs published by OEHHA are for use by the State Water Resources Control Board (SWRCB) in establishing primary drinking water standards (California Maximum Contaminant Levels, or CA MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, MCLs adopted by SWRCB consider economic factors and technological feasibility. State law requires that MCLs be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory and represent only non-mandatory goals. Under federal law, CA MCLs established by SWRCB must be at least as stringent as the federal MCL if one exists.

CONTENTS

List of Commonly Used Abbreviations

ACTH – adrenocorticotropic hormone
ADD – acceptable daily dose
ALP – alkaline phosphatase
ALT – alanine aminotransferase
ASF – age sensitivity factor
AST – aspartate aminotransferase
ATP – adenosine triphosphate
ATSDR – Agency for Toxic Substances and Disease Registry
AUC – area under the curve
BMD – benchmark dose
BMDL – lower 95% confidence limit of the benchmark dose
BMDS – benchmark dose software
BMI – body mass index
BMR – benchmark response
BUN – blood urea nitrogen
BW – body weight
cAMP – cyclic adenosine monophosphate
CAR – constitutive androstane receptor
CAT – catalase
CBG – corticosteroid binding globulin
CCK – cholecystokinin
CI – confidence interval
CL – clearance
CL_F – fecal clearance
CL_R – renal clearance
CORT – corticosterone
CSF – cancer slope factor
DART – developmental and reproductive toxicity
DEN – diethylnitrosamine
DNA – deoxyribonucleic acid
DWI – daily water intake
E2 – estradiol
EDS – ethane dimethyl sulfonate
ER – endoplasmic reticulum
ERβ – estrogen receptor beta
FOSE – perfluorooctane sulfonamide ethanol
fT4 – free thyroxine
FTOH – fluorotelomer alcohol
GA – gestational age
GD – gestation day
GFR – glomerular filtration rate
GGT – gamma-glutamyl transpeptidase

GluR2 – glutamate receptor 2
GnRH – gonadotropin-releasing hormone
GSH – glutathione
GSH-Px – glutathione peroxidase
HDL – high-density lipoprotein
HPC – health-protective concentration
HR – hazard ratio
IARC – International Agency for Research on Cancer
IgE – immunoglobulin E
IgG – immunoglobulin G
IL – interleukin
IQR – interquartile range
IVIVE – in vitro to in vivo extrapolation
i.v. – intravenous
KKS – kallikrein-kinin system
KO – knockout
L/kg-day – liters per kilogram body weight per day
LCMRL – lowest concentration minimum reporting level
LDH – lactate dehydrogenase
LDL – low-density lipoprotein
LH – luteinizing hormone
LMS – linearized multistage
LOAEC – lowest-observed-adverse-effect concentration
LOAEL – lowest-observed-adverse-effect level
LXR – liver X receptor
MCL – maximum contaminant level
MCMC – Markov chain Monte Carlo
MDA – malondialdehyde
µg/L – micrograms per liter
µg/ml – micrograms per milliliter
mg/kg-day – milligrams per kilogram body weight per day
MOA – mode of action
ng/L – nanograms per liter
ng/ml – nanograms per milliliter
NHANES – National Health and Nutrition Examination Survey
NIS – sodium-iodide symporter
NK – natural killer
NL – notification level
NMDA – n-methyl-d-aspartate
NO – nitric oxide
NOAEC – no-observed-adverse-effect concentration
NOAEL – no-observed-adverse-effect level
NTP – National Toxicology Program

OAT – organic anion transporter
OEHHA – Office of Environmental Health Hazard Assessment
8-OHdG – 8-hydroxy-2'-deoxyguanosine
OR – odds ratio
OVA – ovalbumin
P4 – progesterone
PBDE – polybrominated diphenyl ether
PBPK – physiologically-based pharmacokinetic
PCB – polychlorinated biphenyl
PCNA – proliferating cell nuclear antigen
PFAS – per- and polyfluoroalkyl substances
PFCA – polyfluorinated carboxylic acid
PFOA – perfluorooctanoic acid
PFOS – perfluorooctane sufonic acid
PHG – public health goal
PK – pharmacokinetic
PND – postnatal day
POD – point of departure
PPAR – peroxisome proliferator-activated receptor
ppb – parts per billion
ppm – parts per million
ppt – parts per trillion
PTFE – polytetrafluoroethylene
PXR – pregnane X receptor
RL – reference level
RNA – ribonucleic acid
ROS – reactive oxygen species
RR – relative risk
RSC – relative source contribution
RXR – retinoic X receptor
SDH – sorbitol dehydrogenase
SGA – small for gestational age
SIR – standardized incidence ratio
SMR – standardized mortality ratio
SOD – superoxide dismutase
SRBC – sheep red blood cells
SWRCB – State Water Resources Control Board
$T_{1/2}$ – half-life
T3 – triiodothyronine
T4 – thyroxine
TBG – thyroxine-binding globulin
TC – total cholesterol
TFE – tetrafluoroethylene

TK – toxicokinetic
TNF-α – tumor necrosis factor alpha
TSH – thyroid stimulating hormone
tT4 – total thyroxine
TTR – transthyretin
UCMR3 – US EPA's Third Unregulated Contaminant Monitoring Rule
UF – uncertainty factor
V_d – volume of distribution
WT – wild-type

SUMMARY

For more than a half-century, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) were widely used in industrial applications and consumer products, notably, PFOA in nonstick cookware and PFOS in stain and water-repellant fabrics and in fire-fighting foams. The manufacture of these chemicals was phased out in the US following concerns about their extreme persistence in the environment and their detection in virtually all human blood serum samples. Although levels in the environment have declined from their peak around the year 2000, PFOA and PFOS continue to be present in the environment and are found in California drinking water. Because exposure to these chemicals is so prevalent and elimination times are so long, it is critical to understand the toxicity associated with these compounds, and their impacts on human health.

Public Health Goals for PFOA and PFOS

Scientific studies show adverse health effects of PFOA and PFOS in people exposed at environmental levels, and similar effects in laboratory animals. There is evidence from epidemiologic studies that exposure to PFOA increases the risk of kidney cancer. Human exposure to PFOA is also associated with liver and immune system toxicity and increased total cholesterol, and there is suggestive evidence of an association with risk of preeclampsia and pregnancy-related hypertension. PFOS is associated with immune system toxicity and increased total cholesterol in humans, with suggestive evidence of an association with risk of preeclampsia and pregnancy-related hypertension. The effects seen in humans are supported by studies in laboratory animals, which show that PFOA and PFOS can cause liver toxicity, immunotoxicity, thyroid toxicity, developmental/reproductive toxicity, and cancer.

This draft document presents proposed public health goals (PHGs) for PFOA and PFOS in drinking water, based on the most sensitive health effects. A PHG is the concentration of a contaminant in drinking water that is estimated to pose no significant health risk to individuals consuming the water on a daily basis over a lifetime. The proposed PHG for PFOA is based on kidney cancer in humans, while the proposed PHG for PFOS is based on liver and pancreatic tumor data from rat studies. This draft document also identifies health-protective concentrations (HPCs) for noncancer effects of PFOA and PFOS. The dose-response data from human studies were sufficient for derivation of the HPCs for these compounds, with the most sensitive noncancer endpoints being liver damage for PFOA and clinically relevant increased total cholesterol for PFOS.

Table S1 shows the proposed PHGs and HPCs for PFOA and PFOS.

Table S1. Proposed Public Health Goals and Health-Protective Concentrations

Chemical Name	PHG (ppt)	PHG Effect(s)	HPC (ppt)	HPC Effect
Perfluorooctanoic acid	0.007	Kidney cancer (human data)	3	Increased risk of liver damage (human data)
Perfluorooctane sulfonic acid	1	Cancer (animal data)	2	Increased total cholesterol (human data)

HPC, health-protective concentration; PHG, public health goal; ppt, parts per trillion (equivalent to nanograms per liter or ng/L)

PHGs are not regulatory requirements, and are based solely on protection of public health without regard to cost impacts or other factors. PHGs form the basis of California's Maximum Contaminant Levels (MCLs) for drinking water, which are established by the State Water Resources Control Board (SWRCB), and each MCL must be set as close to the corresponding PHG as is economically and technologically feasible. PHGs are developed for chemical contaminants based on the best available data in the scientific literature and using the most current principles, practices, and methods used by public health professionals, and on comprehensive analyses of information on the toxicology of each compound.

Derivation of the PHGs and HPCs

As noted above, a number of adverse health effects have been observed in studies in humans and laboratory animals exposed to PFOA and PFOS. The proposed PHGs are based on the cancer endpoint because it is the most sensitive effect. PHGs are set at a level where the cancer risk is estimated to be one per one million persons exposed over a lifetime. The PHGs, as well as the noncancer HPCs, also include considerations of sensitive and highly exposed populations.

The proposed PHGs and noncancer HPCs are different from the reference levels supporting the notification levels OEHHA recommended to SWRCB in 2019.[1] This is due to a number of factors, including the availability of new studies, the use of human data when possible, and improved toxicokinetic analyses.

Systematic Literature Search

The proposed PHGs and noncancer HPCs are based on epidemiologic and laboratory animal studies OEHHA identified through systematic literature searches described in appendices to this document. In addition, OEHHA identified studies published before 2016 from the reference lists of assessments published by the US Environmental Protection Agency (US EPA, 2016b; US EPA, 2016d), the National Toxicology Program (NTP, 2016), the International Agency for Research on Cancer (IARC, 2017a), and the Agency for Toxic Substances and Disease Registry (ATSDR, 2018a). Finally, in 2019, OEHHA issued a data call-in to request information from the public. For each pertinent study identified in this manner, OEHHA evaluated the methods and quality in order to base the PHGs and noncancer HPCs on studies of sufficient quality.

Toxicokinetic Analyses to Address Persistence in Humans

PFOA and PFOS strongly bioaccumulate in humans and to a much lesser degree in animals. To address the issue of the persistence of PFOA and PFOS in the human body in its dose-response analyses, OEHHA used serum concentrations as a measure of the internal exposure. Fortunately, all the available human toxicity studies OEHHA selected to use in the dose-response analyses report serum concentrations for PFOA or PFOS. OEHHA converted serum

[1] In 2019, OEHHA developed reference levels of 0.1 ppt for PFOA based on pancreatic and liver tumors in rats and 0.4 ppt for PFOS based on liver tumors in rats. However, the cancer-based reference levels were lower than could be reliably detected in drinking water using currently available technologies. Thus, OEHHA recommended that SWRCB set the notification levels for PFOA and PFOS at the lowest levels that could be reliably detected in drinking water (5.1 ppt for PFOA and 6.5 ppt for PFOS). OEHHA also developed noncancer reference levels of 2 ppt for PFOA based on liver toxicity in mice and 7 ppt for PFOS based on immunotoxicity in mice (OEHHA, 2019).

levels to chronic intake doses (amount of chemical consumed per unit body weight) using toxicokinetic (TK) approaches. In establishing human equivalent doses associated with health effects observed in animal studies, OEHHA also used a TK approach to address the large differential in chemical half-life between animals and humans.

OEHHA conducted an extensive review of the literature and analyzed different TK approaches in order to select the best method to address this matter. The TK approaches OEHHA selected for use rely on the application of a clearance factor. OEHHA conducted a comprehensive review of the literature before it selected the most appropriate clearance value to use in its analyses. A human clearance factor of 2.8×10^{-4} L/kg-day was developed for PFOA and 3.9×10^{-4} L/kg-day for PFOS. This improves the approach used previously (OEHHA, 2019).

PFOA Health Effects Basis for PHG and HPC

Kidney cancer in humans as the sensitive endpoint for the PHG: A large case-control study of PFOA and kidney cancer in 10 study centers across the US was recently published by the National Cancer Institute (Shearer et al., 2021).[2] This prospective population-based study, along with previous epidemiologic studies in an area of high environmental exposure (Barry et al., 2013; Vieira et al., 2013) and in an occupational setting (Steenland and Woskie, 2012), provide strong evidence that PFOA causes kidney cancer in humans. The occupational study by Raleigh et al. (2014) did not report an association between PFOA and kidney cancer, but used an inhalation exposure model that was not validated. Lack of information on potential confounding, a potentially inappropriate comparison group, and limited statistical power are other possible reasons why this study may have missed a true association. Multiple analyses performed by the researchers of all four studies show that the positive results for kidney cancer and PFOA are very unlikely to be due to chance. Also, evidence of dose-response patterns, a criterion commonly used for evaluating causality, is seen in each of the four studies, and the prospective nature of these studies assures that PFOA exposure came before kidney cancer development, which helps rule out the possibility of reverse causality.

OEHHA performed a number of evaluations and detailed analyses on whether the biases or problems frequently seen in epidemiologic studies could have caused the positive associations reported. This includes evaluating potential problems related to participant recruitment and selection, in categorizing participants' PFOA exposure, and in classifying people with and without kidney cancer. Overall, these analyses show that each of these issues was either very minor or likely to have led to underestimates, rather than overestimates, of kidney cancer risk from PFOA exposure.

A number of other factors are known to cause or are suspected to cause kidney cancer, and these could potentially confound the relationship between PFOA and kidney cancer. The studies used in OEHHA's dose-response analyses accounted for the most important of these (e.g., sex, age, tobacco smoking, and being overweight or obese) with statistical adjustments or stratified analyses. For all other factors, including the presence of other PFAS, all available evidence shows that they were either not prevalent enough or their associations with PFOA or kidney cancer were not strong enough to cause major confounding.

[2] Paper became available online in September, 2020 at: https://academic.oup.com/jnci/article/113/5/580/5906528

Overall, the body of evidence on PFOA and kidney cancer from human studies meets the modified Hill (1965) criteria that are commonly used to evaluate causality. The carcinogenicity of PFOA in humans is supported by the results of animal cancer bioassays. A recent NTP study in rats concluded there is "clear evidence of carcinogenic activity of PFOA" in male rats and "some evidence of carcinogenic activity of PFOA" in female rats (NTP, 2020). It is also supported by in vivo and in vitro mechanistic studies.

Dose-response analysis of the human kidney cancer data for the PFOA PHG: The epidemiologic studies by Shearer et al. (2021) and Vieira et al. (2013) include data sufficient for quantifying cancer risks. Both studies involved large sample sizes and therefore had good statistical power to identify true effects. Both studies had a good range of PFOA exposures, included large numbers of participants with exposure levels close to those seen in the general US population, and adjusted or stratified for other major kidney cancer risk factors. In these two studies, PFOA exposure was assessed using either directly measured serum PFOA levels in each individual (Shearer et al., 2021), which are good indicators of long-term PFOA exposure, or individual PFOA serum levels estimated using a validated exposure model (Vieira et al., 2013). Evaluations show that the impacts of exposure misclassification from either of these methods are likely to be mostly minor and unlikely to have caused the elevated risks reported in either study.

The cancer slope factors derived from these studies are within about six-fold of each other. To make maximum use of both these strong studies, the geometric mean of the cancer slope factors, 0.0026 per ng/kg-day, is used to derive the proposed PHG of 0.007 parts per trillion (ppt) for PFOA (Table S1).

Human liver toxicity as the basis for the noncancer HPC for PFOA: Several epidemiologic studies provide sufficient data to derive the noncancer dose-response benchmarks for immunotoxicity, liver toxicity and increased cholesterol health effects. The proposed noncancer HPC for PFOA is based on an increased risk of liver toxicity, as indicated by elevated alanine aminotransferase (ALT) levels (Gallo et al., 2012) exceeding clinically based reference levels used by the International Federation of Clinical Chemistry and Laboratory Medicine. The study involved residents of the Mid-Ohio Valley who lived near a chemical plant known to have emitted PFOA into the surrounding environment, and whose ALT increased with increasing serum levels of PFOA. This is the basis of an acceptable daily dose of 0.87 ng/kg-day and a proposed noncancer HPC of 3 ppt for PFOA (Table S1).

PFOS Health Effects Basis for PHG and HPC

PFOS PHG based on cancer endpoint: Although there are a few epidemiologic studies that show some association of PFOS with breast, liver, and bladder cancer, the results are mixed or the sample sizes are small. OEHHA did not identify any epidemiologic studies of PFOS that could be used for quantifying cancer risk in humans. Thus, the proposed PHG for PFOS is based on cancer data in laboratory animals. The cancer slope factor of 15.6 per mg/kg-day, derived from liver and pancreatic tumors in male rats exposed through the diet for two years (Butenhoff et al., 2012b), is used to derive the proposed PHG of 1 ppt for PFOS (Table S1).

Increased cholesterol in humans as the basis for the noncancer HPC for PFOS: Sensitive noncancer endpoints for PFOS are immunotoxicity and alterations in lipid metabolism. Total cholesterol appeared to be a somewhat more sensitive endpoint, with the large study by

Steenland et al. (2009) of residents near the West Virginia facility who drank contaminated water and had elevated blood serum levels of PFOS. Based on the relationship in this study between PFOS and serum lipids (Steenland et al., 2009), a point of departure for PFOS was derived for an increased risk of elevated total cholesterol above the clinical reference level published by the American Heart Association. This is the basis of an acceptable daily dose of 0.64 ng/kg-day and a proposed noncancer HPC of 2 ppt for PFOS (Table S1).

Evaluation of Exposure Parameters

Calculation of the PHGs entails making assumptions about the amount of water people consume and, in the calculation of the HPCs for noncancer effects, an additional assumption regarding the relative source contribution, that is, the proportion of exposures to a chemical attributed to tap water as part of total exposure from all sources, including food.

Drinking water intake: OEHHA used age-specific water ingestion estimates derived from a nationwide survey of food and beverage intake from approximately 20,000 individuals (see OEHHA (2012)). These age-specific intake rates are normalized to body weight. This enables addressing the higher rate of ingested water (per unit body weight) of the young. In considering levels of water intake to assume for the PFOA and PFOS assessment, OEHHA reviewed the literature that could inform drinking water trends and intake in California and performed a detailed evaluation of US EPA's recently updated chapter on water consumption in its *Exposure Factors Handbook* (US EPA, 2019) that used nationwide survey data from 2005-2010.

OEHHA found that US EPA's updated drinking water intake rates may not be representative of California's residents due to a number of factors such as greater numbers of jobs performed in outdoor settings (e.g., farm work and construction) for longer periods throughout the year, and lifestyle and recreation, which all require proper hydration, particularly in California's hot summer climate. Thus, OEHHA retained the peer reviewed drinking water intake rates that it developed in 2012. A lifetime-weighted average drinking water intake rate of 0.053 L/kg-day was used in deriving the PHGs and HPCs.

Relative source contribution for PFOA and PFOS: OEHHA applied US EPA's Exposure Decision Tree Approach (US EPA, 2000) and found a lack of information about sources of exposure to support a value other than the default value of 20%. This determination is consistent with other regulatory bodies, including US EPA, Health Canada, and the State of New Jersey.

Conclusion

PFOA and PFOS are extremely persistent in the environment and are present in virtually all human blood serum samples. New analyses in this assessment have improved the estimates of clearance rates for PFOA and PFOS. Clearance in humans is much slower than in laboratory animals, with half-lives of several years in humans versus several days in animals. There is mounting evidence that environmental levels of PFOA and PFOS can adversely affect human health, and studies in laboratory animals support the effects observed in humans exposed to these chemicals through the environment. Large, well-conducted epidemiologic studies form the basis for the proposed PHG for PFOA and HPCs for PFOA and PFOS, while a well-conducted study in laboratory animals is the basis for the proposed PHG for PFOS.

1. INTRODUCTION

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (or perfluorooctane sulfonate, PFOS) are organic acids that consist of a chain of eight carbons, where all the hydrogens on the carbon chain have been replaced with fluorines. The two chemicals differ in that PFOA has a carboxylic acid at one end of the carbon chain, whereas PFOS has a sulfonic acid group. Both chemicals belong to a large class of chemicals called per- and polyfluoroalkyl substances (PFAS), of which PFOA and PFOS are the best studied.

The strong carbon-fluorine bonds of these compounds make them resistant to biological and environmental degradation. As such, PFAS confer stain, grease, heat, and water resistant properties to consumer products when they are applied, which has made the commercial use of these chemicals desirable. Historically, PFAS have been used in myriad products, including fabrics, carpets, cookware, cleaning products, and aqueous fire-fighting foam.

Due to their resistance to biological and environmental degradation, most PFAS have long half-lives in humans and the environment. Coupled with many industrial uses, the environmental persistence of PFAS has resulted in widespread exposure. Even though PFOA and PFOS were voluntarily phased out in the United States (PFOS in 2002 and PFOA by 2015), exposure to these chemicals still occurs due to their environmental persistence, their use in imported goods, and the use of products manufactured before the phase-out. Additionally, PFOA and PFOS can result from the breakdown of other PFAS. PFOA and PFOS have been detected in California drinking water, despite the absence of manufacturing of these compounds in California. Furthermore, Biomonitoring California reports that PFOA and PFOS were found in the serum of >99% and >97%, respectively, of participants across all study cohorts. This indicates that exposure to these chemicals is widespread in California.

Numerous adverse health effects in humans and laboratory animals have been associated with exposure to PFOA and PFOS, including liver toxicity, immunotoxicity, thyroid toxicity, reproductive/developmental toxicity, effects on lipids and cholesterol, and cancer. In 2019, OEHHA derived cancer reference levels (RLs) for PFOA and PFOS in drinking water (OEHHA, 2019).

- 0.1 ng/L (nanograms/liter) or parts per trillion (ppt) for PFOA, based on pancreatic and liver tumors in male rats;
- 0.4 ng/L (or ppt) for PFOS, based on liver tumors in male rats.

OEHHA also developed RLs for noncancer effects as follows:

- 2 ng/L (or ppt) for PFOA, based on liver toxicity in female mice;
- 7 ng/L (or ppt) for PFOS, based on immunotoxicity in male mice.

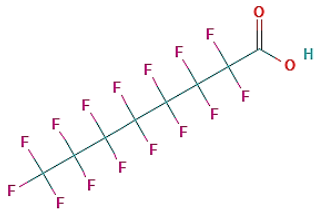
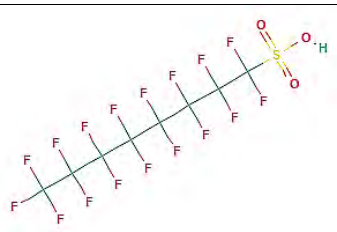
OEHHA reviewed previous evaluations by US EPA (2016a, 2016b, 2016c, 2016d), New Jersey Drinking Water Quality Institute (2017, 2018), and ATSDR (2018a) and concluded those evaluations comprehensively and effectively identified the published literature prior to their respective dates of publication. Thus, in deriving Public Health Goals (PHGs) for PFOA and PFOS, OEHHA performed a systematic literature search and thoroughly evaluated human, animal, and mechanistic toxicity studies published from 2016 onward. Additionally, OEHHA conducted rigorous evaluations of toxicokinetics, drinking water intake rates, and the relative

source contribution of drinking water, and used its own health risk assessment methodology when deriving health-protective concentrations in this document.

1.1. Physical and Chemical Properties

The physical and chemical properties for PFOA and PFOS can be found in Table 1.1.1. The structures of PFOA and PFOS can be linear or branched and formation of these structures is dependent on the manufacturing process. Both chemicals are solid at room temperature and have low vapor pressure. PFOA and PFOS are stable and are resistant to environmental degradation. In solution, PFOA exists as the perfluorooctanoate anion and is a strong acid. PFOS also exists as an anion in solution; however, pKa values can only be estimated.

Table 1.1.1. Physical and Chemical Properties of PFOA and PFOS

	Perfluorooctanoic acid (PFOA)	**Perfluorooctane sulfonic acid (PFOS)**
Formula	$C_8HF_{15}O_2$	$C_8HF_{17}O_3S$
CAS No.	335-67-1	1763-23-1
Structure		
Molecular weight (g/mole)	414.09	500.13
Physical state at ambient temperature	White powder	White powder
Melting point (°C)	54.3 (HSDB, 2020a) 55 (US EPA, 2020)	≥400 (potassium salt) (EFSA, 2008)
Boiling point (°C)	192 (HSDB, 2020a) 189 (US EPA, 2020)	249 (HSDB, 2020b) 258–260 (US EPA, 2016b; US EPA, 2016d)
Density (g/cm^3)	1.792 (20 °C) (HSDB, 2020a)	No data
Solubility in water (mg/L)	3,300 (25 °C) (HSDB, 2020a)	680 (OECD, 2002) 570 (potassium salt in pure water) (ATSDR, 2021) 519 (20 ± 0,5°C) (EFSA, 2008) 680 (24 - 25°C) (EFSA, 2008)
Vapor pressure (mm Hg)	0.525 at 25 °C (measured) (Hekster et al., 2003) 0.962 at 59.25 °C (measured) (Kaiser et al., 2005)	2.0×10^{-3} at 25 °C estimated US EPA EPI Suite) (HSDB, 2020b)

	Perfluorooctanoic acid (PFOA)	**Perfluorooctane sulfonic acid (PFOS)**
Henry's Law constant (atm. m^3/mol)	9.08×10^{-2} (25 °C, estimated US EPA EPI Suite) (HSDB, 2020a)	4.1×10^{-4} (25 °C, estimated US EPA EPI Suite) (HSDB, 2020b) 3.05×10^{-9} atm. m^3/mol pure water (potassium salt) (EFSA, 2008)
Log K_{ow}	4.81 (estimated US EPA EPI Suite) (HSDB, 2020a) Ammonium perfluorooctanoate (APFO): 0.7 (EFSA, 2008)	4.49 (estimated US EPA EPI Suite) (HSDB, 2020b)
Acidity, pKa	-0.5-4.2 (HSDB, 2020a) 2.8 (US EPA, 2016b) 2.5, 2 to 3 (Prevedouros et al., 2006)	<1.0 (estimated) (HSDB, 2020b) -3.3 (calculated value for acid) (Brooke et al., 2004)

2. METHODOLOGY

Development of a PHG for a chemical in drinking water entails a four-part process.

2.1. Systematic Literature Search and Toxicological Evaluation

The toxicological evaluation of a chemical starts with a review of the available literature. A systematic literature search is conducted, using a comprehensive search string and multiple scientific databases (Pubmed, Embase, Scopus, etc.). Details on the literature search are provided in Appendix 1. Briefly, a PECO (population, exposure, comparator, outcome) statement that outlines inclusion/exclusion criteria is developed for initial reference screening. The title and abstract of each reference are screened by a minimum of two reviewers, and each reference is included or excluded based on criteria outlined in the PECO statement. A separate search was conducted specifically for human epidemiology studies as outlined in Appendix 7. Subsequently, a full-text review of included references is conducted to ensure that the studies are relevant for PHG development.

2.2. Study Evaluation

2.2.1. Animal Studies

The findings from studies that meet the PECO criteria are critically evaluated and the quality of each study is assessed. OEHHA's criteria for study quality evaluation include the following:

- appropriate number of animals per dose group
- an untreated control group plus a minimum of two dose groups
- appropriate exposure duration
- relevant route of exposure
- appropriate test species
- appropriate statistical analysis
- biological significance of endpoints
- adequate reporting.

2.2.2. Human Studies

For epidemiology studies, several factors were evaluated when assessing study quality, including study design, factors related to bias, exposure and outcome methods, and confounding. Specific details for OEHHA's study quality evaluation for human epidemiology studies are provided in Chapters 5 and 6, and in Appendix 7.

2.3. Evaluation of Health Hazards

For each major toxicity endpoint (e.g. hepatotoxicity, immunotoxicity, reproductive toxicity, cancer, etc.), relevant studies are reviewed to identify the health hazards induced by chemical exposure. This review considers factors such as consistency across multiple studies, exposure-reponse relationships, biological plausibility, and relevance to human health. Furthermore, consideration is given to the potential molecular and cellular mechanisms by which toxicity is induced (modes of action).

2.4. PHG Derivation

After a review of the toxicity studies of suitable quality and identification of relevant hazards, the most sensitive endpoints from studies determined to be relevant to human health are selected, and analyses of the dose-response relationships are performed. The adverse effect or a physiological change that leads to an adverse effect that occurs at the lowest dose is selected as the critical effect from which a PHG is derived.

If a chemical has been identified as a human or animal carcinogen, health-protective water concentrations are determined for both cancer and noncancer effects and the lowest value is selected as the PHG.

2.4.1. Deriving Health-Protective Concentrations for Noncancer Effects

Calculation of a health-protective concentration for noncancer effects involves a four-step approach: determination of the point of departure (POD), estimation of an acceptable daily dose (ADD), determination of the relative source contribution (RSC) and calculation of a health-protective drinking water concentration (C).

Point of Departure (POD)

The POD is the dose of a chemical (in units of milligrams per kilogram of body weight per day, mg/kg-day) from a study in animals or humans that is used as a starting point for calculation of the ADD. The POD is typically determined by fitting a mathemathical model to the dose-response data. OEHHA generally uses the publicly available Benchmark Dose Software (BMDS) program developed and maintained by the US Environmental Protection Agency (US EPA; https://www.epa.gov/bmds). BMDS fits mathematical models to the data and determines the dose (benchmark dose or BMD) that corresponds to a pre-determined level of response (benchmark response or BMR). The BMR is typically set at 5% above the background or the response of the control group for dichotomous data. For continuous data, a BMR of one standard deviation from the control mean is typically used when there are no data to indicate what level of response is biologically significant (US EPA, 2012). In order to account for the uncertainty of the data, the model also calculates the 95% lower confidence limit of the BMD, called the BMDL (L stands for the lower confidence limit). For PHG development, OEHHA uses the BMDL as the POD for the calculation of a health-protective drinking water concentration when the data are amenable to BMD modeling. When data are not amenable to BMD modeling, OEHHA uses the no-observed-adverse-effect level or concentration (NOAEL or NOAEC), or lowest-observed-adverse-effect level or concentration (LOAEL or LOAEC) approach in identifying the POD.

Application of BMD modeling for noncancer effects mitigates some of the limitations of the NOAEL/LOAEL approach, including:

- dependence on dose selection and sample size;
- inability to account for uncertainty and variability of experimental results due to the characteristics of the study design;
- the need to use an uncertainty factor when a NOAEL cannot be determined in a study; and
- inability to account for the shape of the dose-response curve.

Acceptable Daily Dose (ADD)

The ADD is the estimated maximum average daily dose of a chemical (in mg/kg-day) that can be consumed by a human for an entire lifetime without adverse effects. This is similar to the term "reference dose" used by US EPA. To determine the ADD, the POD is adjusted by factors that account for uncertainties and variabilities in the risk assessment, such as differences between animals and humans, and differences among humans in response to a chemical exposure. These factors are combined into a composite uncertainty factor (UF).

Uncertainty and Variability Factors (UF)

When developing health-protective levels for noncancer effects based on animal toxicity studies, OEHHA generally applies a combined UF of 300 (OEHHA, 2008).

These UFs are:

- 10 for interspecies extrapolation, accounting for possible differences in the way laboratory animals and humans respond to the chemical, consisting of
  - $\sqrt{10}$ for toxicokinetics
  - $\sqrt{10}$ for toxicodynamics
- 30 for intraspecies variability, which accounts for some human subpopulations, such as children and the elderly, possibly being more sensitive to the chemical than the general population, consisting of
  - $\sqrt{10}$ for toxicodynamics
  - 10 for toxicokinetics.

These default factors are applied unless data support an alternative value. A table of default UFs for ADD derivation is presented in Appendix 2. Additional adjustments may be included depending on the limitations of available data.

The ADD is calculated using the following equation:

$$\mathrm{ADD} = \mathrm{POD} \div \mathrm{UF}.$$

Relative Source Contribution (RSC)

The RSC is the proportion of exposures to a chemical attributed to tap water, as part of total exposure from all sources (including food and air). The RSC values typically range from 20% to 80% (expressed as 0.20 to 0.80), and are determined based on available environmental monitoring data. For certain PHGs, the RSC can be as high as 1.0 (tap water is the only source of the chemical) when it is deemed appropriate. OEHHA uses this approach to ensure that the PHG identifies a level of a drinking water contaminant that would pose no significant health risk after taking into account exposures to all other sources (see Appendix 4 for details).

Daily Water Intake Equivalent (DWI)

To calculate a PHG for a chemical, the ADD is converted to a concentration in drinking water that accounts for the total exposure to the chemical that people receive from using tap water. It includes intake from ingestion as well as inhalation and dermal contact with the chemical in tap water from household uses (e.g., drinking, cooking, bathing, and showering). Inhalation exposure can take place when the chemical volatilizes out of the water during cooking or showering. Dermal absorption of the chemical can occur during bathing and other household uses of tap water.

DWI is expressed in units of liters or liter equivalents per kilogram of body weight per day (L/kg-day or L_{eq}/kg-day, respectively). Liter equivalents represent the equivalent of the amount of tap water one would have to drink to account for the exposure to a chemical in tap water through oral, inhalation, and dermal routes. However, due to the physicochemical properties of PFOA and PFOS, inhalation and dermal exposure through household uses of tap water are expected to be negligible.

For oral intake rates, the PHG program uses age-specific water ingestion estimates (OEHHA, 2012) derived from a nationwide survey of food and beverage intake from approximately 20,000 people (US Department of Agriculture's Continuing Survey of Food Intake of Individuals 1994-1996, 1998 dataset). These age-specific intake rates, normalized to body weight and expressed as L/kg-day, indicate that drinking water ingestion per unit body weight is higher in infants than in adults. Previous PHGs using ingestion rates of 2 L/day for adults and 1 L/day for a 10 kg child are being updated with these more refined estimates. While US EPA has recently updated drinking water ingestion rates in their Exposure Factors Handbook (US EPA, 2019), OEHHA has not adopted these new ingestion rates for reasons described in Appendix 3.

Derivation of the Health-Protective Concentration (C)

Following the determination of the ADD, the health-protective concentration (C, in milligrams per liter, mg/L or in micrograms per liter, µg/L) in drinking water can be derived by incorporating the DWI and RSC of the chemical:

$$\mathrm{C} = (\mathrm{ADD} \times \mathrm{RSC}) \div \mathrm{DWI}.$$

2.4.2. Deriving Health-Protective Concentrations for Cancer Effects

Calculation of a health-protective concentration for cancer effects involves a three-step approach: determination of a POD from which a cancer potency can be determined, estimation of an average daily dose, and calculation of a health-protective drinking water concentration (C).

Cancer Dose-Response Analyses and Cancer Potency Derivation

Standard methods for estimation of lifetime theoretical cancer risks are employed in the development of cancer potencies based on animal studies (US EPA, 2005; OEHHA, 2009; US EPA, 2012). The estimated cancer potency, also referred to as the cancer slope factor (CSF), is a measure of the carcinogenic potential of a compound. It is often reported in units of 1/(mg/kg-day) or $(\mathrm{mg/kg\text{-}day})^{-1}$ and is derived by fitting a linear low-dose extrapolation using US EPA's BMDS Multistage-Cancer model (US EPA, 2012) to the tumor incidence data from an animal carcinogenicity bioassay.

Method for Calculating Cancer Potency

Development of cancer potency estimates from animal bioassays includes consideration of:

- the quality, suitability, and sensitivity of the available animal bioassay studies; for example, the thoroughness of experimental protocol, the temporal exposure pattern, the degree to which dosing resembles the expected manner of human exposure, the duration of the study, the purity of test material, the number and size of exposed groups, and the extent of tumor occurrence
- the cancer sites and types from the selected experiments most appropriate for characterizing the cancer potency; where there are multiple sites with significant tumor findings in a selected experiment, a multisite analysis is performed to describe the overall carcinogenic potential
- whether a dose-response model that assumes the absence of a carcinogenic threshold dose should be used or whether there are compelling mechanistic data to support an alternative approach
- interspecies scaling of animal cancer potency to human cancer potency
- physiologic, TK and metabolic information for possible use in extrapolating from test animals to humans, from high to low dose, and from one exposure route to another.

Calculating Average Daily Dose

A mathematical model is fit to dose-response data from animal studies. For studies that do not involve daily administration of a fixed mg/kg body weight amount, an average daily dose (in units of mg/kg-day) is calculated. This is done by adjusting the administered or nominal dose, accounting for days of dosing during the week and total dosing weeks during the experimental period. For studies using variable doses, the weighted mean dose is calculated considering the dosing frequency and duration of the various administered doses.

Dose-Response Model

Information on the mode of action (MOA) involved in the carcinogenesis of a chemical is evaluated to determine whether human cancer risk should be estimated using the default assumption of low dose linearity or otherwise. Unless there is sufficiently compelling evidence, OEHHA uses a non-threshold approach and a linearized multistage (LMS) cancer model to calculate the chemical's cancer potency, expressed as the CSF. This is accomplished by using the BMDS Multistage-Cancer model developed by US EPA (BMDS version 2.7). The model calculates the lifetime probability of developing a tumor (p) induced by an average daily dose (d) using the following equation:

$$p(d) = \beta + (1- \beta) \times \exp[-(q_1 d + q_2 d^2 + ... + q_i d^i)].$$

The q_i are parameters of the model, which are taken to be constants and are estimated from the animal cancer bioassay data. As recommended by US EPA (2012), $q_i \geq 0$ for all i. For example, with four dose groups, the Multistage-Cancer model can have a maximum of four parameters, β, q_1, q_2, and q_3. When dose is expressed in units of mg/kg-day, q_1 is given in units of $(\text{mg/kg-day})^{-1}$. The q_1 parameter is, for small doses, the ratio of excess lifetime cancer risk to the average daily dose received. The parameter β provides the basis for estimating the

background lifetime probability of the tumor (i.e., when dose d is zero, the probability of cancer, p, is equal to β).

The Multistage-Cancer model defines the probability of developing a tumor at a single site. For carcinogens that induce tumors at multiple sites and/or in different cell types at the same site in a particular species and sex, US EPA's BMDS can be used to derive maximum likelihood estimates (MLEs) for the parameters of the multisite carcinogenicity model by summing the MLEs for the individual multistage models from the different sites and/or cell types. This multisite model provides a basis for estimating the cancer potency of a chemical that causes tumors at multiple sites.

Adjusting for Human-Animal Differences

In the absence of reliable TK information, the human cancer slope factor (CSF_{human}) is estimated by assuming the chemical dose per body weight scaled to the three-quarters power produces the same degree of effect in different species. Under this assumption, the CSF_{animal} is multiplied by the ratio of human to animal body weights raised to the one-fourth power when animal cancer potency is expressed in units of $(mg/kg\text{-}day)^{-1}$:

$$CSF_{(human)} = CSF_{(animal)} \times (\text{body weight}_{(human)} \div \text{body weight}_{(animal)})^{1/4}$$

When data are available, separate oral and inhalation cancer potencies may be calculated and they are applied to each specific exposure route. Since it is unusual to have a cancer bioassay through dermal exposure, OEHHA generally uses the oral cancer potency for estimating cancer risk through the dermal route. Similarly, when an inhalation cancer potency is not available, the oral cancer potency is used to estimate cancer risk through the inhalation route. If only an inhalation cancer potency is available, then it will be applied to all routes when determining the PHG.

Accounting for Increased Susceptibility During Early-in-Life Exposures

When determining cancer risk, OEHHA applies age sensitivity factors (ASFs, unitless) to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2 years of age, and a factor of 3 is applied for exposures that occur from 2 through 15 years of age. However, analysis of recent PFOA bioassay data from the National Toxicology Program (NTP, 2020) indicates that perinatal exposure (from gestation day (GD) 6 through postnatal day (PND) 21) does not increase cancer risk, compared to later-in-life exposures, in rats (see Section 5.7.2 for details). Thus, ASFs are not applied in the calculation of cancer based health-protective concentrations for PFOA and, due to structural and toxicological profile similarities, for PFOS as well.

Derivation of the Health-Protective Concentration (C)

The health-protective water concentration (C) for carcinogenic effects can be calculated using the following equation:

$$C = R \div (CSF \times DWI)$$

where,

R = default risk level of one in one million, or 10^{-6}

CSF = cancer slope factor, in $(mg/kg\text{-}day)^{-1}$

DWI = lifetime average daily water intake rate.

Water consumption rates are described in the noncancer methodology section, and the underlying principles do not change when examining cancer endpoints.

3. PRODUCTION, USE, AND ENVIRONMENTAL OCCURRENCE

3.1. Production and Use

PFOA and PFOS are produced by two main methods: by electrochemical fluorination (ECF) or by telomerization (US EPA, 2016b; US EPA, 2016d). Historically, the ECF method has been the main source for PFOA and PFOS production (Prevedouros et al., 2006; Paul et al., 2009). In addition to the linear form of PFOA or PFOS, 20-30% of the PFOA or PFOS produced by the ECF method are branched isomers (Paul et al., 2009; Beesoon et al., 2011). In contrast, primarily linear isomers of PFOA and PFOS are produced in the telomerization process, and the relative fraction of the linear isomer in the analyzed sample can be used to trace the manufacturing origin of the compound mix in the environment or human serum (US EPA, 2016b; US EPA, 2016d).

The main use of PFOA has been as a processing aid in the manufacturing of fluoropolymers such as polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (Prevedouros et al., 2006). PTFE was widely used as the functional component of non-stick pans, but partly as a result of the US EPA PFOA Stewardship Program[3] and Canada's PFAS Environmental Performance Agreement,[4] this and other uses of PFOA have been steadily declining in North America (Vierke et al., 2012). As the processing aid, PFOA acts to solubilize fluoromonomers to facilitate polymerization (Prevedouros et al., 2006). PFOA was also added to aqueous polymer dispersions used for paints, photographic film additives and in the textile finishing industry (OECD, 2006; Vierke et al., 2012). Additionally, PFOA and other polyfluorinated carboxylic acids (PFCAs) are indicated in patents of various consumer products, including floor polishes, cleaning formulations, hair care products, medical inhalers, fuel additives, air fresheners and textile treatments (references summarized in Prevedouros et al. (2006)). In one case study, high levels of PFOA and PFOS in the serum samples of one Canadian family were attributed to repeated carpet treatments in their house (Beesoon et al., 2012). PFOA was used as a component of aqueous fire-fighting foams from approximately 1965-1975, and this use has contributed to environmental contamination near related facilities such as airports and military bases (Prevedouros et al., 2006; Ahrens et al., 2015; Darlington et al., 2018).

PFOS has been used in the production of firefighting foams, semiconductors, hydraulic fluids and photolithography (D'Eon and Mabury, 2011b). The major consumer product-related uses for PFOS include water-repellant treatment for clothes, stain and dirt-resistant treatment for carpets, and oil and grease-repellant treatments for paper and packaging (Paul et al., 2009). PFOS-containing fire-fighting foams continue to be held in stock (OECD, 2006). Specific industrial uses of PFOS and its salts included as a mist-suppressing agent (i.e., fume suppressant) in the mining industry, as an antireflective agent in the photographic industry, as photo-resistant and antireflective coatings, as an anti-erosion additive in the aviation industry, and as a surfactant (fume suppressant) in the metal plating industry (OECD, 2006). PFOS is found as an impurity in food packaging (Bagley et al., 2017). Both PFOA and PFOS appear to be used in certain pesticide products (OECD, 2006; Liu et al., 2017c).

[3] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-20102015-pfoa-stewardship-program
[4] https://www.canada.ca/en/environment-climate-change/services/environmental-performance-agreements/list/perfluorocarboxylic-acids-overview.html

PFOA and PFOS can accumulate in the environment as impurities or degradation products of other PFAS (Prevedouros et al., 2006; Paul et al., 2009).

3.2. Environmental Occurrence and Human Exposure

PFOA and PFOS are manmade compounds and are not known to occur naturally. The historical worldwide production of PFOA was estimated at a total of 3,600-5,700 tons from 1951-2004, and the estimated direct global environmental emissions were 400-700 tons over the same period (Prevedouros et al., 2006). One of the major PFOA production sites in the US was the DuPont Washington Works plant, located in West Virginia near the Ohio border. The historical worldwide production of PFOS was estimated at 96,000 tons from 1970-2002, and estimated total global emissions were 6,800-45,250 tons during that time period (Paul et al., 2009). Other reports estimated lower PFOS production and emissions (Armitage et al., 2009; Wang et al., 2017b). The main PFOS manufacturing site in the US was at the 3M Company in Decatur, Alabama (Wang et al., 2017b). PFOA and PFOS are resistant to degradation in the environment (Parsons et al., 2008; Sáez et al., 2008). While manufacturing and direct use of PFOA and PFOS have declined in Western countries, these compounds have continuing presence in the environment. China continues production of PFOA and PFOS, and yearly environmental release from all sources was estimated at 40 tons of PFOA and 70 tons of PFOS (Liu et al., 2017c).

The presence of PFOA and PFOS in groundwater and surface water is of major concern. These chemicals can reach groundwater and surface waters via direct emissions of liquid waste and leaching of contaminated sites, such as landfills and airfields. In addition, due to their unique physicochemical properties, PFOA and PFOS can also reach groundwater from air emissions followed by deposition from air to soil with precipitation and migration through soil to groundwater (Davis et al., 2007). Both DuPont Washington Works and 3M Decatur plants caused PFOA and PFOS contamination of the drinking water in the nearby communities.

Air

Due to their ionic nature, PFOA and PFOS have low volatility (US EPA, 2014). Ionic PFAS such as PFOA and PFOS have low vapor pressures and predominantly bind to particles (Abbott et al., 2007; Haug et al., 2011b; Vierke et al., 2011). When directly introduced into the atmosphere, particle-bound PFAS settle to the ground following rain events (Barton et al., 2007; Davis et al., 2007). When dissolved in water, PFOA and PFOS tend to concentrate at the air-water interface, and for this reason, aerosols formed with the action of ocean waves have much higher PFOA levels than in the water (McMurdo et al., 2008). Aerosol transport by air currents likely contributes to the long-range transport of PFAS, which may explain the presence of these chemicals in the organisms in the Arctic (Martin et al., 2004a; Smithwick et al., 2006). PFOS levels remained constant in the particle phase of the air in the Arctic in 2006-2012 (Hung et al., 2016).

Commonly, PFAS air studies focused on precursors of PFOA and PFOS (Dreyer et al., 2009; Cai et al., 2012a; Del Vento et al., 2012; Wang et al., 2015b). Precursor compounds FTOHs (fluorotelomer alcohols) and FOSEs (perfluorooctane sulfonamide ethanols) play an important role in the mechanisms of air transport of PFOA and PFOS. These substances are used as grease-proofing agents in various consumer products such as carpets and food packaging and, due to their volatility, are often present at higher concentrations in the air than PFOA and PFOS. Once in an organism, FTOHs and FOSEs can break down to PFOA and PFOS, and FTOHs in

indoor office air significantly predicted PFOA serum concentrations in office workers (Fraser et al., 2012). Precursors can also degrade to PFOA and PFOS in the environment due to microbial action (Lee et al., 2010; Liu and Mejia Avendaño, 2013). Due to their neutral nature, these precursor compounds are much more efficiently transported into the air from wastewater and landfills (Ahrens et al., 2011; Vierke et al., 2011), and could also contribute to long-range transport of PFOA and PFOS (Jahnke et al., 2007a; Schenker et al., 2008).

PFAS air concentrations are generally higher in urban areas compared to rural areas. PFOA and PFOS concentrations in the gas fractions of outside air in an urban area (Albany, New York) averaged 2.86 (range, 1.89-6.53) picograms per cubic meter (pg/m^3) and 1.42 (0.94-3) pg/m^3, respectively (Kim and Kannan, 2007). For the particle fraction of the outside air, mean PFOA and PFOS concentrations were reported at 1.57 (range, 0.76-4.19) pg/m^3 and 0.66 (range, 0.35-1.16) pg/m^3, respectively, in samples collected in 2006 (Kim and Kannan, 2007). In two urban areas in Japan during 2000-2001, PFOS outdoor air concentrations were 0.6-5.3 pg/m^3 (Sasaki et al., 2003). In a different study of urban areas in Japan, with samples collected from 2001-2003, PFOA air concentrations were 2.0-263 pg/m^3 and PFOS concentrations were 0.7-5.2 pg/m^3 (Harada et al., 2005b). The exposure due to the high PFOA air levels (geometric mean of 263 pg/m^3) observed in the Kyoto area were comparable with the estimated exposure from food intake in the area. In rural areas of northwestern Europe, PFOA and PFOS concentrations in the outdoor air were 6.4 and 0.34 pg/mg^3, respectively (combined means from available data), and for urban areas, these values were 232 and 33 pg/m^3, respectively (Barber et al., 2007; Egeghy and Lorber, 2011). In an urban area in Germany (Hamburg), PFOA and PFOS in the outdoor air were present at <0.2-2.6 pg/m^3 and 0.4-1.6 pg/m^3, respectively (Jahnke et al., 2007b).

Reports on PFOA and PFOS in indoor air are scarce. Based on the observation of twenty-fold higher indoor levels of EtFOSA (ethylperfluorooctanesulfonamide) and MeFOSEA (methylperfluorooctanesulfoamidethylacrylate) compared to outdoor levels (Shoeib et al., 2005), some exposure models assumed that the indoor concentrations of PFOA and PFOS would also be twenty-fold higher than their respective outdoor levels, pegging the estimates at about 30-50 ng/m^3 (Egeghy and Lorber, 2011; Lorber and Egeghy, 2011). Mean PFOA levels in the particulate phase of indoor air in Tromso, Norway was 4.4 ng/m^3 (Barber et al., 2007). In a case study of a family with very high PFAS exposure due to repeated carpet treatments, PFOA and PFOS indoor air concentrations in the dust samples in a residence were as high as 150 and 1,100 ng/g respectively (Beesoon et al., 2012).

Soil

PFOA and PFOS can bind effectively to different types of soil and sediment, and the organic content in the solid phase appears to be the main determinant of binding (Higgins and Luthy, 2006). High organic content in sewage sludge results in absorption of PFOA and PFOS from wastewater (Zareitalabad et al., 2013), with negative implications for downstream sludge uses, such as for fertilizer in agricultural settings. Based on a review of available studies for PFOA and PFOS, Zareitalabad et al. (2013) reported average soil-adsorption coefficients ($\log K_{OC}$) of 2.1 log(l/kg) and 3.0 log(l/kg), respectively. In the FAO (Food and Agriculture Organization of the United Nations) soil mobility classification scheme, these correspond to the moderately mobile class (US EPA, 2009).

Strynar et al. (2012) analyzed sixty 'background' soil samples (i.e., without known PFAS contamination sources) from around the world and noted that the US soils had generally higher

PFAS background levels than some of the other countries. The authors hypothesized, that this would correlate with the relatively higher PFAS serum levels in US residents (Strynar et al., 2012). Among the reported results, PFOA was present at 1.56-31.7 ng/g in soil samples from North Carolina, Kentucky, Texas and Indiana, and PFOS was present at 0.606-2.55 ng/g. The levels of PFOA and PFOS in the agricultural soil in the Chicago area were greatly increased with the addition of bio-solids, with a maximum detected PFOS level of 483 ng/g (Sepulvado et al., 2011). The concentration of certain precursors, such as MeFOSAA (2-N-methylperfluorooctane sulfonamidoacetic acid) was high in the applied sludge but not in the augmented soil, indicating likely conversion to PFOS upon application (Sepulvado et al., 2011).

Global soil analysis of samples from each continent revealed PFAS accumulation in the Northern hemisphere (Rankin et al., 2016). The highest PFOS concentrations were detected in Europe, and the highest PFOA level occurred in one sample in Asia. Among the North American soil samples, three were from California, which contained 11-101 pg/g PFOA and 3.2-14.2 pg/g PFOS (Rankin et al., 2016). The soil level data of Rankin et al. (2016) were modeled to predict the global soil load of PFOA and PFOS (Washington et al., 2019), which was about 1,000 metric tons for either compound.

Indoor dust contained very high levels of PFOA and PFOS, at 100-500 ng/g for samples from the UK, Canada, France, Germany, Japan and US, while homes from Sweden, Norway, Thailand and Kazakhstan demonstrated much lower levels (Kubwabo et al., 2005; Kato et al., 2009; Goosey and Harrad, 2011; Haug et al., 2011b). Levels were also high in the dust from cars, classrooms and offices in the UK (Goosey and Harrad, 2011). High levels were found in the dust from the daycare centers in the US (mean values, 142 and 201 ng/g for PFOA and PFOS, respectively) and Sweden (~40 ng/g for either) (Strynar and Lindstrom, 2008; Björklund et al., 2009). Harrad et al. (2010) reviewed available data and suggested that carpeting and use of floor wax could be contributing to the presence of PFAS in the house dust.

Water

PFOA and PFOS occur in groundwater and surface waters, and in public drinking water in the US and around the world. PFOA and PFOS were included in US EPA's Third Unregulated Contaminant Monitoring Rule (UCMR3) which extensively analyzed sources of drinking water across the US from 2013-2015, with method reporting limits (detection limit) for PFOA and PFOS at 20 and 40 ng/L, respectively (US EPA, 2017). Out of 4,920 public water systems (PWS) analyzed, PFOA was detected in 117 (2.4%) and PFOS in 95 (1.9%). PFOA and PFOS were more frequently detected in the groundwater than surface water and were strongly correlated with the presence of major industrial sites (Hu et al., 2016). Although having tested widely across the US, the low detection rates under UCMR3 could be due to the relatively high detection limits of 20 ng/L for PFOA and 40 ng/L for PFOS. Several UCMR3-tested areas in California had 20-70 ng/L PFOA and/or 40-200 ng/L PFOS in drinking water (Hu et al., 2016).

In the subset of UCMR3 results for California,[5] 0.83% of water samples contained PFOA or PFOS at the level of or greater than the detection limit. The average PFOA concentration among these samples was 28 ng/L, and the average PFOS concentration was 57 ng/L.

[5] https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/PFOA_PFOS, accessed September 2020

The results of the more recent drinking water monitoring program carried out by SWRCB are summarized in Table 3.2.1.[6] This program comprised four rounds of testing in 2019-2020. Due to lower detection limits, the detection rate was higher compared to the UCMR3 dataset, and the average values are lower. The program utilized US EPA Method 537.1, with lowest concentration minimum reporting levels (LCMRLs) of 0.82 ng/L for PFOA and 2.7 ng/L for PFOS (Shoemaker and Tettenhorst, 2018).

Table 3.2.1. PFOA/PFOS public water system testing results in California (2019-2020)

Dates	PFAS	Samples (N)	% detected	Arithmetic mean including non-detects (ng/L)	Arithmetic mean excluding non-detects (ng/L)	Geometric mean excluding non-detects (ng/L)
04/01/19-06/30/19	PFOA	570	40	5.71	14.4	8.71
07/01/19-09/30/19	PFOA	653	43	5.31	12.4	8.16
10/01/19-12/31/19	PFOA	920	33	4.79	14.5	9.01
01/01/20-06/30/20	PFOA	771	38	5.27	13.9	7.89
04/01/19-06/30/19	PFOS	570	45	11.5	25.5	14.6
07/01/19-09/30/19	PFOS	653	47	11.5	24.5	14.0
10/01/19-12/31/19	PFOS	920	40	10.4	26.1	13.3
01/01/20-06/30/20	PFOS	772	42	9.7	22.8	11.8

In a different study, the median of 57 reported PFOA concentrations in US surface waters was 41 ng/L, and for PFOS (N=56), 42 ng/L (Zareitalabad et al., 2013). These values were highest among ten nations analyzed, and for comparison, PFOA and PFOS median values for China (N=86, 83) were 1.8 ng/L and 0.89 ng/L, respectively, while for Canada (N=74, 70) PFOA and PFOS median values were 0.1 ng/L and 2.2 ng/L, respectively (Zareitalabad et al., 2013). These surface water data were based on several reports (Hansen et al., 2002; Boulanger et al., 2005; Giesy et al., 2006; Nakayama et al., 2007).

In a summary of published studies, PFOA and PFOS levels in river water (including rivers from Europe and China) were generally within 1-10 ng/L and 0.1-10 ng/L, respectively (Zhao et al., 2015b). Higher PFOA levels (22-260 ng/L) were observed in the Yangtze river (So et al., 2007).

Another contributor to PFOA and PFOS in surface waters is stormwater runoff. PFOA and PFOS were detected in 100% of stormwater runoff samples in the Minneapolis, MN, area at concentrations up to 30.6 and 155.8 ng/L, respectively (Xiao et al., 2012). These levels were significantly higher than rainfall concentrations (8.4 and 6.7 ng/L for PFOA and PFOS, respectively), indicating leaching of PFOA and PFOS from urban surfaces during the storm events. In a different study conducted in New York state, PFOA and PFOS concentrations in runoff reached 29.3 and 14.6 ng/L, respectively, and were also higher than concentrations in rain or snow (Kim and Kannan, 2007).

The discharge from municipal wastewater treatment plants (WWTPs) appears to be a major contributor to increased PFOA and PFOS concentrations in surface water (Becker et al., 2008). PFOA and PFOS in wastewater discharges are persistent despite wastewater treatment

[6] https://www.waterboards.ca.gov/pfas/drinking_water.html, accessed September 2020, accessed September 2020

(Sinclair and Kannan, 2006; Schultz et al., 2006a; Becker et al., 2008). Certain wastewater primary treatments, such as activated sludge treatment, can result in increased levels, presumably as a result of degradation of precursor compounds such as fluorotelomer alcohols (Sinclair and Kannan, 2006; Schultz et al., 2006b; Eriksson et al., 2017a). Oxidation and microbial processes can also contribute to transformation of precursor compounds to PFOA and PFOS (Houtz and Sedlak, 2012).

PFOA and PFOS are ubiquitously present throughout the world's oceans, and their concentrations in surface ocean water can reach several hundred pg/L (Ahrens et al., 2009; Ahrens et al., 2010; Benskin et al., 2012; Cai et al., 2012b; Zhao et al., 2012; González-Gaya et al., 2014; Zhao et al., 2015b). As the result of bioaccumulation, PFOA and PFOS are present in marine organisms, and consumption of seafood can be a major source of PFAS in humans.

Biota and Food

PFOA and PFOS have been described as proteinophilic compounds (Kelly et al., 2009; Xia et al., 2013), and as a result they readily distribute into living organisms. PFAS did not significantly correlate with several classes of lipophilic compounds such as PCBs (polychlorinated biphenyls), DDTs (dichlorodiphenyltrichloroethanes) and PBDEs (polybrominated diphenyl ethers) in a bioaccumulation study in fish and birds in the Barents Sea food web, consistent with the distinct physicochemical properties of PFAS (Haukås et al., 2007).

The marine environments in the industrialized parts of the world, e.g., the Baltic Sea, are of particular concern for PFOA and PFOS pollution, as high levels were found in fish, birds and marine mammals (Kratzer et al., 2011; Gebbink et al., 2016; de Wit et al., 2020). Gebbink et al. (2016) reported dramatic trophic magnification of PFOS in the food chain, but PFOA levels were fairly similar in the living organisms in the ecosystem. This study also reported enrichment of the linear PFOS isomer up the food chain, with approximately 90% of total PFOS in the top organisms (Gebbink et al., 2016). Accordingly, fish and seafood are among the top contributors to PFOA and PFOS in the diet (Haug et al., 2010; Noorlander et al., 2011).

Trophic magnification of PFOA and PFOS and selective enrichment of linear PFOS (n-PFOS) were also found in the food web of Taihu Lake, China (Fang et al., 2014). In this study, n-PFOS concentrations in fish were 10-90 ng/g. High biomagnification of PFOS was also observed in the eastern Arctic food webs (Tomy et al., 2004; Haukås et al., 2007). In this ecosystem, PFOA did not appear to be subject to trophic magnification (Tomy et al., 2004). Martin et al. (2004b) observed trophic magnification of PFOS in the Lake Ontario food chain but suggested that conversion of PFOS precursors to PFOS could contribute more to trophic magnification than PFOS bioaccumulation, at least in this particular ecosystem.

Metabolic conversion from precursor compounds can be a major source of PFAS exposure in marine organisms (Gebbink et al., 2016). Pilot whales, as well as crustaceans, lack the enzyme necessary for conversion of PFOSA (perfulorooctane sulfonamide) to PFOS, and contained much higher levels of PFOSA than PFOS in a study in the North Atlantic (Dassuncao et al., 2017). Moreover, PFOSA levels in whale muscle demonstrated a sharp decline from 2001 onward, whereas PFOS levels in whale muscle were increasing. Based on these observations and exposure models, this study concluded that high PFOS levels in most marine organisms, as well as recent declines of PFOS in marine organisms other than whales could be primarily driven by exposure to PFOS precursor(s) (Dassuncao et al., 2017). Due to the efficient and reversible retention by soil, PFOA and PFOS are readily taken up by plants and are subject to

further bioaccumulation in livestock (Ghisi et al., 2019). Stahl et al. (2009) observed a statistically significant concentration-dependent carryover of PFOA and PFOS from spiked soil (0.25-50 mg/kg) to maize, oats, wheat and potatoes. In this study, PFOA levels in foodstuffs exceeded the background levels at 0.25 mg/kg spiked concentration in soil (lowest studied), while PFOS in plants exceeded background levels at 1 mg/kg. PFOA and PFOS were also detected in lettuce, tomatoes, carrots, peas, celery and radishes (Blaine et al., 2013; Blaine et al., 2014; Bizkarguenaga et al., 2016). Cereals and cereal products were the main source of PFOA in the Norwegian diet, followed by meat and dairy, and with much smaller contribution from fruits and vegetables among other food groups (Haug et al., 2010). In a different study, PFOA contribution from vegetables (18 pg/kg-day) was comparable to that of meat products (22 pg/kg-day) (Gebbink et al., 2015b).

Animals appear to bioaccumulate PFOA and PFOS, as discussed in more detail in Chapter 4 of this document. As a result of feed and drinking water contamination, meat, eggs and dairy contain high levels of PFAS. Generally, animal products (meat, eggs, fish and dairy) tend to dominate dietary intake of PFOS, while for PFOA, cereals and vegetables have a comparable contribution (Tittlemier et al., 2007; Haug et al., 2010; Noorlander et al., 2011; Vestergren et al., 2012; Gebbink et al., 2015b). Although food packaging materials and non-stick cookware contain PFAS, it is unclear whether they significantly contribute to PFOA and PFOS intake with food (Jogsten et al., 2009).

The dietary studies discussed in this section were not specific to California or US diet, and the contribution of diet to overall exposure for California residents is further discussed in Appendix 4 of this document.

Human Exposure

Humans are exposed to PFOA and PFOS through diet, drinking water, ingestion of dust, inhalation of indoor and outdoor air, and through metabolic conversion of PFAS precursors, such as FOSEs and FTOHs. Appendix 5, Table A5.1 summarizes exposure studies that analyzed different routes of exposure (Egeghy and Lorber, 2011; Haug et al., 2011a; Lorber and Egeghy, 2011). The major exposure contribution in adults is food (71-87%), followed by drinking water (7.5-23%). The contribution of PFOA in ingested dust is also significant in adults (14.7-19.8%), whereas that of PFOS is smaller (1.6-5.5%). The biggest difference in infants and young children compared to adults is larger contribution from ingested dust, attributed to increased floor level playing activity and hand to mouth transfer. These exposure assessments did not consider indirect exposure resulting from exposure to PFOA and PFOS precursors in the environment, and the subsequent conversion to PFOA and PFOS in the organism. The contribution from precursor compounds to the overall PFOA and PFOS exposure is generally thought to be low (<10%) based on exposure assessments that estimate the 50th percentiles of exposures from different routes (Vestergren et al., 2008; Gebbink et al., 2015a; Gebbink et al., 2015b). However, in the high-exposure scenario, based on the 95th percentile of exposure via all routes, conversion from precursors was estimated to contribute up to 55% and 80% of overall exposure to PFOA and PFOS, respectively (Vestergren et al., 2008). Based on the study of temporal trends of PFOA, PFOS and precursors in diet, consumer products and human serum, it was suggested that precursor compounds may significantly contribute to the overall PFOA exposure in the Norwegian population (D'Eon and Mabury, 2011b).

Additional exposure studies found comparable values to those presented in Appendix 5, Table A5.1 (Noorlander et al., 2011; Vestergren et al., 2012; Gebbink et al., 2015a; Balk et al., 2019).

However, some studies predicted higher background exposure to PFOA and PFOS (Trudel et al., 2008; Fromme et al., 2009; Karrman et al., 2009). For example, Trudel et al. (2008) estimated PFOA exposure levels for an intermediate exposure scenario for infants, children and adults at 9.8, 7.6 and 2.5 ng/kg-day, respectively. For PFOS, exposure levels for an intermediate exposure scenario for infants, children and adults were at 54.6, 22.1 and 15.3 ng/kg-day, respectively. These values, particularly for PFOS, are higher compared to the studies in Table A5.1 in Appendix 5. While different exposure routes were analyzed, individual contributions to overall exposure were not specified in Trudel et al. (2008).

Occupational PFOA exposure can be much higher, e.g., 158 ng/kg-day for male adults in one estimate, and driven by inhalation (Vestergren and Cousins, 2009). In the general population, air pollution can provide an exposure contribution equal to that of diet (Harada et al., 2005b).

Contaminated drinking water can also become the main source of exposure. Vestergren and Cousins (2009) estimated that at 519 ng/L or higher, the contribution of drinking water to the overall PFOA exposure would be greater than 75%. The cutoff of 519 ng/L was from reported PFOA drinking water concentrations in Arnsberg, Germany (Hölzer et al., 2008). These exposure conditions were also met in exposure studies in Little Hocking and Lubeck (US), where the PFOA concentration in drinking water was 3,550 and 500 ng/L, respectively, and with PFOS exposure studies in Ronneby, Sweden, where the PFOS concentration in drinking water was 8,000 ng/L (Thompson et al., 2010; Li et al., 2018c). The PFOA exposure from drinking water in the Veneto region of Italy was close, with a median of 319 ng/L (Pitter et al., 2020).

Biomonitoring

Due to their ubiquitous presence in the environment, PFOA and PFOS have been detected in close to 100% of human serum samples. The available publications reporting PFOA and PFOS serum levels are too numerous to provide a comprehensive review. In the US, the National Health and Nutrition Examination Survey (NHANES) compiles the data for PFAS levels, including PFOA and PFOS, in serum for the general population. Data from this survey are available independently and in published reports (Calafat et al., 2007a; Calafat et al., 2007b; Kato et al., 2011; Jain, 2018). In 2013-2014, the weighted average PFOA serum concentration was 2.0 ng/ml, and for PFOS the weighted average serum concentration was 4.1 ng/ml (Jain, 2018). Mirroring PFOA and PFOS trends in manufacturing and uses serum concentrations increased through the 90s and then declined. The published studies on the time trends of PFOA and PFOS serum levels are summarized in Appendix 5, Table A5.2. While decreases in serum concentrations were observed in all studies from the US, Western Europe and Japan over 2000-2015, one study from South Korea (Seo et al., 2018) did not find a clear trend within the 2006-2015 period. Time trend studies from other countries and parts of the world are lacking, and it is possible that the decrease in PFOA and PFOS serum concentration observed over the last decade would be specific to industrial countries, where use and environmental pollution has been decreasing.

Several studies analyzed PFOA and PFOS serum levels in California residents, they are summarized in Table 3.2.2. These include studies conducted by the Biomonitoring California[7] program as well as other published studies of Californians. Generally, the PFOA and PFOS serum levels are consistent with NHANES data. Studies reporting time trends in the PFOA and

[7] https://dev.biomonitoring.ca.gov/

PFOS serum concentrations in California also found decreases from earlier peaks (Wang et al., 2011; Olsen et al., 2012; Kim et al., 2020).

Table 3.2.2. PFOA and PFOS biomonitoring studies in California

Study	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[a]	Reference
CHDS[b]	Northern California	Pregnant women, archived samples (N=105)	PFOA	Arithmetic means 1960s[c]: 0.30 1980s: 3.17 2009: 2.21	Wang et al. (2011)
			PFOS	Arithmetic means 1960s[c]: 45.90 1980s: 30.60 2009: 9.44	
CHARGE[b]	Northern California	Women (mothers) (N=450)	PFOA[d]	2009: 1.66; 2010: 1.37 2011: 1.26; 2012: 1.14 2013: 1.02; 2014: 0.94 2015: 0.80; 2016: 0.68	Kim et al. (2020)
			PFOS[d]	2009: 4.86; 2010: 4.1 2011: 3.78; 2012: 3.42 2013: 3.02; 2014: 2.8 2015: 2.42; 2016: 2.12	
Red Cross plasma samples[b]	Los Angeles	Adult plasma donors (N=100)	PFOA[d]	2000/01: 4.0 2006: 2.7 2010: 1.9	Olsen et al. (2012)
			PFOS[d]	2000/01: 35.0 2006: 14.3 2010: 7.7	
MIEEP	San Francisco	Pregnant women (N=49-77)	PFOA	2010-2011: 0.47 (95th pctl: 2.14)	Website,[e] Morello-Frosch et al. (2016)
			PFOS	2010-2011: 2.55 (95th pctl: 7.25)	
FOX	California	Firefighters (N=101)	PFOA	2010-2011: 3.75 (95th pctl: 9.54)	Website,[e] Dobraca et al. (2015)
			PFOS	2010-2011: 12.5 (95th pctl: 24.7)	
CTS	California	Female teachers (N=1,257-1,759)	PFOA	2011-ongoing: 2.46 (95th pctl: 6.22) 2011-2015: 2.996 (mean)	Website,[e] Hurley et al. (2018)
			PFOS	2011-ongoing: 6.80 (95th pctl: 19.5) 2011-2015: 8.539 (mean)	Website,[e] Hurley et al. (2018)
		Subset of CTS subjects with identifiable source of drinking water (N=1,333)	PFOA	2011-2013: 2.45 (1,263 subjects with non-detectable PFOA[c] in drinking water); 3.47 (70 subjects with detectable levels of PFOA in drinking water)	Hurley et al. (2016)
			PFOS	2011-2013: 6.76 (1,240 subjects with non-detectable PFOS[c] in drinking water); 8.51 (93 subjects with detectable PFOS in drinking water)	
BEST-1	Central Valley	Adults (N=110)	PFOA	2011-2012: 1.97 (95th pctl: 4.7)	Website[e]
			PFOS	2011-2012: 7.00 (95th pctl: 25.8)	

Study	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[a]	Reference
BEST-2	Central Valley	Adults (N=337)	PFOA	2013: 1.49 (95th pctl: 4.57)	Website[e]
			PFOS	2013: 5.21 (95th pctl: 17.6)	
MAMAS	California	Pregnant women (N=200)	PFOA	2012-2015: 1.24 (95th pctl: 2.81)	Website[e]
			PFOS	2012-2015: 4.2 (95th pctl: 12.3)	
ACE 1	San Francisco Bay Area	Chinese adults (N=96)	PFOA	2016: 1.41 (90th pctl: 2.85)	Website[e]
			PFOS	2016: 6.51 (90th pctl: 19.3)	
ACE 2	San Francisco Bay Area	Vietnamese adults (N=99)	PFOA	2017: 1.69 (90th pctl: 3.06)	Website[e]
			PFOS	2017: 7.47 (90th pctl: 22.9)	
CARE-LA	Los Angeles county	Adults (N=425)	PFOA	2018: 1.04 (95th pctl: 3.06)	Website[e]
			PFOS	2018: 2.13 (95th pctl: 8.33)	
CARE-2	Riverside, San Bernardino, Imperial, Mono, and Inyo counties	Adults (N=358)	PFOA	2019: 0.98 (95th pctl: 2.70)	Website[e]
		Adults (N=357)	PFOS	2019: 2.40 (95th pctl: 8.72)	

ACE, Asian/Pacific Islander community exposures; BEST, biomonitoring exposures study; CARE-LA, California regional exposure study, Los Angeles county; CHARGE, childhood autism risk from genetics and environment study; CARE-2, California Regional Exposure Study, Region 2; CHDS, child health and development studies; CTS, California teachers study; FOX, firefighter occupational exposures project; MAMAS, measuring analytes in maternal archived samples; MIEEP, maternal and infant environmental exposure project

[a] Geometric mean if not indicated otherwise; pctl, percentile

[b] CHDS (except 2009 data), CHARGE and Olsen et al. (2012) studies were not part of the Biomonitoring California program

[c] Non-detects were as assigned a value of LOD (limit of detection)/$\sqrt{2}$ for each analyte

[d] Graphical data were quantified using GetData Graph Digitizer software (version 2.26)

[e] https://biomonitoring.ca.gov/, last accessed May 2021

4. TOXICOKINETICS

PFOA and PFOS strongly bioaccumulate in humans and, to a much lesser degree, in animals. For such compounds, dose-response analysis is usually based on an internal dose metric such as serum concentrations, and the results are converted to applied doses using toxicokinetic (TK) approaches. All available human toxicity studies report serum concentrations for PFOA or PFOS, necessitating an adequate TK approach to convert human dose-response data to an oral dose, which serves as the basis for the PHG. Similarly, animal studies that report applied doses would require conversion to serum concentrations prior to dose-response analysis, and those that report serum levels would require conversion to human equivalent doses in deriving an acceptable daily dose. This chapter summarizes available TK information for these compounds in humans and animals (Sections 4.1-4.5), considers available physiologically-based pharmacokinetic models (Section 4.6) and analyzes in more detail the estimation of serum clearance for these compounds (Sections 4.7-4.9). As will be explained in the text, applying a clearance factor is a relatively simple method to convert serum concentrations to applied doses, and has been used in this capacity by US EPA for its PFOA and PFOS assessments (US EPA, 2016b; US EPA, 2016d). After reviewing this approach in detail, OEHHA provides updated clearance estimates for PFOA and PFOS (Section 4.9).

4.1. Species Differences: PFOA and PFOS Serum Half-lives

PFAS, including PFOA and PFOS, demonstrate dramatic differences in TK[8] properties among species, and in some cases, between sexes, as demonstrated for half-life ($T_{1/2}$) estimates in Table 4.1.1. Except for human $T_{1/2}$ values, data are from a recent review that summarized available studies in rat, mouse and monkey (Pizzurro et al., 2019). For human $T_{1/2}$, values in the table were chosen by US EPA for their PFOA and PFOS assessments. Human $T_{1/2}$ values are discussed in detail in Section 4.7. Additionally, PFOA $T_{1/2}$ was 5.5-7 hours in the rabbit, 10.6-20.1 days in the dog and 2.7-5.6 days in the Japanese macaque (Hanhijärvi et al., 1988; Kudo and Kawashima, 2003; Harada et al., 2005a).

Table 4.1.1. PFOA and PFOS serum half-lives in species (Pizzurro et al., 2019)

	PFOA		**PFOS**	
	female	**male**	**female**	**male**
Rat	1.9-4.6 h (<25mg/kg) 16.2 h (25 mg/kg) 24 h (50 mg/kg)	1.5-15 d (<25 mg/kg) 6.5 d (25 mg/kg) 4.4 d (50 mg/kg)	24-83 d	26-82 d
Mouse	1.2 d (20 mg/kg-d[a]) 15.6 d (1 or 10 mg/kg)	21.7 d (1 or 10 mg/kg)	38 d (1 mg/kg-d) 30 d (20 mg/kg-d)	43 d (1 mg/kg-d) 36 d (20 mg/kg-d)
Cynomolgus monkey	32.6 d	20 d	110-200 d	132-200 d
Human	2.3 years (US EPA, 2016b)		5.4 years (US EPA, 2016d)	

d, days; h, hours
[a] 17 days (multi-dose treatment)

[8] The terms “toxicokinetic” and “pharmacokinetic” have been used interchangeably in past PHG documents. In this document, OEHHA is using the term toxicokinetic, unless specifically referring to physiologically based pharmacokinetic models, for consistency.

Human PFOA $T_{1/2}$ is ~ 200x that of the rat (male) and ~40x that of the mouse. Human PFOS $T_{1/2}$ is ~ 40x that of the rat or mouse. Such dramatic differences in half-lives indicate that interspecies dose extrapolations have to be done using TK considerations, such as clearance rates or PBPK (physiologically based pharmacokinetic) models. Another interesting aspect of interspecies TK differences is the sex difference for the rat PFOA $T_{1/2}$ values, with much faster elimination in the female rat (Table 4.1.1). Other species, including human, do not demonstrate sex differences in PFOA $T_{1/2}$ values. The observation of sex differences in PFOA elimination in the rat allowed characterization of the underlying mechanisms of renal reabsorption involving specific membrane transporters. While absorption, distribution and metabolism of PFOA and PFOS demonstrate interspecies similarity, differences in excretion appear to underlie interspecies differences in PFAS kinetics.

4.2. Absorption

PFOA and PFOS are well absorbed with oral administration in animal studies. In male CD rats, 93% of the oral gavage dose (11 mg/kg) was absorbed after 24 hours (unpublished report cited in US EPA (2016b)). Similarly, oral absorption efficiency was at least 92-93% in male Sprague Dawley rats exposed to 5 or 20 mg/kg of PFOA via gavage (Cui et al., 2010). In the same study, absorption of PFOS in male rats was approximately 98% for either 5 or 20 mg/kg oral gavage doses (Cui et al., 2010). The PFOA absorption efficiency in male C57BL/6 mice was 98% over 48 hours following a gavage dose of approximately 7.5 mg (Jandacek et al., 2010). In a PFOA mouse study, the area under the concentration-time curve (AUC) was measured over 24 hours for serum concentrations following a single intravenous (i.v.) or oral gavage dose, and the resulting i.v. to oral AUC ratio was close to 1, indicating 100% absorption efficiency (Fujii et al., 2015). In male Sprague Dawley rats, the absorption of a single oral dose at 4.2 mg/kg PFOS was estimated to be >95% (Chang et al., 2012). PFOS was fully absorbed in female white New Zealand rabbits following an oral gavage dose of 0.2 µg/kg (Tarazona et al., 2016). When PFOS was alternatively introduced by i.v. or oral gavage to male or female Sprague Dawley rats at 2 mg/kg, the plasma concentration curves overlapped at ≥2 hours in either sex, indicating 100% absorption (Huang et al., 2019a).

In humans, PFOA was rapidly absorbed with oral doses of 50-1,200 mg in a clinical trial of cancer patients, as indicated by peak plasma concentrations at 2-4 hours in most study participants (Elcombe et al., 2013; IARC, 2017a; Convertino et al., 2018). However, in a sizable fraction of the 43 subjects in this study, peak plasma concentrations were steadily increasing over the initial 24 hours of measurement, indicating a slower absorption/distribution process and significant kinetic variability in this population (Elcombe et al., 2013). No studies of human PFOS oral absorption were identified. PFOA and PFOS human exposure models demonstrated good correlation of predicted and observed serum concentrations, with the assumption of high oral absorption efficiency (Haug et al., 2011a; Noorlander et al., 2011; Vestergren et al., 2012).

Several PFOA and PFOS exposure models, originating with Trudel et al. (2008) used PFOA/PFOS oral absorption efficiency values of 0.66, 0.8 and 0.91 to represent low, intermediate and high exposure scenarios, respectively (Trudel et al., 2008; Gebbink et al., 2015a; Balk et al., 2019). This scale was derived from the distribution of recovery of [^{14}C]PFOA (carbon-14 radioisotope labeled PFOA) doses (as percentage of total administered) in urine and tissues among both sexes of rat, mouse, hamster and rabbit (Hundley et al., 2006). This study did not consider elimination via bile excretion/fecal route, and produced much lower PFOA absorption estimates, e.g., 77% and 57% in male and female mice, respectively, than most other reports. Therefore, the resulting assumption of lower human absorption efficiency, such

as reported in Trudel et al. (2008) is not supported by most available data. Based on available human and animal studies, oral absorption of PFOA and PFOS appears to be equal to or greater than 90%.

PFOA was dermally absorbed in rabbits and rats (Kennedy Jr, 1985; US EPA, 2016b). In an unpublished study, O'Malley and Ebbens (1981) observed increased mortality in female and male New Zealand white rabbits at 1,000 and 2,000 mg/kg PFOA applied dermally for 14 days, while no animals died at 100 mg/kg (US EPA, 2016b).

Dermal permeability coefficients (K_p) for PFOA were $9.49 \pm 2.86 \times 10^{-7}$ cm/h and $3.25 \pm 1.51 \times 10^{-5}$ cm/h, for human and rat skin respectively, as determined in vitro (Fasano et al., 2005).

In an unpublished study, Hinderliter (2003) demonstrated effective absorption of PFOA in rats through inhalation exposure to a PFOA aerosol (US EPA, 2016b). Plasma PFOA concentrations increased proportionally to the applied dose, and reached higher levels in male rats, presumably due to sex-specific excretion differences.

4.3. Distribution

PFOA and PFOS are widely distributed in the body with preferential accumulation in the liver, plasma and kidney (Appendix 6, Table A6.1). Liver appears to have the highest levels of PFOA in rats and male mice, but in female mice plasma concentrations are higher than those in the liver. In two monkey and two human studies, liver had lower PFOA concentrations than serum (Griffith and Long, 1980; Butenhoff et al., 2004a; Maestri et al., 2006; Mamsen et al., 2019). For PFOS, all species but humans had strong liver accumulation, and in human studies, evidence was mixed, with highest levels of PFOS in the liver in two studies (Olsen et al., 2003c; Maestri et al., 2006). Taken together, these data suggest toxicokinetic differences between species.

PFOA and PFOS can cross the placenta and accumulate in the fetus, with liver as one of the fetal target organs (Mamsen et al., 2019). PFOA and PFOS can transfer via lactation in humans (reviewed in Pizzurro et al. (2019)), resulting in decreased body burden in the mother and increased blood concentrations in the infant. This may be of concern, particularly for PFOA, since concentrations in the infant plasma can become much higher relative to maternal plasma. In a German study of 53 mother-infant pairs randomly sampled from the general population and of which 37 infants were exclusively breast-fed, the median concentration of PFOS was nearly the same in the maternal and infant serum at 6 months of age, while PFOA concentrations were 4.6-fold higher in the infants, on average (Fromme et al., 2010). In infants from a Faroe Islands study, PFOS levels in plasma increased on average at a rate of 29.2% per month over the first 18 months of life, while PFOA levels increased on average at a rate of 27.8% per month (Mogensen et al., 2015a). Overall the values increased approximately 4-fold for either compound, the majority of increase was during the first 11 months and correlated with breast-feeding. In the same study, at the age of 5 years, the PFOA and PFOS values decreased relative to the peak at 11-18 months.

The effects of breastfeeding on increasing PFOA and PFOS concentrations at 1 year of age were also reported in a study of 101 German infants (Abraham et al., 2020). However, in a cross-sectional study of 300 children in Texas, plasma concentrations of PFOA or PFOS steadily increased for 0-3, 3-6, 6-9 and 9-13 years of age groups, indicating that a possible early life spike in plasma concentrations would have dissipated by 3 years of age (Schecter et al., 2012). This could be partly due to growth dilution. Several recent kinetic and PBPK models

addressed infant kinetics of PFAS (Verner et al., 2016; Brochot et al., 2019; Goeden et al., 2019).

In general, PFAS distribution in the body has been thought to be driven by protein binding (Kennedy et al., 2004; Cheng and Ng, 2017). High levels of PFOA and PFOS accumulation in plasma and liver are likely driven by binding to specific proteins, such as serum albumin and liver fatty acid-binding protein (L-FABP). Published reports on PFOA and PFOS binding to proteins are summarized in Appendix 6, Table A6.2. PFOA and PFOS bind to albumin with particularly high affinity, often with the dissociation constant at ~10^{-6} M. Detailed analysis of PFOA-albumin binding energies indicates non-covalent binding with likely involvement of Van-der-Waals forces and hydrogen bonds (Qin et al., 2010).

Binding of PFOA and PFOS to certain targets, such as plasma albumin, L-FABP and transthyretin appears to be of the same magnitude as binding constants reported for their endogenous ligands (Luebker et al., 2002; D'Eon et al., 2010; MacManus-Spencer et al., 2010). Displacement of endogenous ligands from carrier or transporter proteins has been hypothesized as one of the possible mechanisms of action in PFOA/PFOS toxicity.

While binding to blood proteins is important, PFOA and PFOS do not appear to distribute to red blood cells or lipoproteins to a significant extent in either humans or rats (Johnson et al., 1984; Kärrman et al., 2006; Ehresman et al., 2007; Kudo et al., 2007; Butenhoff et al., 2012c; Hanssen et al., 2013; Jin et al., 2016). In contrast, PFOA and PFOS appear to be efficiently distributed to the liver, likely due to the demonstrated L-FABP binding (Luebker et al., 2002).

At physiological pH, PFOA and PFOS are charged and therefore, would not be able to cross membranes via passive transport. Several transporter proteins are likely to be involved in PFOA and PFOS transport (Appendix 6, Table A6.3). Transporter studies primarily involved cell-cultures (in vitro system), in which kinetics of PFAS absorption into cells or across a cell barrier were compared to that of a negative control, such as cells lacking the transporter of interest. In some studies, such as Kummu et al. (2015), the efficiency of in vitro transport was correlated with transporter expression levels for human organ samples.

Important physiological roles for PFAS-active transporters have been proposed and include:

- in the kidney, secretion and/or reabsorption of PFAS, which drives overall PFAS kinetics; transporters are suggested to be responsible for species/sex kinetic differences;
- in the liver, bile acid transporters (Zhao et al., 2015a) appear to mediate re-uptake of PFAS following secretion in bile, likely contributing to enterohepatic circulation of these compounds;
- in the placenta, organic anion transporter (OAT) 4 may be involved in transport of PFAS into the fetus (Kummu et al., 2015).

There are limited animal studies on distribution of PFOA and PFOS isomers. When administered orally as a mixture of isomers to rats, both PFOA and PFOS demonstrated organ-specific and sex-specific differences among isomers after 38 days following a single dose or after 38 days of daily treatments (Benskin et al., 2009; De Silva et al., 2009). While some isomers demonstrated higher levels of accumulation in some organs, the underlying mechanisms or similarity with human systems remain unclear. Branched PFOS isomers were eliminated faster than n-PFOS (linear form) in rats given PFAS-spiked food for 77 days (Ross et al., 2012).

In humans, n-PFAS isomers appear to be enriched (relative to other isomers and the source of exposure) and with longer half-lives compared to other isomers (Zhang et al., 2013b; Zhou et al., 2014). Longer retention of linear isomers could be due to tighter binding to blood proteins (Beesoon and Martin, 2015; Gao et al., 2015b).

4.4. Metabolism

PFOA and PFOS are not known to be metabolized (EFSA, 2018). Based on the distribution and excretion of ionic and non-ionic fluorine in female rats administered an unspecified dose of PFOA (mix of linear and branched isomers) and followed for 96 hours, Ophaug and Singer (1980) hypothesized that PFOA did not undergo Phase I metabolism and was excreted intact. Single dose i.v. studies in rats of both sexes and in vitro microsomal incubations with radioactively labelled PFOA did not detect covalently modified or Phase II conjugated metabolites of PFOA (Vanden Heuvel et al., 1991; Goecke et al., 1992; Kuslikis et al., 1992). For example, only parent [^{14}C]PFOA was excreted in urine and bile of male and female rats treated with a single i.v. dose of 9.4 µmol/kg PFOA and followed for 6 hours (bile) or up to 28 days (male rats) (Vanden Heuvel et al., 1991). Consistent with the general lack of metabolism, only 1.3-5.2% of ^{14}C activity was recovered in the expired air from mice, rats and hamsters over 120 hours after a single oral gavage dose of [^{14}C]PFOA (10 mg/kg) (Hundley et al., 2006). Based on elution properties of PFOA excreted in rats exposed to a single intraperitoneal (i.p.) dose (50 mg/kg) and followed for 96 hours, Ylinen et al. (1989) also concluded that the compound would not be conjugated with glucuronic or amino acid groups, indicating no Phase II metabolism. While PFOA metabolism studies in humans and studies with PFOS are lacking, it is generally assumed that both compounds are inert to metabolism in humans and are excreted intact (US EPA, 2016b; US EPA, 2016d).

4.5. Excretion

Excretion pathways of PFOA and PFOS include:

1) Renal or urinary excretion, which occurs in all mammalian species and appears to be dominant in fast eliminators, e.g., in the case of PFOA elimination in the female rat.
2) Fecal or gastrointestinal excretion appears to play a more important role in slow eliminators, such as humans; likely subject to enterohepatic circulation.
3) Elimination pathways via pregnancy and lactation in human females (Wong et al., 2014).

Several studies in rats determined that renal excretion was higher than fecal excretion for PFOA and PFOS. With a single i.p. dose of PFOA (20 mg/kg), cumulative elimination in urine (50-75% of the dose) was more than 20-fold higher than excretion in feces, in both female and male rats (Kudo et al., 2001). During subchronic exposure in male rats, the daily amount of PFOA excreted in urine was ≥2-fold higher than the amount excreted in feces over 28 days at either 5 or 20 mg/kg-day doses (Cui et al., 2010). For PFOS, approximately similar amounts were excreted in urine and feces for the first 10 days at either 5 or 20 mg/kg-day doses, with progressively relatively higher amounts of PFOS excreted in urine at 10+ days (Cui et al., 2010). Ohmori et al. (2003) found that in male and female rats injected with a single dose of PFOA or other perfluorocarboxylic acids (48.65 mmol/kg), increasing PFAS chain length correlated with longer $T_{1/2}$ and lower renal clearance. Regression of renal clearance versus total clearance for all data points (PFAS/sex combinations) produced good fit (r^2 = 0.981) and a slope of 0.48, suggesting that renal clearance would account for approximately half of overall excretion. Additional rat studies are consistent with these general conclusions (Ophaug and Singer, 1980;

Kojo et al., 1986; Vanden Heuvel et al., 1991; Luebker et al., 2005a; Katakura et al., 2007; Benskin et al., 2009; Chang et al., 2012; Gao et al., 2015a).

In male and female mice administered a single dose of PFOA and followed for 24 hours, urinary clearance was 7.6x higher than fecal clearance with an i.v. dose (0.31 µmol/kg), and 2.5x higher with a gavage dose (3.13 µmol/kg) (Fujii et al., 2015). Similar to rats (Ohmori et al., 2003), increasing chain length of perfluoroalkyl carboxylate (C7-C14) resulted in progressively lower urinary clearance and progressively higher fecal clearance, with approximately similar amounts eliminated via urine or feces for PFDA (perfluorodecanoic acid, C10).

In humans, multiple reports directly measured PFOA and PFOS renal clearance in occupationally exposed subjects and in the general population (Table 4.5.1). All studies were in Asia (China, Japan). Despite a wide range of observed serum concentrations, renal clearance values for both PFOA and PFOS were generally narrowly distributed. The only exception was the PFOA clearance value from the Zhang et al. (2013b) study, which at 0.79 ml/kg-day was higher than the rest of the values, with the next highest value for PFOA clearance at 0.09 ml/kg-day. Considering it an outlier, the geometric mean of the remaining six studies for PFOA renal clearance was 0.059 ml/kg-day (using an average for two values in Harada et al. (2005a)). For PFOS, the geometric mean of six studies was 0.016 ml/kg-day.

Table 4.5.1. Human renal clearance (CL_R) studies for PFOA and PFOS

Reference	Chemical	Population	CL_R (ml/kg-day)[a]	C_{serum} (ng/ml)[a]
Fu et al. (2016)	PFOA	Occupational (N=302)	0.067 (9×10^{-5}-2.4)	1,052
	PFOS		0.01 (5×10^{-5}-0.54)	5,624
Gao et al. (2015b)	PFOA	Occupational (N=36)	0.09 (0.01-2.17)	2.66-10,515
	PFOS		0.01 (0.0002-0.07)	37.9-36,625
Zhang et al. (2015)	PFOA	General adult (N=54)	0.071 (adults)	2.47
	PFOS		0.026 (adults)	8.62
Fujii et al. (2015)	PFOA	General adult (N=10)	0.044	5.96
Zhou et al. (2014)	PFOA	Fishermen (N=16-39)	0.075 (0.02-0.263)	43.5 (34.7-52.4)
	n-PFOS		0.015 (0.001-0.092)	8,940 (7,280-10,600)
Zhang et al. (2013b)	n-PFOA	General adult (N=7-20)	0.79 (0.48-1.1)	3 (0.24-28)
	n-PFOS		0.031 (0.021-0.042)	15 (0.91-50)
Harada et al. (2005a)	PFOA	General adult (N=20)	0.033 (M) 0.027 (F)	7.9-12 (M) 7.6-14 (F)
	PFOS		0.012 (M) 0.019 (F)	12.6-26.3 (M) 11.2-23.5 (F)

[a] Range indicated in parenthesis when reported
C_{serum}, concentration in serum; n-PFOA, n-PFOS, linear isomers

Han et al. (2012) compared available PFOA renal clearances (CL_R) for different species and sexes to their corresponding glomerular filtration rates (GFR). The adapted table for this comparison is presented in Appendix 6 (Table A6.4), with added values for male and female rat, and an OEHHA-derived human clearance value. Assuming the unbound fraction in blood (f_u) as 0.02, comparison of the $GFR \times f_u$ (amount passively accessible to tubular secretion) and CL_R (amount eventually excreted) values provides insight into whether the compound is actively secreted or reabsorbed during the renal filtration process. This comparison demonstrated dramatic interspecies differences, with net renal tubular secretion in some systems (rabbit, female rat) to account for very high clearance values, and a wide range of reabsorption efficiencies with other species (Appendix 6,Table A6.4). According to this calculation, human

kidney reabsorbed 99.8% of PFOA. This mechanism would explain the exceptionally long human $T_{1/2}$ relative to animals.

Renal reabsorption was found to be hormone-dependent in rats (Ylinen et al., 1989; Vanden Heuvel et al., 1992; Kudo et al., 2002). Multiple receptors at the basolateral and apical membranes of tubular cells have been implicated in this process (Kato et al., 2002; Kudo et al., 2002; Katakura et al., 2007; Yang et al., 2009a; Weaver et al., 2010; Han et al., 2012). Renal reabsorption has been consistently incorporated into PBPK models of PFAS.

While more PFOA and PFOS would be expected to be eliminated in feces than in urine in humans, measurements in feces were below the detection limit in the three available studies (Beesoon et al., 2012; Genuis et al., 2013; Fujii et al., 2015). PFAS are more readily detected in the urine compared to feces due to the significantly lower detection limits. However, in one study both PFOA and PFOS could be detected in stool samples following treatment with cholestyramine in eight individuals with high PFAS body burdens (Genuis et al., 2013), providing evidence for the presence of enterohepatic circulation of these compounds. Cholestyramine is a resin used to immobilize certain lipophilic compounds in the gastrointestinal (GI) tract, preventing their reabsorption and therefore, interrupting their enterohepatic cycle.

Further evidence for enterohepatic circulation of PFOA and PFOS came from the measurements of their biliary clearance in humans. Harada et al. (2007b) measured PFOA and PFOS in the bile samples from four elderly patients, and estimated mean biliary clearance as 1.06 and 2.98 ml/kg-day, respectively. These values are dramatically higher than the urinary clearances, and would also greatly exceed the overall plasma clearances given the kinetic assumptions used in that study. Therefore, the authors concluded that a large fraction of PFOA and PFOS secreted with bile would be reabsorbed via an enterohepatic circulation mechanism, and estimated that the reabsorbed fractions were 0.89 and 0.97 for PFOA and PFOS, respectively. Using similar methodology, Fujii et al. (2015) reported biliary clearance for PFOA as 0.044±0.01 ml/kg-day (mean ± standard deviation, N=5). Both studies reported very close ratios of PFOA concentration in bile to its concentration in serum, 0.21 (Harada et al., 2007b) and 0.25 (Fujii et al., 2015). Harada et al. (2007) estimated the bile to serum ratio for PFOA was 0.60 (N=4).

Enterohepatic circulation for PFAS appears to be observed in other species. Vanden Heuvel et al. (1991) reported a fairly high level of excretion in bile for [^{14}C]PFOA in bile duct-cannulated rats exposed to a single i.v. dose (9.4 µmol/kg) and followed for 6 hours. There was no difference between male and female rats, and in male rats, bile excretion likely contributed to elimination through feces, which after 28 days added up to approximately 35% of the total dose. These results were consistent with another rat study (Kudo et al., 2001).

In mice treated with increasing doses of PFOA (12.5, 25, 50 µmol/kg-day) via oral gavage for 4 weeks, Minata et al. (2010) observed a high degree of PFOA concentration in bile. At the low dose, the concentration in bile was 2.8-fold higher than in blood, and at mid and high doses, 16.7-fold and 33.9-fold higher, respectively. Such high excretion rates in bile combined with the relatively long $T_{1/2}$ estimates in mouse (Table 4.1.1) would also imply a high level of re-absorption of PFOA although the report did not address this question.

Additional PFAS elimination routes in humans include pregnancy, lactation, birth, and menstrual loss of blood in females. Several studies modeling human biomonitoring data observed better concordance of predicted and observed serum concentrations for females when terms for loss

through menstrual blood and lactation were included in the overall elimination constant (Wong et al., 2014; Gomis et al., 2017). Table 4.5.2 lists individual terms of the elimination constant used in Gomis et al. (2017), calculated using published parameters, with the resulting values in the second column and overall percent contribution to total elimination in the third column. This study estimated lower $T_{1/2}$ values for both PFOA and PFOS compared to most other studies, which would result in higher plasma elimination.

Table 4.5.2. Elimination rate (Gomis et al., 2017) by term (year^{-1})[a]

$$k_{elim}(t_{age}) = \frac{B'_w(t_{age})}{B_w(t_{age})} + \frac{ln2}{T_{1/2}} + \frac{M_e}{V_d \times B_w} + \frac{BM_t \times I_m \times U}{V_d \times B_w}$$

Formula term	Corresponding elimination rate (y^{-1})		% of total
Growth dilution $\frac{B'_w(t_{age})}{B_w(t_{age})}$	0 in adults		
Plasma elimination $\frac{ln2}{T_{1/2}}$	PFOA	0.33	37.3
	PFOS	0.21	54.8
Loss to menstrual blood $\frac{M_e}{V_d \times B_w}$	PFOA	0.057	6.57
	PFOS	0.049	12.9
Loss to lactation $\frac{BM_t \times I_m \times U}{V_d \times B_w}$	PFOA	0.50	56.1
	PFOS	0.12	32.3

[a] for an average adult woman 30-40 years old**;** body weight (B_w) = 74.8 kg (US EPA, 2011a); half-life ($tT_{1/2}$) 2.1 years (PFOA, American women), 3.3 years (PFOS, American women); volume of distribution (V_d) 200 ml/kg (PFOA), 235 ml/kg (PFOS); menstrual blood loss (M_e) 868 ml/year; serum:milk transfer ratio (BM_t), 0.029 (PFOA), 0.0085 (PFOS); average volume of milk (I_m) 700 ml/day; unit correction factor (*U*) 365 days/year.

The overall comparison for different terms in the elimination rate for an adult woman indicates that contribution of elimination via menstrual blood may not be significant, while PFAS loss through lactation would be sizable. The model assumes only one pregnancy and birth per woman-lifetime, and a lactation period of 6 months, therefore the overall effect of lactation on the life-averaged elimination rate would be higher for a woman who has more than one child. One limitation of this approach was using fixed previously reported V_d estimates, which may not be optimal, as described in Section 4.8 of this document.

4.6. Physiologically-Based Pharmacokinetic Models

A large number of physiologically based pharmacokinetic (PBPK) models have been developed for PFOA and PFOS, and they are summarized in Table A6.5 in Appendix 6. The models were developed for different species, including humans and differ in complexity. The majority of models incorporate the renal reabsorption loop, which was first hypothesized as underlying the sex differences in PFOA kinetics between fast-eliminating female rats and slow eliminating male rats. It is assumed that renal transporters (Appendix 6, Table A6.3) mediate transfer of PFAS, including PFOA and PFOS, at renal interfaces, and that the net effect of secretion and reabsorption could explain the species differences in PFAS elimination (Appendix 6, Table A6.4). The majority of PFAS PBPK models describe renal reabsorption with Michaelis-Menten kinetics, with observed data-optimized K_m and V_{max} parameters.

Andersen et al. (2006) developed the first model with renal reabsorption, in the monkey. The model contains only three compartments: central compartment (~plasma), tissue compartment and filtrate compartment for renal elimination. The reabsorption loop was modeled as the back-flow from the filtrate to the central compartment. Six parameters in the model were optimized based on observed data, including reabsorption parameters, compartmental transfer constants, V_d and the proportion of free compound in blood. The resulting model described well the PFOA kinetics of a single dose (10 mg/kg), as well as kinetics of the approach to steady state during repeat dosing and following the discontinuation of daily treatment (20 mg/kg-day). A similar approach was used for PFOS, and while a single dose (2 mg/kg) demonstrated good convergence with the observed data, predictions for repeated treatment experiments (0.03, 0.15, 0.74 mg/kg-day) were much higher than the observed values in an independent validation study.

There were two important directions of PFAS PBPK modelling that started with the Andersen et al. (2006) model. In the first direction, Tan and coworkers and later, Loccisano and coworkers, continued to add compartments and expand to other species, successively developing a five-compartment model for rat and monkey (Tan et al., 2008), an eight-compartment model for rat (Loccisano et al., 2012a), a nine-compartment model for monkey and human (Loccisano et al., 2011), and culminating in the addition of gestational and lactational compartments for rat (Loccisano et al., 2012a) and human (Loccisano et al., 2013). The Loccisano et al. (2011) human model was further optimized at the organ level (Fabrega et al., 2014) using contemporaneously published human cadaver data. These sequentially developed models re-used certain parameters developed in previous iterations. One of the limitations of the rat models is the use of limited TK studies for optimization and validation and reliance on unpublished data. Another limitation of several models is using a time-dependent function for some parameters, such as V_d and free fraction in blood. While time-dependence was employed for a better fit of the observed kinetic data (particularly for longer treatments), the biological basis underlying such an adaptation of a physiological parameter is unclear and lacks experimental justification.

The second important derivation of the Andersen et al. (2006) model was the development of the complex probabilistic optimization of the same basic structure for two compounds (PFOA, PFOS) and three species (rat, mouse, monkey). This approach (Wambaugh et al., 2013) was used by US EPA in the risk assessment of PFOA and PFOS (US EPA, 2016b; US EPA, 2016d). Some of the optimized parameter values have very wide confidence intervals that span orders of magnitude, indicating a high level of uncertainty. The model optimization for rat and mouse relied on a limited number of kinetic studies, even though multiple studies are available in the literature. Certain parameter values, such as the filtrate compartment volume had biologically implausible values, highlighting the fact that the model was not physiologically based. It appears that, at least for some species, the model may have limited predictive power outside the range of concentrations used in the optimization process. For example, OEHHA ran the Wambaugh et al. (2013) model with kinetic data from a 28-day oral study in mice (Li et al., 2017b) that has doses below the range of doses used for optimization of the model and found that at the lowest PFOA dose (0.05 mg/kg-day), the model predicted approximately 10 times higher serum concentration than was reported (Appendix 6, Table A6.6). The code for the model was obtained from the authors and was adapted for Berkeley Madonna, a mathematical modeling software package.

Rodriguez et al. (2009) developed simplified 2- to 3-compartment models for PFOA in mice. The model for non-pregnant mice included a more complex renal recirculation circuit with an

additional compartment for renal plasma. The models that include gestation and lactation in mice did not include renal reabsorption and the dam was modeled as a single compartment. The model relied on data from only three published studies for optimization and validation. The model had a tendency to overestimate plasma levels. However, in OEHHA's comparison of mouse models (Appendix 6, Table A6.6), this model performed well at predicting plasma concentration at 0.05 mg/kg-day in the 28-day oral study by (Li et al., 2017b). The model code was adapted for Berkeley Madonna.

Unlike traditional PBPK methods that optimize a certain number of parameters for a better fit of observed data, Cheng and Ng (2017) developed a complex 19-compartment PFOA model for rat that utilized 72 independent parameters without fitting any parameters to data. Instead, parameter values and ranges were developed through expert knowledge and used for Monte-Carlo analysis of uncertainty ranges for predicted serum concentrations. The fit to the observed data was excellent and the uncertainty ranges were narrow. However, the study only modeled a single dose scenario and was validated with limited data. The model was re-iterative and not differential equation-based, which made it computationally challenging. While this appears to be the most biologically-informed approach of all published models to date, its utility for risk assessment is limited because it cannot handle repeated dose exposures.

Worley and Fisher (2015) developed a novel PFOA rat model with detailed mechanism of active transport in the renal reabsorption loop. This included active transport from filtrate to proximal tubule cells via apical transporters, active transport from kidney to proximal tubule cells via basolateral transporters and active flux from proximal tubule cells to blood. The transporter kinetics were described using Michaelis-Menten kinetics or first-order transfer, and in some cases was informed by in vitro parameters adjusted to the in vivo system (in vitro to in vivo extrapolation or IVIVE). The model was calibrated for single dose oral gavage and i.v. experiments and demonstrated good agreement with observed serum, liver and urine concentrations. However, the model relied on a limited number of studies for development and validation, including unpublished data. This model was further developed into a PFOA human model (Worley et al., 2017a) and a PFOS rat, mouse, monkey and human model (Chou and Lin, 2019). The human PFOA model only considered human serum data from a single study for optimization and a single study for validation, despite including many non-serum compartments (Worley et al., 2017a). The model has not been validated with additional human kinetic data that are available.

The multi-species PFOS model of Chou and Lin (2019) and the related read-across report (Chou and Lin, 2020) is an attempt to develop a comprehensive PBPK model framework for use in risk assessment. The main contribution of this research was to develop uncertainty distributions for different parameters and species, which were used in a somewhat traditionally defined PFOS model with more detailed GI and kidney compartments and simplified compartment for the rest of the body. Many starting parameters in the model were values optimized to data in the related Loccisano models, and since Bayesian-Markov chain Monte Carlo (MCMC) optimization did not appear to change these original values much (within 20% of the prior values), the overall parameter space was not very different compared to the Loccisano models. However, unlike the Loccisano suite of models for PFOS, free fraction in blood was modeled as a constant. The models demonstrated generally good agreement of predicted and observed values for the rat, mouse and monkey. For humans, the model failed to accurately predict organ levels (liver, kidney). The organ predictions for rats were generally accurate for one study used for validation. Similar to other PFAS PBPK models, this model relied on the same handful of animal studies and did not evaluate the vast majority of published data.

Although this model was further used for human risk assessment (Chou and Lin, 2020), its utility would be limited given poor predictive power at the organ level.

Two simplified human models have been reported for predictions of gestational and lactational transfer (Verner et al., 2016; Goeden et al., 2019). Verner et al. (2016) modeled mother and child as two compartments, with placental transfer (gestational) and lactational transfer (post-birth) parameters, and values for V_d and $T_{1/2}$, lactation, and growth characteristics summarized from available literature. Evaluating the Verner model with available plasma data in infants and children (Fromme et al., 2010; Granum et al., 2013; Mogensen et al., 2015a) provided acceptable fit (R^2 = 0.5-0.6 for regression of individual predictions). The model predicted a peak in infant plasma levels at cessation of breastfeeding, e.g., at 6 months for the Fromme et al. (2010) study. For PFOA, the child/mother plasma ratio reached 4.5 at the 50th percentile, 7.8 at the 95th percentile and a maximum 15.3. For PFOS these values were <1, 3 and 7, respectively. Much lower ratios for PFOS were attributed by the authors to less efficient transfer to milk.

The Goeden et al. (2019) model for PFOA was similar the the Verner model overall. Apart from slightly different parameter values, the main differences were: consideration for bottle-fed infants, adjusting V_d for age, and a detailed break-down of age-dependent water consumption rates. The model also considered central tendency as well as upper percentiles for its parameter values. This model demonstrated good fit of predicted to observed plasma data (e.g., R^2 = 0.7 for PFOA based on Fromme et al. (2010) data). The model also predicted a peak in plasma concentration in breast-fed infants at the age of termination of breast-feeding. At their peak, PFOA concentrations in infants were 6-fold higher than maternal serum at delivery.

Both human lactational models predicted an increase in plasma concentration, particularly for PFOA during the first year of life attributed to breast-feeding. This could be important for relevant developmental endpoints. However, it is important to emphasize that the exact level in infants depends on the mother's exposure and the context of exposure.

In addition to more complex models, multiple PFOA and PFOS exposure studies utilized a one-compartment model, typically to back-calculate exposure from serum concentrations (Washburn et al., 2005; Fromme et al., 2007; Trudel et al., 2008; Karrman et al., 2009; Vestergren and Cousins, 2009; Haug et al., 2010; Niisoe et al., 2010; Thompson et al., 2010; Egeghy and Lorber, 2011; Lorber and Egeghy, 2011; Shin et al., 2011; Lorber et al., 2015; Zhang et al., 2015; Gomis et al., 2017; Balk et al., 2019; Zhang et al., 2019b). These models had different assumptions for absorption efficiency, V_d and $T_{1/2}$ values; some of these issues are discussed later in this document.

There are several common conclusions regarding PFOA and PFOS PBPK models. Most models have been developed in series, with the rat PFOA model as the first. The rat PFOA models relied on the same limited number of studies, some unpublished, for optimization and validation, and at the expense of multiple other published kinetic studies. None of the published animal models have been extensively tested against published studies with kinetic data to determine overall prediction efficiency and dose range applicability, i.e., whether the models can predict serum concentrations outside the range used for their optimization. US EPA used the Wambaugh et al. (2013) model in their PFOA and PFOS risk assessment, but as it happens the studies used for the optimization of the model were also the critical studies chosen for dose-response in the toxicity assessment (US EPA, 2016b; US EPA, 2016d). It remains unclear, whether this or another model can be effectively applied to a study not used in the model

development, especially if the predicted doses or plasma concentrations are outside the range used for model optimization.

Despite well-established species differences in PFOA and PFOS toxicokinetics, physiological parameters optimized in a PBPK model or measured in vitro for one species are often used for a PBPK model in another species without adjustment. This is usually motivated by the lack of data in the species of interest but can also mask possible interspecies differences in physiological parameters. In order to better fit observed data, some models incorporated biologically implausible mechanisms, such as making certain physiological parameters time-dependent. Finally, although several human PBPK models were developed, only one was optimized at the organ level, with limited success (Fabrega et al., 2014), underscoring the need for more detailed human data.

Use of PFOA and PFOS PBPK models in risk assessment

The fact that there are dramatic differences in PFOA/PFOS half-lives between species necessitates incorporation of kinetic considerations in extrapolating dose from animal studies to humans. Even when evaluating human studies, which are often based on serum concentrations, kinetic considerations are important in conversion of the point of departure to an exposure concentration. Due to well understood challenges in this area, most animal and human PFOA and PFOS toxicity studies include plasma or serum level measurements in the reports, as detailed in Chapters 5 and 6 of this document.

The available PFOA and PFOS animal PBPK models have not been validated with the majority of published kinetic data and their use for the purposes of risk assessment would require an extensive review. OEHHA's work with two mouse models (Appendix 6, Table A6.6) suggested that models can err dramatically outside their optimized range and specifically at lower doses, which are of particular interest for POD determinations. However, since most animal studies of interest have reported serum concentrations, use of this metric appears to be least uncertain for the PFOA/PFOS assessment, and still fairly precise compared to modeled average concentrations when half-lives are much longer than the treatment interval.

In most animal species with half-lives at approximately 1 month (Table 4.1.1), day-to-day fluctuations in serum concentrations would be small compared to the steady state concentration, and using reported serum concentrations would add the least uncertainty to the dose-response analysis. Female and male mice at lower PFOA dose (1 mg/kg) demonstrate fairly long half-lives of 15-20 days (Table 4.1.1), and a similar argument applies, i.e., using reported serum concentrations would add the least uncertainty. While male rats demonstrate shorter PFOA $T_{1/2}$ estimates of 1.5-15 days (Table 4.1.1), fluctuations in serum concentrations and the resulting difference of the modeled average and reported serum concentration are expected to be small relative to other uncertainties in this risk assessment. In this case, using the reported serum concentration would provide a slightly more conservative dose metric of adverse effect, assuming levels were measured prior to daily dosing in a repeated dose experiment.

Due to the long half-lives of PFOA and PFOS in humans, a simple one-compartment model with first order elimination appears to be most effective in predicting serum concentration in humans (US EPA, 2016b; US EPA, 2016d). The use of this model ultimately requires determination of the clearance rate (CL), and three methods for CL calculation are considered in the following sections of this document. The first method for CL determination, previously employed by US

EPA for PFOS (US EPA, 2016d), involves independent estimates for $T_{1/2}$ and V_d, which are then used to calculate CL. The second method analyzes epidemiologic data in specific exposure situations, which allows direct CL calculation. This method was effectively used by US EPA for PFOA CL determination (US EPA, 2016b). The third method considers enterohepatic circulation of PFOA and PFOS as the limiting CL factor, and provides upper-bound CL estimates based on limited experimental data. The following sections of this document provide detailed review of published parameter estimates ($T_{1/2}$ and V_d) and application of these three methods to CL calculation.

4.7. Half-life ($T_{1/2}$) Estimate for PFOA and PFOS in Humans

Humans demonstrated longer half-lives than animals, on the order of years versus days or weeks. The available data are summarized in Table 4.7.1 for PFOA and in Table 4.7.2 for PFOS. For the calculation of the CLs, US EPA used $T_{1/2}$ estimates of 2.3 years for PFOA (Bartell et al., 2010) and 5.4 years for PFOS (Olsen et al., 2007). The Bartell et al. (2010) PFOA study was based on tracing the drop in serum concentrations in a subset of the C8 Panel following discontinued exposure from drinking water. Importantly, it was established that C8 Panel exposures were likely long enough to reach steady-state levels in exposed subjects (an important assumption in TK modeling). In this exposure scenario, very high initial exposures resulted in high initial serum concentrations and the ongoing exposures from other sources, such as diet, would have little effect on the time-dependent decrease of plasma concentrations. In a reanalysis of the C8 Panel data, with more subjects and longer follow-up time, the Li et al. (2017e) study produced a slightly higher $T_{1/2}$ value of 2.7 years, which appears to be the best available estimate for PFOA $T_{1/2}$ to date. The same robust analytical method applied to PFOA TK data from Ronneby in Sweden, also produced a PFOA $T_{1/2}$ estimate of 2.7 years (Li et al., 2017e; Li et al., 2018c). Thus, the estimated PFOA $T_{1/2}$ of **2.7 years** reported by Li et al. (2017e) and Li et al. (2018c) is deemed the most appropriate value for use by OEHHA.

Other comparable studies of drinking water exposure to PFOA, such as for the Arnsberg and Decatur sites (Brede et al., 2010; Worley et al., 2017b), produced PFOA $T_{1/2}$ estimates of 2.3-3.9 years, which are close to 2.7 years. Brede et al. (2010) investigated paired plasma concentrations following detection of elevated PFOA levels in drinking water in Ansberg, Germany. This study had shorter follow-up (2 years), mixed composition of subjects, including childen (who demonstrated TK properties distinct from adult participants), unclear exposure history and importantly, significantly lower water consumption in exposed individuals compared to controls. These factors would increase the uncertainty of the final estimated $T_{1/2}$ compared to those derived from the Li et al. (2017e, 2018c) studies.

Worley et al. (2017b) analyzed decreased PFOA levels in environmentally exposed subjects in Decatur, Alabama. While the follow-up was 6 years, the study involved relatively few participants (45, vs. 455 in the reanalysis of C8 Panel data by Li et al. (2017e)), exposure history was not clear, and most importantly, PFOA $T_{1/2}$ was estimated using a one-comaprtment TK model that required an assumption of a specific PFOA V_d value. As described in the following section, identifying a correct PFOA V_d value may pose its own problems, and therefore, this indirect $T_{1/2}$ estimation method would introduce additional levels of uncertainty.

While most studies estimate PFOA $T_{1/2}$ within 2.4-4.8 years, the encompassed values are mostly derived in situations of relatively high PFOA exposure, such as those occurring in Little Hocking, Ohio, in the C8 Panel studies. However, high starting PFOA exposures may not correctly predict kinetic behavior at lower environmental concentrations, and specifically with the

overall approximation of a first-order elimination model, high and low exposure scenarios may provide different $T_{1/2}$ estimates. Consistent with this hypothesis, Seals et al. (2011) found a longer $T_{1/2}$ value (8.5-10.1 years) in the C8 Panel participants with lower PFOA exposure.

Other types of PFOA $T_{1/2}$ studies, such as occupational studies or analysis of disappearance trend in the general population (Table 4.7.1) produce somewhat higher estimates, likely due to underestimating or ignoring ongoing exposures. Studies that include considerations of urinary clearance (Zhang et al., 2013b; Fu et al., 2016) are based on the premise of the predominance of renal elimination, and moreover, depend on a chosen V_d value. Similarly, the $T_{1/2}$ determination based on dynamic population modeling by Gomis et al. (2017) relied on an a priori chosen V_d value. As discussed in Section 4.8, estimation and subsequent use of a V_d value could bring an additional level of uncertainty to toxicokinetic analysis. Finally, Dourson et al. (2019), based on previously available kinetic data from controlled dosing of human cancer patients at relatively high levels, assumed that the steady state for PFOA exposure would be achieved by approximately 36 weeks and estimated plasma concentration at that point. Then, using the steady state assumptions, they determined the resulting $T_{1/2}$. In addition to a number of other issues, this evaluation assumed and did not experimentally determine the time to steady state. While the underlying kinetic data could be useful for further analysis, OEHHA does not consider the PFOA $T_{1/2}$ reported in the Dourson et al. (2019) study to be of sufficient quality or representative of environmental exposure.

Table 4.7.1. Human half-life estimates for PFOA

Reference	Population	N	$T_{1/2}$ (years)	Method
Burris (2002) as cited in Harada et al. (2005a)	Occupational	9	4.4	3M study, unpublished
Olsen et al. (2007)	Occupational	26	3.5^{GM}	Retired workers followed for 5 years
Spliethoff et al. (2008)	General (infants)	2,640	4.1	Disappearance $T_{1/2}$ due to declining levels
Costa et al. (2009)	Occupational	16	4.8	Former workers followed for 2-29 years
Brede et al. (2010)	Arnsberg, 2y	138	3.3	Drinking water pollution, decline
Bartell et al. (2010)	C8 Panel, 1y	200	2.3	Drinking water pollution, decline
Seals et al. (2011)	C8 Panel	1,573	2.9	Cross-sectional study, higher exposure
			8.5-10.1	Cross-sectional study, lower exposure
Zhang et al. (2013b)	General	66	2.8	Calculated from urinary clearance
Gomis et al. (2016)	Occupational	4	2.4	Accounted for ongoing exposure
Fu et al. (2016)	Occupational	207	11.7^{GM}	Calculated from urinary clearance
Gomis et al. (2017)	General	120	1.8-2.4	Population-based cross-sectional model
Li et al. (2017e)	Ronneby; C8 Panel, 4y	455	2.7	Drinking water pollution, decline
Worley et al. (2017b)	Decatur	45	3.9	Drinking water decline, one-compartment model with V_d assumption
Li et al. (2018c)	Ronneby	106	2.7	Drinking water pollution, decline
Dourson et al. (2019)	Clinical trial	NA	<0.7	Assumed steady state at given dose
Xu et al. (2020a)	Occupational	17	1.5-1.8	Airport workers followed for 5 months

^{GM} geometric mean; NA, not applicable.

The PFOS $T_{1/2}$ of 5.4 years used by US EPA was determined based on elimination kinetics in retired workers (Olsen et al., 2007). Several disappearance studies in the general population, which analyzed declining levels of PFOS in industrialized countries, produced lower values of 3.3-4.8 years (Yeung et al., 2013b; Gomis et al., 2017). Using rigorous analysis and high and discontinued environmental exposure data, Li et al. (2018c) estimated PFOS $T_{1/2}$ at **3.4 years**. This value is consistent with most recent reports (Table 4.7.2), and is chosen by OEHHA as the best estimate based on available evidence.

Table 4.7.2. Human half-life estimates for PFOS

Reference	Population	N	$T_{1/2}$ (years)	Method
Burris (2002) as cited in Harada et al. (2005a)	Occupational	9	8.7	3M study, unpublished
Olsen et al. (2007)	Occupational	26	5.4	Retired workers followed up for 5 years
Spliethoff et al. (2008)	General (infants)	2,640	4.4	Disappearance $T_{1/2}$ due to declining levels
D'Eon and Mabury (2011b)	Review of studies	NA	5.4	Disappearance $T_{1/2}$ due to declining levels
Glynn et al. (2012)	General	413	8.1	Disappearance $T_{1/2}$ due to declining levels
Olsen et al. (2012)	General	600	4.3	Cross-sectional, population based
Yeung et al. (2013b)	General	420	4.3-4.8	Disappearance $T_{1/2}$ due to declining levels
Zhang et al. (2013b)	General	66	22^{GM}	Calculated from urinary clearance
Fu et al. (2016)	Occupational	207	4.1^{GM}	Calculated from urinary clearance
Gomis et al. (2017)	General	120	3.3-5	Population-based cross-sectional model
Li et al. (2017e)	C8 Panel, 4y	455	3.7	Drinking water pollution, decline
Worley et al. (2017b)	Decatur	45	3.3	Probabilistic model of C8 Panel data
Li et al. (2018c)	**Ronneby**	**106**	**3.4**	**Drinking water pollution, decline**
Xu et al. (2020a)	Occupational	17	1.7-2.9	Airport workers followed for 5 months

^{GM} geometric mean; NA, not applicable.

Data are available on PFOA and PFOS plasma levels in California residents in several Biomonitoring California studies, with samples collected as early as 2011-2012 and as recent as 2018 (https://biomonitoring.ca.gov; these data have been reported in several publications (Hurley et al., 2016; Hurley et al., 2018; Kim et al., 2020). These studies demonstrated decreases in serum PFOA and PFOS levels in California residents over the 2009-2016 period. Kim et al. (2020) reported that in California mothers of young children (N=450), average PFOA serum concentration decreased from 1.65 ng/ml in 2009 to 0.7 ng/ml in 2016 and for PFOS, from 4.8 ng/ml in 2009 to 2 ng/ml in 2016. Assuming no additional input (disappearance method), these rates of decrease would correspond to $T_{1/2}$ estimates of about 6 years for either compound, higher than the chosen $T_{1/2}$ values derived by Li et al. (2017e) and Li et al. (2018c). Similar to the conclusion of the Seals et al. (2011) study, higher values could be due to lower exposure levels and/or continuous background exposures.

4.8. PFOA and PFOS Volume of Distribution (V_d) and Clearance Rate (CL)

Published estimates for PFOA and PFOS V_d values are presented in Table 4.8.1. These mainly comprise two types of studies, the first being studies with original TK experimental data and assessments, and the second being applied studies, such as those modeling human exposure, which derived novel V_d values based on previously published data and suited to the specific assumptions of the study.

Table 4.8.1. Estimates of V_d of PFOA and PFOS

Reference	Data Source	PFAS	Species	V_d (ml/kg)	Method
Harada et al. (2003)	Seacat et al. (2002)	PFOS	monkey (cynomolgus) 26 weeks	300 (corrected to 541 by OEHHA)[a]	Subchronic oral study, 1-compartment, ss assumption, although calculation appears to be incorrect[a]
Kemper et al. (2003) unpublished	same study	PFOA	rat	211 - 264	Single i.v. or oral dose, non-compartmental; reported in Vestergren and Cousins (2009)
Ohmori et al. (2003)	same study	PFOA	rat (m)	196	Single i.v. dose, serum concentrations fitted to 2-compartment model
			rat (f)	201	
Butenhoff et al. (2004a)	same study	PFOA	monkey (m)	181	Single i.v. dose, non-compartmental analysis; $V_d = \frac{Dose \times AUMC}{AUC^2}$
			monkey (f)	198	
Washburn et al. (2005)	Noker (unpublished)	PFOA	monkey (m)	1,810-5,210	Subchronic oral study, 1-compartment, steady state[b]; $V_d = \frac{Dose}{k_e \times C_{ss}}$
			monkey (f)	2,460-6,340	
	Butenhoff et al. (2002)		monkey (m)	1,260-3,730	
	Butenhoff et al. (2004b)		rat (m)	270	
			rat (f)	430	
	Palazzolo (1993), unpublished		rat (m)	100-550	
Vestergren and Cousins (2009)	Griffith and Long (1980)	PFOA	monkey (rhesus) 90 days	1,480-4,470	Subchronic oral studies; steady state with 1-compartment elimination ($T_{1/2}$=25 days); $V_d = \frac{Dose}{k_e \times C_{ss}}$
	Butenhoff et al. (2004a)	PFOA	monkey (cynomolgus) 6 months	1,300-4,470	
Niisoe et al. (2010)	Harada et al. (2007b)	PFOA	human	300	Mass balance for excretion through bile, assuming 0.88 efficiency for reabsorption and 3.5-year $T_{1/2}$
	Vestergren and Cousins (2009)	PFOA	human	464-780	95% confidence of the regression line for human intake-serum concentration; steady state assumption
Thompson et al. (2010)	Emmett et al. (2006), Dupont	PFOA	human	170	Steady state with 1-compartment elimination ($T_{1/2}$=2.3 years); $V_d = \frac{Dose}{k_e \times C_{ss}}$
Chang et al. (2012)	same study	PFOS	rat (m)	649-765	Oral or i.v. single dose in jugular-cannulated rat
			rat (f)	521-586	
			rat (m)	666-1,228	Single oral dose with long follow-up; data fit to non-compartmental model;
			rat (f)	468-484	
			mouse (m)	263-290	
			mouse (f)	258-261	

Reference	Data Source	PFAS	Species	V_d (ml/kg)	Method
			monkey (m) (cynomologus)	202	$V_d = \frac{Dose \times AUMC}{AUC^2}$
			monkey (f) (cynomologus)	274	
Fujii et al. (2015)	same study	PFOA	mouse (m)	180	Single i.v. dose, fitted to 2-compartment model; $V_d = Dose/C(0)$
			mouse (f)	150	
Kim et al. (2016b)	same study	PFOA	rat (m)	112	Single i.v. dose; $V_d = \frac{Dose \times AUMC}{AUC^2}$
			rat (f)	171	
		PFOS	rat (m)	383	
			rat (f)	351	
		PFOA	rat (m)	106	Single oral dose; $V_d = \frac{Dose \times AUMC}{AUC^2}$
			rat (f)	154	
		PFOS	rat (m)	280	
			rat (f)	289	
Iwabuchi et al. (2017)	same study	PFOA	rat (m)	150	Single oral dose, V_d calculated as ratio of dose to AUC and k_e; k_e from first order elimination model
		PFOS	rat (m)	960	
Huang et al. (2019a)	same study	PFOS	rat (m)	681[c]	Single i.v. dose, serum concentrations fitted to 2-compartment model
			rat (f)	421[c]	
			rat (m)	78.5-524[c]	Single oral dose, serum concentrations fit to 2-compartment model
			rat (f)	55.4-315[c]	
			rat (m)	299[c]	5-day gavage study, serum concentrations fit to 2-compartment model
			rat (f)	222[c]	
Dzierlenga et al. (2020)	same study	PFOA	rat(m)	153[c]	Single i.v. dose, serum concentrations fit to 2-compartment model
			rat(f)	207[c]	
			rat(m)	154-202	Single oral dose, serum concentrations fit to 1-compartment model
			rat(f)	79.2-342[c]	Single oral dose, serum concentrations fit to 2-compartment model

AUC, area under the curve; AUMC, area under the first moment curve; k_e, elimination constant; ss, steady state.

[a] The calculation is based on two C_{ss} points (Seacat et al., 2002): at 0.03 mg/kg-day, C_{ss}=16 mg/L (ppm) and at 0.15 mg/kg-day, C_{ss} = 80 mg/L (ppm). At steady state, intake = elimination, i.e., $Dose = V_d \times C_{ss} \times k_e$. Assuming that $k_e = ln2/T_{1/2}$, the equation can be rewritten as $V_d = Dose \times T_{1/2}/(C_{ss} \times ln2)$. With $T_{1/2}$ of 200 days, at 0.03 mg/kg-day: $V_d = 0.03 \times \frac{200}{16 \times 0.693} = 541\ ml/kg$, and at 0.15 mg/kg-day: $V_d = 0.15 \times \frac{200}{80 \times 0.693} = 541\ ml/kg$ as well, different from the reported 300 ml/kg. Furthermore, the assumption of steady state may not be quite correct in this case, since $T_{1/2}$ is estimated as 200 days, about equal to the duration of the study. In a 1-compartment model described as $C_{ss} = \alpha \times (1 - e^{-k_e \times t})$, serum concentration would equal ½ of the C_{ss} at $t = T_{1/2}$. Therefore, V_d could be further increased two-fold.

[b] For use in the human 1-compartment model (Washburn et al., 2005), V_d values derived from subchronic studies were much higher than 0.2 L/kg, based on a single dose rat study; however, "Discussion with Dr. Joseph Rodricks and Dr. John Butenhoff, the corresponding author of the cynomolgus monkey

toxicokinetic paper (Butenhoff et al., 2004a), confirmed that the fractional volumes of distribution based on the subchronic monkey study may be preferable."
[c] Sum of the central and peripheral compounds in a 2-compartment model.

A third type of study analyzed the fit of kinetic models to animal data and identified V_d or a comparable property through an optimization algorithm (Andersen et al., 2006; Wambaugh et al., 2013). These computational models describe the body as a single compartment or a series of compartments, and optimize the model parameters, which describe PFAS distribution and excretion, to fit TK datasets, such as plasma concentration profiles. Andersen et al. (2006) developed a simplified model for PFOA and PFOS in the monkey using kinetic data from a single i.v. dose study and from 6-month oral studies (Butenhoff et al., 2002; Seacat et al., 2002; Butenhoff et al., 2004a). In this model, PFAS was distributed between the central and peripheral compartments, and was excreted through a separate renal compartment with a renal reabsorption loop. The central compartment was described by its volume of distribution (V_{dc}). The peripheral (tissue) compartment was described in terms of compartmental transfer rate from central to tissues (k_{12}) and compartmental transfer rate from tissue back to central (k_{21}). Although the V_d for the tissue compartment was not formally defined, it can be calculated using the following formula (Wambaugh et al., 2013):

$$V_{dt} = \frac{k_{12} \times V_{dc}}{k_{21}}$$

The overall V_d can then be estimated as the sum of V_{dc} and V_{dt}. Table 4.8.2 presents parameter values estimated for chronic oral exposure in monkeys, and the resulting V_{dt} and V_d values.

Table 4.8.2. OEHHA-derived estimates of V_d from the computational model of Andersen et al. (2006)

PFAS	V_{dc} (ml/kg)*	k_{12} (1/h)*	k_{21} (1/h)*	V_{dt} (ml/kg), calculated	V_d (ml/kg), calculated
PFOA	140	3.3	0.1	4,620	4,800
PFOS	220	3.3	0.1	7,260	7,500

*Values reported in Andersen et al. (2006)

The resulting V_d values are much higher than the estimates for the central compartment due to the high predicted distribution of PFOA and PFOS to tissues. Similar to other V_d estimates obtained as optimized parameters in kinetic models of PFAS, these V_d values are subject to many uncertainties, and are less reliable than V_d values directly estimated from experimental data.

Previously used PFOA and PFOS CL and V_d estimates

To calculate PFOA and PFOS clearance rates (CL), US EPA utilized a two-step method:

1) identify V_d and $T_{1/2}$ from animal or human studies;
2) calculate CL using the formula $CL = V_d \times (\frac{ln2}{T_{1/2}})$, based on steady state assumption.

The calculated values for PFOA and PFOS CLs were 1.4×10^{-4} L/kg-day and 8.1×10^{-5} L/kg-day, respectively (US EPA, 2016b; US EPA, 2016d). Half-lives of 2.3 and 5.4 years were used, respectively, as described above. However, selection of V_d values poses new questions in light of the recently available data.

US EPA estimated a human PFOA V_d value of 170 ml/kg. It was derived from an epidemiologic study (C8 cohort) assuming steady state and $T_{1/2}$ of 2.3 years (Thompson et al., 2010). US EPA noted that this value is similar to estimates of V_d in monkey, obtained from a single dose experiment (Butenhoff et al., 2004a). A V_d of this magnitude would indicate primarily extracellular distribution of PFOA, and it has been criticized as likely too low when compared to higher V_d values obtained from subchronic monkey experiments with repeated dosing (Washburn et al., 2005; Vestergren and Cousins, 2009). It has been suggested that V_d estimates based on subchronic monkey studies would be preferable for chronic exposures in humans (Washburn et al., 2005).

Citing the lack of credible studies for a PFOS V_d, US EPA adopted the following strategy. Starting with the PFOA V_d of 170 ml/kg, a factor of 1.35 was applied based on the observation in the modeling paper of Andersen et al. (2006) that the optimized V_{dc} value for PFOS was 20-50% higher than the PFOA value. Thus, US EPA estimated a human PFOS V_d value of 230 ml/kg. Relying on a modeling study would not be optimal since compartment volumes are only some of the optimized parameters and the accuracy of their values can be compromised in order to obtain the best data fit. Moreover, as explained in the previous section, V_{dc} values determined in this study were not representative of the V_d, but rather of volume of the central compartment, which would have a much smaller contribution to the overall V_d in this model Finally, multiple published PFOS V_d studies are now available, obviating the need for indirect considerations.

Consideration of subchronic monkey studies for V_d estimates

Several subchronic monkey studies reported measurements of PFOA and PFOS steady state levels (Butenhoff et al., 2002; Seacat et al., 2002; Butenhoff et al., 2004a) that may be more appropriate for estimating V_d values than those obtained from single dose animal experiments (Table 4.8.1).

For example, in the Butenhoff et al. (2004a) study, daily oral doses of 3, 10 and 20 mg/kg administered to 4-6 monkeys/group over 6 months resulted in steady state serum concentrations of 81±40, 99±50 and 156±103 µg/ml, respectively. Based on these values and the steady state assumption, Vestergren and Cousins (2009) estimated V_d as 1,300-4,470 ml/kg, which is substantially higher than 181-198 ml/kg reported in the original study derived from a single-dose experiment (10 mg/kg) in male and female monkeys (Butenhoff et al., 2004a). Although serum concentration results for subchronic experiments were reported, the authors did not calculate the corresponding steady state V_d. However, they noted that the plasma levels at steady state were lower than expected, which would drive the corresponding V_d values higher. The authors suggested that this could be due to incomplete absorption (due to the fact that fecal PFOA dropped dramatically when the dietary exposure stopped), and secondly, due to the possibility that some PFOA retained in the body could be trapped in the enterohepatic loop and therefore be absent from the plasma pool. Both of these reasons appear plausible and none of the available subchronic monkey studies included controls to account for less than complete absorption and enterohepatic circulation. The uncertainty in the outcome of this approach also sheds light on the limitations of the steady state assumption. Similar to the monkey, enterohepatic circulation plays an important role in PFOA and PFOS PK in humans, necessitating an alternative enterohepatic circulation-based approach to estimating V_d and clearance rate.

Updated PFOA V_d estimate

Exposure to PFOA from the PFAS manufacturing plant at the border of Ohio and West Virginia (C8 Panel site) appears to have occurred at a constant level during 1985-2005 (Shin et al., 2011), after which the main source of exposure ceased and plasma levels started declining. Assuming PFOA $T_{1/2}$ at 2.3-2.7 years, the exposure duration was sufficient to reach steady state (about eight half-lives). Therefore, at the end of this period, V_d can be calculated using the following formula, where C_{ss} is plasma concentration at steady state:

$$V_d = Dose \times T_{1/2} \div (C_{ss} \times ln2).$$

Thompson et al. (2010) used this approach and calculated the applied dose, as daily amount of PFOA in consumed drinking water, averaged at two locations in the C8 region: in Lubeck, where PFOA in drinking water was 500 ng/L (500 ppt), and in Little Hocking, where PFOA in drinking water was 3,550 ng/L (3,500 ppt). These values are consistent with an independent report from the C8 Panel studies (Shin et al., 2011). However, predating the majority of C8 Panel studies, Emmett et al. (2006) was used as the source of serum concentration value for Little Hocking: specifically, 448 ng/ml in subjects using 'Little Hocking system water only' as their drinking water source (N=291). Besides this specified category, Emmett et al. (2006) reported mean serum concentrations for Little Hocking in general (N=478) and Belpre (N=14), another nearby location, as 478 and 321 ng/ml, respectively. For Lubeck, the average plasma concentration for non-occupationally exposed individuals (N=12) was 68 ng/ml (Emmett et al., 2006).

Reported serum values from the C8 Science Panel studies, which were exposure and health studies conducted in the same area one year later and on a large scale, are different from those reported by Emmett et al. (2006). Frisbee et al. (2009) reported 227.58, 42.96 and 92.36 ng/ml for Little Hocking, Belpre and Lubeck, respectively, with 82-87% population coverage (percent that participated in the study). While the Frisbee et al. (2009) C8 Panel samples were taken a year later than Emmett et al. (2006), such a large difference in values is unexpected given the long $T_{1/2}$ of PFOA. The more likely cause is the difference in sample selection, which in the Emmett et al. (2006) study included preliminary selection of households based on a certain expected level of air and water exposure supplemented with some number of volunteered samples. This selection may have introduced unaccounted for bias toward higher than average levels in plasma. In contrast, the C8 Panel studies, including Frisbee et al. (2009) aimed for full population coverage and randomization, and as such, have reported better average values for the population.

Applying the Thompson et al. (2010) approach to the calculation of V_d, using serum concentrations reported in Frisbee et al. (2009), and assuming a $T_{1/2}$ of 2.3 years, a reported average water consumption 1.4 L, absorption efficiency (f_a) 0.91 and a reported average body weight (BW) 71.8 kg, the resulting V_d, averaged between Little Hocking and Lubeck, equals **225 ml/kg**, as follows:

$$V_d = \frac{C_w \times 1.4\ L/d \times f_a \times T_{1/2}}{C_{serum} \times ln2 \times BW}$$

Little Hocking:

$$V_d = \frac{3{,}550 \times 1.4 \times 0.91 \times 839.5}{227.58 \times ln2 \times 71.8} = 335\ ml/kg$$

Lubeck:

$$V_d = \frac{500 \times 1.4 \times 0.91 \times 839.5}{92.36 \times ln2 \times 71.8} = 116\ ml/kg$$

Average: 225 ml/kg or 0.225 L/kg.

Similar to the original analysis, the calculation assumes 0.91 absorption efficiency. The resulting value for V_d is not much higher than the previously used 170 ml/kg (US EPA, 2016b) and appears much lower than the values derived from the subchronic monkey studies. The reasons for this discrepancy remain unclear, although one possible reason could be the choice of the PFOA $T_{1/2}$ value.

Updated PFOS V_d and CL estimates

Most published animal studies (Table 4.8.1) produced higher directly measured V_d estimates for PFOS than the value of 220 ml/kg derived by EPA using a kinetic model-based approximation. The only analysis relying on subchronic data (Harada et al., 2003) produced a V_d of 541 ml/kg when the calculation was corrected, as described in the footnote in Table 4.8.1. This calculation was performed with the assumption of steady state, even though the duration of exposure was only about one $T_{1/2}$. In a one-compartment model, plasma concentration at one $T_{1/2}$ would only reach 50% of the steady state level. Applying this adjustment results in a V_d estimate of 1,080 ml/kg. Substituting this V_d value and the PFOS $T_{1/2}$ value of 3.4 years into the clearance formula, the updated CL estimate is obtained:

$$CL = V_d \times (\frac{ln2}{T_{1/2}}) = 6.0\times10^{-4}\ \text{L/kg/day}.$$

Li et al. (2018c) described a PFOS exposure case from drinking water that was somewhat similar to the PFOA C8 Panel exposure scenario (Little Hocking and Lubeck). In 2013, the PFOS concentration in outgoing water from the two water works in Ronneby, Sweden was 8,000 ng/L, while median serum concentration in Ronneby residents exposed for at least 10 years was 372 ng/ml (Li et al., 2018c; Silva et al., 2020). While exposure was occurring from the mid-1980s, exact time-dependent levels are unknown. However, assuming that steady state was reached in 2013, V_d can be calculated as follows:

$$V_d = \frac{C_w \times 1.4\ L/d \times f_a \times T_{1/2}}{C_{serum} \times ln2 \times BW} = \frac{8{,}000 \times 1.4 \times 0.9 \times 1{,}241}{372 \times ln2 \times 70} = 693\ \text{ml/kg}.$$

In this calculation, daily water consumption was 1.4 L/day, absorption efficiency 0.9, $T_{1/2}$ was 3.4 years, and the default average body weight of 70 kg was applied.

The PFOS V_d estimate derived from human epidemiologic data produced a higher value (693 ml/kg) than that of PFOA (225 ml/kg). This can be due to experimental uncertainty or true chemical differences.

4.9. Alternative Approaches and Calculation of Clearance (CL)

Given the wide range for PFOA V_d estimates, additional approaches to data analysis should be considered. Moreover, direct determination of clearance may help to decrease uncertainty due to separate determinations of $T_{1/2}$ and V_d.

PFOA CL and V_d estimates based on biliary clearance

This approach has been previously described (Fujii et al., 2015). It is assumed that the PFOA overall CL (which can be expressed as the function of $T_{1/2}$ and V_d), is the sum of renal (CL_R) and fecal (CL_F) clearances. While CL_F cannot be measured directly due to experimental limitations, it can be expressed as PFOA biliary clearance adjusted for biliary reabsorption (f_b). Biliary reabsorption for PFOA would likely be similar to the overall absorption efficiency, which based on animal studies, is $f_b \geq 0.9$, as described in Section 4.2. The resulting formulas for the plasma clearance (CL) and V_d are:

$$CL = CL_R + (1 - f_b) \times \frac{C_{bile}}{C_s} \times Bile\_flux$$

$$V_d = \frac{T_{1/2}}{ln2}(CL_R + (1 - f_b) \times \frac{C_{bile}}{C_s} \times Bile\_flux).$$

Bile flux was estimated as 5 ml/kg-day (Davies and Morris, 1993). In two available studies with human patients, the ratio of bile to serum concentrations ($\frac{C_{bile}}{C_s}$) for PFOA was 0.21 and 0.25, with an average 0.23 (Harada et al., 2007b; Fujii et al., 2015). As described Section 4.5, the geometric mean of human CL_R for 6 studies was 0.060 ml/kg-day. Inputting these values in the formulas results in **CL ≤1.75x10^{-4} L/kg-day**, and V_d ≤248 ml/kg. Similar to other approaches, this method predicts a somewhat lower V_d for PFOA. The main limitations include the reliability of human CL_R and biliary clearance studies.

PFOA CL determination from exposure studies

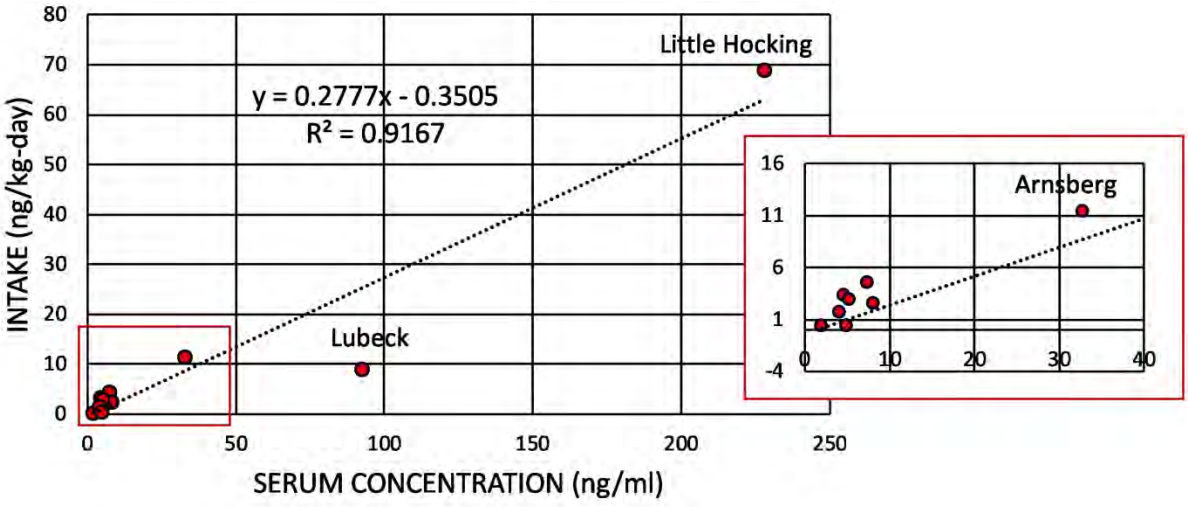
As an extension of the steady state-based approach used to estimate V_d based on Little Hocking and Lubeck data, as described above, other exposure studies that reported matching serum concentrations can be added to the dataset, and all data points can be fitted to a linear regression. In addition to the studies of exposure through polluted drinking water (Little Hocking, Lubeck, Arnsberg), several studies approximated exposure through all routes and some dietary studies reported diet as the primary exposure route. All these exposure assessments are assumed to occur at steady state levels. Details of the studies included in the dataset are provided in Table 4.9.1. The serum data for the Thompson et al. (2010) entry was updated as described in Section 4.8.

Table 4.9.1. PFOA Exposure studies that accounted for primary route(s) of exposure and reported plasma or serum concentrations

Reference	Population	Exposure	Intake (ng/kg-day)	Serum (ng/ml)
Fromme et al. (2007)	Bavaria, 2005	Diet	3.3 (females)	4.6
			4.4 (males)	7.4
Trudel et al. (2008)	North America, Europe, 1999-2007	All routes	2.5 (North America)	8.1
			2.9 (Europe)	5.3
Brede et al. (2010)	Arnsberg, Germany (men)	Drinking water	11.4	32.8
Thompson et al. (2010), Frisbee et al. (2009)	C8, Lubeck	Drinking water	9	92.4
	C8, Little Hocking		69	228
Haug et al. (2011a)	Norway, 2008	All routes	0.27	2
Lorber and Egeghy (2011)	USA, 2003-2004	All routes	1.6	4.1
Vestergren et al. (2012)	Sweden, 1999	Diet	0.35	5

The result of the linear regression for this data set is presented in Figure 4.9.1. The slope of the regression line in this graph is in fact clearance, which equals 0.28 ml/kg-day or **2.8×10^{-4} L/kg-day**.

Figure 4.9.1. Regression of PFOA exposure studies



PFOS CL determination based on the exposure through drinking water in Ronneby, Sweden

For PFOS, OEHHA considered a similar regression approach to that described in the previous section for PFOA, using all exposure studies. However, other than the scenario of increased intake from drinking water in Ronneby, Sweden (Li et al., 2018c), the rest of the available exposure assessments were at the lower range, rendering the regression approach non-informative. Therefore, analysis of the Ronneby data sets was more straightforward.

Three studies have been published that characterized exposure of Ronneby residents to PFOS in drinking water (Li et al., 2018c; Andersson et al., 2019; Silva et al., 2020). During approximately 1985-2013, about one third of households in Ronneby, Sweden received drinking water contaminated with PFAS from a nearby military airport. While the exact levels of

exposure throughout this period are not known, PFOS concentration in drinking water was 8,000 ng/L at the end of the exposure period (Li et al., 2018c). Extensive biomonitoring started approximately 6 months after the end of the exposure, and during the 2014-2016 time frame, 3,418 residents of Ronneby participated. The categorization of PFOS exposure within this population is presented in Table 4.9.2. Overall, this group comprised 7% of the Ronneby population in 2013, with a slightly higher percentage of females, 55% of study participants vs. 47% in the overall population of Ronneby (Andersson et al., 2019).

Table 4.9.2. Exposure[#] to PFOS in residents of Ronneby, Sweden

Reference	N	Exposure characterization	Descriptive statistic	C_{serum} ng/ml
Li et al. (2018c)	3,418	Original group: general population of Ronneby	mean±st.dev. median	245±234 176
Andersson et al. (2019)	2,347	Exposed for at least one year during 1985-2013	median[a]	222
	2,003	Exposed for at least one year during 2005-2013	median[a]	261
Silva et al. (2020)	1,845	Exposed for at least one year ending in 2013, <2 year olds excluded	median[b]	279
	1,176	Exposed for at least 10 years ending in 2013	median[b]	372
	506	Exposed for at least 29 years ending in 2013	median[b]	485

st.dev., standard deviation.
[#] Exposure is defined as residence at an address serviced by the affected water system.
[a] Separate values reported for men and women, averaged values presented here were calculated based on the reported numbers for each category.
[b] Separate values reported for men and women, averaged values presented here were calculated based on the reported percentages for each category.

According to the data presented in Table 4.9.2, the PFOS median serum concentrations increased with longer presumed exposure, which was determined as residence at an address serviced by the affected water system. The exposed individuals are more likely to attain the steady state (assumption for CL calculation) when exposed for longer periods of time, in this case for ≥10 years. This subgroup still contains a large number of subjects (N=1,176), increasing confidence in the choice of median as a reporting metric. In age composition, 64.6% of this group were 19-65 years, which is the population subgroup likely to be described by the steady state model. However, 24.3% were 66-94 years (Silva et al., 2020), the population subgroup with further increasing PFOS serum levels (Li et al., 2017e) that cannot be described by the steady state model. Such increases in older subjects have been described in other population studies of PFAS, and could be possibly attributed to declining kidney function with age, and as a result, decreased PFAS elimination. The fraction of these subjects in the '≥10 years' group is relatively low, and the resulting effect on serum concentration would be low.

In contrast, the fraction of 66-94 years comprises 45.7% of the '≥ 29 years' exposure group (Silva et al., 2020), and would violate the steady state assumption, were this group selected for analysis. Although longer confirmed exposure would be generally considered better for this type of analysis, the high fraction of the older age group and the overall lower number of participants (N=506) were among the reasons not to consider this subpopulation for analysis. Using these data (66 to94-year-olds) for clearance estimate would require a different kinetic

model and better understanding of the age-dependent changes in PFOA and PFOS excretion that currently exists.

The PFOS serum level reported for residents exposed for at least 10 years (ending in 2013) was 372 ng/ml (median, N=1,176). The method was the same as described in Section 4.8, except CL and not V_d was estimated, obviating the need for a $T_{1/2}$ estimate:

$$CL = \frac{C_w \times 1.4\ L/d \times f_a}{C_{serum} \times BW} = \frac{8{,}000 \times 1.4 \times 0.9}{372 \times 70} = 0.39 \text{ ml/kg-day} = \mathbf{3.9x10^{-4}\ L/kg\text{-}day}.$$

In this calculation, daily water consumption was 1.4 L/day, absorption efficiency 0.9, and average body weight 70 kg; C_w is the concentration of 8,000 ng/L in water.

PFOS CL and V_d estimates based on biliary clearance

Formulas for CL and V_d based on bile clearance developed above for PFOA in this document can also be applied to PFOS:

$$CL = CL_R + (1 - f_b) \times \frac{C_{bile}}{C_s} \times Bile\_flux$$

$$V_d = \frac{T_{1/2}}{ln2}\left(CL_R + (1 - f_b) \times \frac{C_{bile}}{C_s} \times Bile\_flux\right).$$

Bile flux was estimated as 5 ml/kg-day (Davies and Morris, 1993). The bile to serum ratio was 0.6 based on a study with 4 patients (Harada et al., 2007b). As described Section 4.5, the geometric mean of human CL_R for 6 studies was 0.016 ml/kg-day. Inputting these values in the formulas results in CL ≤ $3.16x10^{-4}$ L/kg-day, or V_d≤616 ml/kg. In conclusion, similar to other approaches, this method predicts a somewhat lower V_d for PFOS. The main limitations include the reliability of human CL_R and biliary clearance studies.

CL conclusions

Table 4.9.3 presents the summary of CL considerations developed in this document, labeled as possible options for this assessment. The corresponding V_d values are also included for comparison purposes. The CLs developed by US EPA (options A-1 and S-1 for PFOA and PFOS, respectively) were calculated using separately defined V_d and $T_{1/2}$ values. For PFOA, US EPA used the exposure data at the site of drinking water contamination in Ohio and West Virginia (C8 Panel) to calculate both V_d and $T_{1/2}$. While OEHHA concurs that these exposure conditions provided the best available system for estimating human V_d at the time, an updated calculation using a better quality report on serum concentrations (Frisbee et al., 2009) resulted in a higher PFOA V_d value (225 ml/kg, option A-2).

An alternative quantitation based on directly measured human CL_R and with consideration of PFOA biliary clearance suggested that PFOA V_d≤250 ml/kg (option A-4). In contrast, PFOA V_d estimates from chronic monkey studies (3,300 ml/kg) are much higher and may not be accurately applied to humans because of experimental limitations or species differences, such as decreased absorption efficiency in chronic gavage experiments in monkeys and inability to account for effects of biliary secretion/enterohepatic circulation of PFOA and extrapolate across species. Utilizing V_d from animal studies would introduce additional uncertainty in the assessment by relying on animal-to-human extrapolation of PK parameters. In this case,

OEHHA believes that using human data would be preferable and result in the least uncertainty; in addition, use of human data allows direct calculation of clearance, bypassing the separate steps of V_d and $T_{1/2}$ determination. In order to increase coverage of available human epidemiologic data, OEHHA performed a regression on human PFOA exposure data from multiple sites and exposure scenarios. The result of this analysis was a PFOA clearance rate of **$2.8x10^{-4}$ L/kg-day** (option A-3), which will be applied to conversion of serum levels to applied dose, as detailed elsewhere in this document. The alternative method considering biliary clearance of PFOA (option A-4) resulted in clearance rate of ≤$1.75x10^{-4}$ L/kg-day, which is fairly consistent with the preferred option A-3, given the underlying uncertainties of the method.

For PFOS, the previously used clearance rate value (option S-1) relied on several assumptions, and this approach should be updated given multiple newly available studies. This document outlines three distinct approaches to the updated PFOS clearance rate. Exposure to high PFOS levels in drinking water that occurred in Ronneby, Sweden, appears to be the best available data set for human exposures, and analysis of this data produced a reported $T_{1/2}$ of 3.4 years and clearance rate of $3.9x10^{-4}$ L/kg-day (option S-2). Using this $T_{1/2}$ together with the V_d from a chronic monkey study (1,080 ml/kg) would result in a CL of $6.0x10^{-4}$ L/kg-day. The alternative method considering biliary clearance of PFOS (option S-3) resulted in a CL ≤$3.2x10^{-4}$ L/kg-day, which is fairly consistent with option S-2. In summary, OEHHA considers option S-2 as the best available estimate for PFOS CL in humans, and will apply the PFOS CL of **$3.9x10^{-4}$ L/kg-day** to conversion of serum levels to applied dose, as detailed elsewhere in this document.

Table 4.9.3. Summary of V_d and CL considerations for PFOA and PFOS

Option	Method	$T_{1/2}$ (years)	V_d (ml/kg)	CL (10^{-4} L/kg-day)	References
PFOA					
A-1	Used by US EPA (2016a), based on human epidemiologic data; steady state	2.3	170	1.4	Emmett et al. (2006); Bartell et al. (2010); Thompson et al. (2010)
A-2	Updated Option A-1; human epidemiologic data; different reference for serum levels	2.3[a]	225	1.9	Frisbee et al. (2009); Bartell et al. (2010); Thompson et al. (2010)
A-3	Regression on exposure-serum data set; human epidemiologic data; steady state	-	-	**2.8**	Fromme et al. (2007); Trudel et al. (2008); Frisbee et al. (2009); Brede et al. (2010); Thompson et al. (2010); Haug et al. (2011a); Lorber and Egeghy (2011); Vestergren et al. (2012)
A-4	Upper-limit estimate based on human renal clearance and biliary clearance/reabsorption	2.7[a]	≤250	≤1.75	Harada et al. (2007b); Fujii et al. (2015); Li et al. (2017e)
PFOS					
S-1	Used by US EPA (2016b); steady state assumption; V_d derived as $1.35 \times V_d^{PFOA}$; human $T_{1/2}$	5.4	230	0.81	Andersen et al. (2006); Olsen et al. (2007); Thompson et al. (2010)

Option	Method	$T_{1/2}$ (years)	V_d (ml/kg)	CL (10^{-4} L/kg-day)	References
S-2	Human epidemiologic data (exposure-serum); steady state assumption	3.4[a]	1,160	**3.9**	Li et al. (2018c); Silva et al. (2020)
S-3	Upper-limit estimate based on human renal clearance and biliary clearance/reabsorption	3.4[a]	≤620	≤3.2	Harada et al. (2007b); (Li et al., 2018c)

[a]Used for V_d calculation only

5. EVIDENCE OF TOXICOLOGICAL EFFECTS

5.1. Immunotoxicity

NTP has previously reviewed the scientific literature on PFOS and PFOA and their relation to immunotoxicity (NTP, 2016). The NTP review included scientific information published up to May 18th, 2016. Based on this evidence, NTP reached the following conclusions for PFOA:

> "The NTP concludes that PFOA is presumed to be an immune hazard to humans based on a high level of evidence that PFOA suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. Although the strongest evidence for an effect of PFOA on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from epidemiological studies that PFOA reduced infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence. The evidence indicating that PFOA affects multiple aspects of the immune system supports the overall conclusion that PFOA alters immune function in humans."

With regards to PFOS, NTP reached the following conclusions (NTP, 2016):

> "The NTP concludes that PFOS is presumed to be an immune hazard to humans based on a high level of evidence that PFOS suppressed the antibody response from animal studies and a moderate level of evidence from studies in humans. Although the strongest evidence for an effect of PFOS on the immune system is for suppression of the antibody response, there is additional, although weaker, evidence that is primarily from studies in experimental animals that PFOS suppresses disease resistance and natural killer (NK) cell activity. The evidence indicating that PFOS suppresses multiple aspects of the immune system supports the overall conclusion that PFOS alters immune function in humans."

NTP's findings are consistent with assessments by US EPA (2016b), New Jersey DWQI (2017) and ATSDR (2018a), which evaluated much of the same immunotoxicity database and described the immunotoxic effects of PFOA and PFOS in animal studies and in humans. These assessments reported effects on the spleen and thymus of rodents (including changes in organ weight and lymphocyte populations), as well as a decreased ability of the immune system to respond to a challenge.

5.1.1. Recent Human Evidence

OEHHA's search strategy and study summary tables for the immunotoxicity of PFOA and PFOS in humans are in Appendix 7. All but two of the immunotoxicity studies OEHHA identified provided results for both PFOA and PFOS. Two studies reported information only for PFOS. This included the study by Xu et al. (2020b), which involved a community where the local water supply was contaminated with PFOS, and the study by Ammitzboll et al. (2019), which appeared to have measured both PFOA and PFOS but only presented immune results for PFOS. Six results were available for antibody responses, with the most common being response to tetanus and diphtheria vaccine. Thirty-five results were available for hypersensitivity-related outcomes including eleven for asthma, nine for eczema, seven for rhinitis, three for immunoglobulin E (IgE) levels, and five for allergy. Thirteen results were available for an infectious disease outcome or related symptom such as gastroenteritis or fever. Three studies provided results for C-reactive protein (CRP) or cytokine levels. Twelve studies

were prospective cohort studies, five were cross-sectional only, and six presented both prospective and cross-sectional results. One study was based on a case-control design but with a cross-sectional assessment of exposure and outcome. Studies were done in a variety of locations including the US, Faroe Islands, Norway, China, and Japan.

Antibody response: Most studies of PFOA and PFOS and antibody response, including those identified by OEHHA and those reviewed by (NTP, 2016), investigated anti-diphtheria and anti-tetanus immunoglobulin G (IgG) levels. The large majority of results on these outcomes are based on two cohorts of pregnant women and their offspring from the Faroe Islands. One of these cohorts involved children born in 1997-2000 (the "1997-2000 birth cohort") and the other involved children born in 2007-2009 (the "2007-2009 birth cohort"). In both studies, concentrations of PFOA and PFOS were measured in maternal serum during pregnancy and in the serum of offspring at various ages after birth. Children from the 1997-2000 birth cohort have been followed up to 13 years of age, and the children from the 2007-2009 cohort have been followed up to 5 years of age. The study by Kielsen et al. (2016) (reviewed in US EPA (2016b)) is the only other study besides the Faroe Islands cohorts to investigate associations between PFOA or PFOS and antibody response to diphtheria or tetanus vaccine. This study was done in twelve adults from Copenhagen, Denmark.

A summary of the results from all studies of PFOA or PFOS and antibody response to tetanus or diphtheria vaccine, either before or after the (NTP, 2016) review, are shown in Table 5.1.1. Most of these results are from various follow-up periods for the 1997-2000 Faroe Islands cohort. All studies presented results in terms of the percentage decrease in antibody levels associated with a two-fold increase in serum PFOA or PFOS levels. As seen in Table 5.1.1, the results vary greatly depending on the timing of the exposure and outcome assessment. However, the large majority of results are consistent with a decline in antibody levels with increasing PFOA or PFOS levels (i.e., the percent change in antibody levels with increasing PFOA or PFOS levels was negative). Many of these results, although not all, show a greater than 10-20% decrease in antibody levels for each two-fold increase in PFOA or PFOS, and several of these results are statistically significant. Overall, 69-85% of all studies' results show at least some decrease in antibody levels with increasing PFOA or PFOS exposure (Table 5.1.2), and in 10-45% of these, the decreases are statistically significant. These associations were seen in both cross-sectional and prospective analyses. The most consistent findings are for diphtheria vaccine response and PFOA, where 85% of results show a decrease in antibody levels with increasing PFOA levels and 45% of these results are statistically significant. In all three cohorts that evaluated diphtheria vaccine response (the Faroe Islands 1997-2000 cohort, the Faroe Islands 2007-09 cohort, and Kielsen et al. (2016)) at least some evidence of an inverse relationship between PFOA and PFOS and antibody response was seen (Table 5.1.1).

NTP (2016) identified several studies that evaluated associations between PFOA and PFOS and antibody response to vaccines other than those for diphtheria and tetanus, including mumps, measles, rubella (MMR), and influenza. Most of these studies found some evidence that IgG levels decreased with increasing PFOA or PFOS serum concentrations, although not all results were statistically significant and non-linear dose-response patterns were seen in some studies (Table 5.1.3). In the two studies published since the NTP (2016) review, the results were mixed. Using data from the US National Health and Nutrition Examination Survey (NHANES), Pilkerton et al. (2018) identified statistically significant inverse associations between both PFOA and PFOS and rubella IgG titers in adults but not in older children (Table 5.1.3). In contrast, in a study of 75 US adults, odds for influenza H1N1 seroconversion were higher in

those with higher PFOA or PFOS serum concentrations, although findings were borderline or not statistically significant (Stein et al., 2016a).

Table 5.1.1. Results of epidemiologic studies of PFOA or PFOS and anti-tetanus or anti-diphtheria vaccine response at different ages. Values are the percent change in IgG levels for each 2-fold increase in PFOA or PFOS concentration (please read the footnotes of this table, they are important for its understanding and interpretation).

PFOA	Outcome									
	Diphtheria					Tetanus				
	5 years pre	**5 years post**	**7 years**	**13 years**	**Adult**	**5 years pre**	**5 years post**	**7 years**	**13 years**	**Adult**
0	-16.2 **-18.9[a]***	-6.2	**-22.8**			-10.5 **-22.2[a]***	14.5	7.4		
1.5 years	4.2*					**-16.3***				
5 years	-6.8 18.3[a]*	-6.1	**-25.2**			-13.3 **-25.3[a]***	-9.7	**-35.8**		
7 years			**-25.4**	-9.2*				-20.5	2.9*	
13 years				**-25.3***					-5.6*	
Adult					-8.2[b]					0.23[b]
PFOS	**5 years pre**	**5 years post**	**7 years**	**13 years**	**Adult**	**5 years pre**	**5 years post**	**7 years**	**13 years**	**Adult**
0	**-38.6** -14.0[a]	-20.6	-10.0			-10.1 -10.8[a]	-2.3	35.3		
1.5 years	17.5					-7.0				
5 years	-16.0 17.1[a]	-15.5	**-27.6**			-11.9 -9.1[a]	**-28.5**	-23.8		
7 years			**-30.3**	-25.6				-9.1	**45.4**	
13 years				-10.5					23.4	
Adult					**-11.9[b]**					-3.6[b]

* Studies published since the NTP (2016) review
"Pre" and "post" refer to pre- and post-vaccination antibody levels
All results are from the 1997-2000 Faroe Islands cohort except as noted below:
[a] 2007-09 Faroe Islands cohort
[b] Kielsen et al. (2016)
Bolded numbers in the column and row headings are the ages when the PFOA or PFOS (left most column) or IgG levels (third row) were measured. "0" represents maternal serum PFOA or PFOS levels measured during gestation; "pre" and "post" are pre- and post-immunization values at age 5.
Bolded results are statistically significant.
Red boxes represent cross-sectional evaluations; all others are prospective.
Example: in the box marked in blue, the upper value of -16.2 is the percent change in pre-vaccination diphtheria IG levels at 5 years of age for each 2-fold increase in PFOA at birth seen in the 1997-2000 Faroe Islands cohort. The lower value is the corresponding results from the 2007-09 Faroe Islands cohort (hence it is marked with an "[a]"). This lower number is statistically significant so it is bolded.
This table does not include the results of Abraham et al. (2020) (reviewed below).

Table 5.1.2. The numbers and percentages of studies of PFOA or PFOS and tetanus or diphtheria antibody levels showing inverse and/or statistically significant associations

			Total results			Inverse association			Statistically significant inverse association			Statistically significant positive association		
Exposure	**Outcome**	**N/%**	**Total**	**Cross**	**Pros**	**Total**	**Cross**	**Pros**	**Total**	**Cross**	**Pros**	**Total**	**Cross**	**Pros**
PFOA	Diphtheria	N	13	6	7	11	5	6	5	2	3	0	0	0
		%[a]							45%	40%	50%	0%	0%	0%
		%[b]				85%	83%	86%	38%	33%	43%	0%	0%	0%
	Tetanus	N	13	6	7	9	5	4	4	1	3	0	0	0
		%[a]							44%	20%	75%	0%	0%	0%
		%[b]				69%	83%	57%	31%	17%	43%	0%	0%	0%
PFOS	Diphtheria	N	13	6	7	11	5	6	4	2	2	0	0	0
		%[a]							36%	40%	33%	0%	0%	0%
		%[b]				85%	83%	86%	31%	33%	29%	0%	0%	0%
	Tetanus	N	13	6	7	10	5	5	1	1	0	1	0	1
		%[a]							10%	20%	0%	10%	0%	20%
		%[b]				77%	83%	71%	8%	17%	0%	8%	0%	14%

"Inverse association" refers to results showing decreasing IgG levels with increasing PFOA or PFOS concentrations; "Positive association" refers to increasing IgG levels with increasing PFOA or PFOS concentrations
Abbreviations: Cross, cross-sectional study; N, number of studies; Pros, prospective study
[a] Percentage of all results indicating an inverse association
[b] Percentage of all results
This table does not include the results of Abraham et al., 2020 (reviewed below)

Table 5.1.3. Epidemiologic studies on associations between PFOA or PFOS and antibody response to other vaccines

Vaccine	Exposure timing	Change in antibodies with PFOA[1]	Change in antibodies with PFOS[1]	Possible sources of heterogeneity	Reference
Rubella	Maternal 0-3 day post delivery	**-0.4 (-0.64 to -0.17)**	**-0.08 (-0.14 to -0.02)**	• developmental exposure metric	Granum et al. (2013)
	Children: current	**-8.9 (-14.6 to -2.9)**	**-13.3 (-19.9 to -6.2)**	• childhood exposure metric booster vaccination	Stein et al. (2016b)
	Adults	**p=0.002**	**p=0.03**	• outcome in all adults combined only F values for decline in IgG reported	Pilkerton et al. (2018)[2]
	Children 12-18 years old: current	p=0.80	p=0.25	• childhood exposure metric only F values for decline in IgG reported	Pilkerton et al. (2018)[2]
Mumps	Children: current	**-6.6 (-11.7 to -1.5)**	**-5.9 (-9.9 to -1.6)**	• childhood exposure metric booster vaccination	Stein et al. (2016b)
Measles	Maternal 0-3 day post delivery	-0.13 (-0.35 to 0.09)	-0.05 (-0.1 to 0.01)	• developmental exposure metric	Granum et al. (2013)
	Children: current	-3.4 (-16.7 to 11.9)	-2.9 (-17.3 to 13.9)	• childhood exposure metric booster vaccination	Stein et al. (2016b)
Influenza	Adult at vaccination	Antibody titer ratio 2nd -0.10 (-0.3 to 0.1) 3rd -0.07 (-0.28 to 0.14) **4th -0.22 (-0.43 to -0.01)** Antibody titer rise **2nd -0.28 (-0.51 to -0.06)** **3rd -0.37 (-0.60 to -0.13)** 4th -0.12 (-0.36 to 0.13)	Antibody titer ratio 2nd -0.06 (-0.26 to 0.14) 3rd -0.02 (-0.18 to 0.23) 4th -0.03 (-0.24 to 0.19) Antibody titer rise 2nd 0.03 (-0.19 to 0.26) 3rd 0.18 (-0.00 to 0.41) 4th -0.04 (-0.28 to 0.21)	• influenza H3N2 outcome is antibody rise or ratio by quartile of PFOA or PFOS compared to the first quartile; negative values indicate a decrease in antibody levels outcome in adults	Looker et al. (2014)

Vaccine	Exposure timing	Change in antibodies with PFOA[1]	Change in antibodies with PFOS[1]	Possible sources of heterogeneity	Reference
	Adult at vaccination	OR = 6.8 (1.0-48.1) p-trend = 0.07 (hemagglutinin inhibition) OR = 1.8 (0.7-4.3) p-trend = 0.27 (immunohistochemistry)	OR = 1.3 (0.2-7.3) p-trend = 0.81 (hemagglutinin inhibition) OR = 2.4 (0.9-6.6) p-trend = 0.12 (immunohistochemistry)	• influenza H1N1 ORs for seroconversion response to FluMist fourth vs. first quartile of PFOA or PFOS outcome in adults	Stein et al. (2016a)

Abbreviations: OR, odds ratio
Results in parentheses are 95% confidence intervals. Bolded results are statistically significant
Results published since the NTP (2016) review are highlighted in red
Data are presented in the format used by the NTP NTP (2016)
[1] Percent change in antibody concentration per 2-fold increase in PFOA or PFOS unless otherwise noted
[2] For Pilkerton et al. (2018), only F values are given
This table does not include the results of Abraham et al. (2020) (reviewed below)

Budtz-Jorgensen and Grandjean (2018) have published a benchmark dose (BMD) analysis of PFOA and PFOS and altered antibody response to tetanus and diphtheria vaccine using data from the 1997-2000 and 2007-2009 Faroe Islands cohorts. Individual data from these cohorts are not publically available. In one set of analyses, prenatal serum PFAS concentrations and IgG levels at age 5 years were assessed using combined data from the 1997-2000 and 2007-2009 cohorts. In another set of analyses, serum PFAS concentrations at age 5 years and IgG levels at age 7 years were assessed. Since data at age 7 years were not available from the 2007-2009 cohort, this later set of analyses only involved data from the 1997-2000 cohort. Analyses were done for each PFOA and PFOS separately, both adjusted and unadjusted for each other. A benchmark response of 5% was used. The benchmark doses were thus the PFOA or PFOS serum concentrations associated with a 5% decrease in anti-tetanus or anti-diphtheria IgG levels after vaccination, and the BMDLs were the lower one-sided 95% confidence intervals of these BMDs. Several dose-response models were evaluated, with the lowest BMD values and the best model fit occurring with the piece-wise linear model. The major characteristic of this model compared to a linear model is that the dose-response slope is allowed to change at the median exposure. Other variables in the models were age, sex, cohort (1997-2000 or 2007-2009), and booster type at age 5 (for analyses of IgG at age 7).

The results of the Budtz-Jorgensen and Grandjean (2018) BMD analyses are shown in Table 5.1.4. The lowest BMDs are for the analyses of PFOA at age 5 years and IgG levels at age 7 years. Adjustment of the PFOA BMD calculations for PFOS led to somewhat higher BMDs in most but not all cases. However, these adjustments overall had only small impacts on BMDLs, likely due to the increased variance that occurred from having two correlated variables (PFOA and PFOS) in the same model. Some of the BMDLs are lower in the analyses involving age 7 IgG levels than in the analyses of age 5 IgG levels. This may be partially due to the fact that the latter involves only the 1997-2000 cohort and therefore a smaller sample size. Overall, the lowest BMDLs were 0.10 and 0.12 ng/ml, which were seen in the unadjusted and adjusted analyses, respectively, of prenatal PFOA exposure and anti-diphtheria IgG levels at age 5 years. Importantly, these BMDLs appear to be well outside the range of PFOA and PFOS values measured in this cohort. That is, although the actual values for the measured PFOA and PFOS concentrations at age 5 were not specified numerically, they were displayed graphically and the lowest measured values appear to be about 6-7 ng/ml for PFOS and 1.2-1.3 ng/ml for PFOA (Grandjean et al., 2012). As such, these BMDLs are approximately 6- to 13-fold lower than the lowest measured value.

Table 5.1.4. Benchmark dose (BMD) modeling results using the piece-wise linear model from the Faroe Islands cohorts (Budtz-Jorgensen and Grandjean, 2018)

	Prenatal PFAS exposure and age 5 IgG (1997-2000 and 2007-2009 cohorts)						Age 5 PFAS exposure and age 7 IgG (1997-2000 cohort only)				
	Unadjusted			Adjusted for other PFAS			Unadjusted			Adjusted for other PFAS	
Tetanus	BMD	BMDL		BMD	BMDL		BMD	BMDL		BMD	BMDL
PFOS	2.59	1.05			1.64		1.45	0.56		3.57	0.72
PFOA	0.25	0.13		0.25	0.13		0.52	0.16		0.67	0.17
Diphtheria											
PFOS	1.62	0.83		2.51	0.95		0.98	0.49		1.21	0.54
PFOA	0.15	0.10		0.21	0.12		0.48	0.17		1.06	0.20

All numbers are in ng/ml serum
Some BMDs were not calculated or not provided by the authors. Explanation not provided.

Infectious diseases: In its review, NTP concluded that there was "low confidence" that PFOA was associated with infectious disease outcomes (NTP, 2016). This rating was based on the small number of studies on any specific outcome and the overall inconsistency of the findings that were available at the time. For most outcomes, OEHHA identified similar weaknesses in the literature published since the NTP review (NTP, 2016). For example, while a statistically significant association between increasing PFOA serum concentrations and increasing risk for fever has been reported (Dalsager et al., 2016), this outcome has not been examined in any other study (either before or after the NTP review). For other outcomes such as colds or gastroenteritis, studies published since the NTP review did not identify clear associations (Appendix 7, Table A7.3). Statistically significant increases with increasing PFOA were seen in three of the four studies of lower respiratory tract infections in children published since the NTP review (Impinen et al., 2018; Impinen et al., 2019; Kvalem et al., 2020), although clear increases were not seen in the large prospective Spanish study by Manzano-Salgado et al. (2019).

NTP also concluded that there was "low confidence" that PFOS was associated with infectious disease outcomes (NTP, 2016). Most of the studies NTP reviewed found no association with outcomes such as gastroenteritis, colds, or flu. One study found an association between PFOS and hospitalizations for any infectious disease, but only in females (Fei et al., 2010). Studies published since the NTP (2016) review on PFOS and gastroenteritis, colds, and cough have not identified clear associations (Table A7.3). However, statistically significant associations have been seen in studies in children for "any infection" (not necessarily hospitalizations) (Goudarzi et al., 2017), fever (Dalsager et al., 2016), gastroenteritis (Impinen et al., 2018), and lower respiratory tract infections (Impinen et al., 2018; Impinen et al., 2019; Kvalem et al., 2020) (Table A7.3). Links between PFOS and fever or lower respiratory tract infections were not examined in any of the studies reviewed by NTP (NTP, 2016).

Hypersensitivity: In their review, NTP (2016) found that that increasing serum concentrations of PFOA in children were consistently associated with asthma, increased total IgE, and several other indicators of immune hypersensitivity. However, NTP rated this evidence as "low confidence" primarily due to the cross-sectional nature of these studies.

Since the NTP (2016) review, at least three more cross-sectional studies in children have found associations between PFOA and asthma (Qin et al., 2017; Timmermann et al., 2017; Averina et

al., 2019). An association was also recently reported between serum PFOA concentrations at age 5 years and whether a child had ever had asthma up to age 13 years but only in children who had not received an MMR vaccination before age 5 (Timmermann et al., 2017). Three of the six studies evaluating possible associations between maternal or cord blood PFOA levels and childhood asthma reported elevated ORs in those with higher PFOA levels although these findings were not statistically significant (Goudarzi et al., 2016; Impinen et al., 2018; Beck et al., 2019) (Table A7.3). The three others reported mixed or negative results.

Most studies on PFOA and eczema published since the NTP (2016) review have not found clear or consistent associations. In a study of cord blood PFOA levels and childhood eczema up to age 24 months, an association was seen in females (OR = 2.52; 95% CI, 1.12-5.68) but not in males (OR not provided) (Chen et al., 2018b). Wen et al. (2019b) reported an elevated OR for eczema (OR = 1.89; 95% CI, 1.10-3.16), with the highest risks in those with the GST-T1null phenotype. None of the other studies on PFOA and eczema published since the NTP review found similar associations. Several of the recent studies assessing rhinitis found ORs greater than 1.2, but none were statistically significant. Recent studies on PFOA and IgE levels or allergy outcomes did not find clear or consistent associations.

For PFOS, NTP concluded that there was low confidence that exposure during childhood is associated with increased hypersensitivity responses based on the human studies they reviewed (NTP, 2016). While noting that several studies did identify associations between PFOS and asthma or serum IgE levels, the cross-sectional nature of these findings led to NTP's rating of low confidence. Most of the studies of PFOS and asthma published since the NTP review have not found clear associations. This includes two prospective studies of maternal serum or cord blood PFOS levels and asthma in young children (Goudarzi et al., 2016; Impinen et al., 2018). Three of the four studies of PFOS and lower respiratory tract infections published since the NTP review reported statistically significant associations between increasing PFOS exposures and this outcome in children (Impinen et al., 2018; Impinen et al., 2019; Kvalem et al., 2020). All of these were prospective studies. Clear or consistent associations were not seen in any of the more recent studies OEHHA reviewed of PFOS and eczema, IgE, or rhinitis.

Other outcomes: With regards to autoimmunity outcomes, NTP (2016) identified two studies that reported associations between PFOA exposures and ulcerative colitis. However, NTP rated this body of evidence as "low confidence" since both studies involved participants from the same study area. In the only study to examine a similar outcome since the NTP review, a clear association was not seen between PFOA and inflammatory bowel disease (Hammer et al., 2019), although the sample size was small (N=37 cases). NTP only identified one study of PFOS and an autoimmunity outcome. This study reported that prenatal concentrations of PFOS were associated with decreases in anti-actin IgG in a test for antibodies to several neural or non-neural antigens in 7-year-old children from the Faroe Islands (Osuna et al., 2014). This finding has not been replicated in another population. Two studies of PFOS and inflammatory bowel disease published since the NTP review have reported mixed results (Hammer et al., 2019; Xu et al., 2020b)(Hammer et al., 2019; Xu et al., 2020).

Abraham et al., 2020: This study was published after OEHHA's main literature search so is not included in the summary tables in Appendix 7. It is reviewed here because it provided information on diminished vaccine response, one of the outcomes OEHHA considered a potential critical effect. Briefly, this study used a cross-sectional design to investigate associations between diminished vaccine response and serum levels of PFOA and PFOS in one-year-old children living near Berlin, Germany. It included 101 children (51 boys and 50

girls) whose parents were recruited using announcements in newspapers and through pediatricians. Serum samples were collected in 1997-98 and stored frozen at -80 °C. The study was originally designed to investigate the effects of dioxins, PCBs, pesticides, heavy metals, and other environmental contaminants on the immune system and other biological parameters. Although not clearly described, it appeared that the researchers attempted to recruit children from industrial areas near Berlin, where exposure to these agents was likely to be high. In fact, levels of lead and several of the other agents were elevated, and serum levels of some of these (specifically PCBs and dioxins) were highly correlated with serum levels of PFOA and PFOS. The researchers reported statistically significant associations between increasing levels of PFOA and decreasing concentrations of diphtheria, influenza, and tetanus antibody. Results were adjusted for time since vaccination and the number of vaccinations. Similar associations were not seen for PFOS. A multivariate analysis using the function 'stepAIC' in the statistical program R with inclusion of dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), ndl-PCBs in addition to PFOA and PFOS revealed a highly significant influence of PFOA only. This study is discussed further in Chapter 6.

Other significant studies identified between January 2, 2020 (i.e., the end date of OEHHAs initial full literature review) and December 31, 2020 are listed in Appendix 7, Table A7.29.

5.1.2. Recent Animal Evidence

In addition to NTP (2016), US EPA (2016b) and New Jersey DWQI (2017) also evaluated the immunotoxicity of PFOA and PFOS. OEHHA evaluated animal studies from 2016 onward, and identified many toxicity endpoints related to immunotoxicity. Observed toxicity included changes in spleen and thymus weights, changes in lymphocyte populations, changes in cytokine levels, and immunosuppression. Studies for PFOA are summarized in Table 5.1.5.

Table 5.1.5. Summary of recent animal toxicity studies of PFOA reporting immune toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Female C57BL/6N PPARα KO and WT mice (6/dose/group)	0, 7.5 or 30 mg/kg-day in drinking water for 15 days	**WT**: ↓ relative spleen and relative thymus weights; **WT and KO**: ↓SRBC-specific IgM antibody responses	LOAEL: 7.5 mg/kg-day for ↓ relative thymus weight in WT mice	DeWitt et al. (2016)
Female C57BL/6N mice (8/dose)	0, 0.94, 1.88, 3.75, or 7.5 mg/kg-day in drinking water for 15 days	↓ dinitrophenyl-ficoll (DNP)-specific IgM antibody response; ↓ relative spleen and thymus weight	NOAEL: 0.94 mg/kg-day for ↓ antibody response	DeWitt et al. (2016)
Female C57BL/6N mice (4/dose/group)	0, 3.75 or 7.5 mg/kg-day in drinking water for 10, 13 or 15 days	changes in splenic lymphocyte subpopulations	LOAEL: 3.75 mg/kg-day	DeWitt et al. (2016)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male ICR mice (5/dose)	Treated mice were sensitized with OVA to induce active systemic anaphylaxis on days 0 and 7. OVA + 100 or 150 mg/kg 3 times on days 9, 11, and 13 orally[b]. Control mice had 150 mg/kg PFOA only or OVA only.	↓ rectal temperature; ↑ serum histamine, TNF-α, IgG1 and IgE levels	LOAEL: 100 mg/kg for ↑ TNF- α and IgE levels in sensitized mice	Lee et al. (2017)
Male Sprague Dawley rats (10/dose)	0, 150, or 300 ppm in feed (0, 14.7, or 29.5 mg/kg-day) for 16 weeks	↓ absolute and relative spleen weight; lymphoid follicle atrophy	LOAEL: 14.7 mg/kg-day for ↓ absolute and relative spleen weight	NTP (2020)
Female Sprague Dawley rats (10/dose)	0, 300, or 1,000 ppm in feed (0, 27.7, or 92.7 mg/kg-day) for 16 weeks	pigment in spleen	LOAEL: 27.7 mg/kg-day	NTP (2020)

[a] LOAEL/NOAEL not applicable for single dose studies.
[b] The specific manner of oral administration was not stated in study.
Abbreviations: GD, gestation day; IgE, immunoglobulin E; IgM, immunoglobulin M; IL-22, interleukin-22; KO, knockout; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; OVA, ovalbumin; PPARα, peroxisome proliferator-activated receptor alpha; SRBC, sheep red blood cells; TNF-α, tumor necrosis factor alpha; WT, wild-type

Immunotoxicity studies for PFOS published from 2016 onward are summarized in Table 5.1.6. Similar immunotoxic effects to PFOA were observed in these recent studies, including changes in spleen and thymus weight, changes in lymphocyte populations, and changes in cytokine levels.

Table 5.1.6. Summary of recent animal toxicity studies of PFOS reporting immune toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant C57BL/6 mice (12/dose)	0, 0.1, 1, or 5 mg/kg-day via gavage from GD 1 - 17; pups examined at 4 and 8 weeks of age	**Male pups:** ↓ absolute spleen and thymus weight; ↓ thymic cellularity; changes in thymic lymphocyte population; **Pups of both sexes:** ↑ absolute liver weight; ↓ splenic cellularity; changes in splenic lymphocyte populations; ↓ splenic lymphocyte proliferation; ↓ splenic NK cell activity; ↓ splenic plaque forming cells; changes in IL-2 and IL-4 levels	NOAEL: 0.1 mg/kg-day for reduction of splenic NK cell activity in males	Zhong et al. (2016)
Male C57BL/6 mice (10/dose)	0, 2.5, 5, or 10 mg/kg-day via gavage for 30 days	↓ absolute spleen weight	NOAEL: 5 mg/kg-day	Xing et al. (2016)
C57BL/6 mice (sex not specified) (4/dose)	0 or 2 mg/kg via gavage for 25 days; mice were infected with *Citrobacter* at day 7	↓ in pathogen clearance at late stage infection; induction of IL-22 from ILC3 and Th17 cells; ↓ mucin	NA[a]	Suo et al. (2017)
Male Sprague Dawley rats (6/dose)	0, 1, or 10 mg/kg-day via gavage for 4 weeks	↑ serum TNF-α and IL-6 levels	LOAEL: 1 mg/kg-day	Han et al. (2018b)
Male ICR mice (5/dose)	Treated mice were sensitized with OVA to induce active systemic anaphylaxis on days 0 and 7. OVA + 50, 100 or 150 mg/kg, 3 times on days 9, 11 and 13 orally.[a] Control mice had 150 mg/kg PFOS only or OVA only.	↓ rectal temperature; ↑ histamine, TNF-α, IgG and IgE levels in sensitized mice	LOAEL: 50 mg/kg for ↑TNF-α and IgE levels	Lee et al. (2018)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male and female Sprague Dawley rats (10/sex/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day for 28 days via gavage	**Males**: ↓white blood cells; ↓neutrophils; ↓ eosinophils; ↓ relative thymus weight **Females**: ↓ relative thymus weight at 1.25 mg/kg-day (not statistically significant at higher doses)	NOAEL: 2.5 mg/kg-day for all endpoints in males	NTP (2019b)

[a] The specific manner of oral administration was not stated in study.
Abbreviations: GD, gestation day; IgE, immunoglobulin E; IgG, immunoglobulin G; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; LOAEL, lowest-observed-adverse-effect level; NK cell, natural killer cell; NOAEL, no-observed-adverse-effect level; OVA, ovalbumin; TNF-α, tumor necrosis factor alpha

5.1.3. Recent Mechanistic Evidence

OEHHA's recent literature search identified only one mechanistic study of PFOA immunotoxicity. Results from an in vitro study investigating the effects of PFOA on allergic inflammation showed that PFOA enhanced histamine release and changes in pro-inflammatory cytokines in RBL-2H3 rat cells through the NF-κB pathway (Lee et al., 2017).

For PFOS, OEHHA identified only a few studies that investigated the mechanisms for immunotoxicity in human cell lines. Yang et al. (2016) reported that PFOS inhibited 11β-hydroxysteroid dehydrogenase 1, an enzyme responsible for converting cortisone to cortisol, in human decidual stromal cells. Furthermore, exposure to PFOS attenuated the cortisone-induced reduction of the pro-inflammatory cytokines, IL-6 and IL-1β (Yang et al., 2016). In human lymphocytes, PFOS caused cytotoxicity by inducing oxidative stress, as evidenced by increased ROS formation, lipid peroxidation, glutathione depletion and activation of caspase-3 (Zarei et al., 2018).

Two recent mechanistic immunotoxicity studies of PFOS in animal cells or tissues were identified. In rat RBL-2H3 cells sensitized with anti-dinitrophenol (DNP), PFOS increased histamine and pro-inflammatory cytokine levels, and activated the NF-κB pathway (Lee et al., 2018). Additionally, a study in dolphins showed that PFOS caused an increase in CD4+ and CD8+ T cell proliferation and induced pro-inflammatory interferon-gamma (IFN-γ) in T cells (Soloff et al., 2017).

5.1.4. Conclusions

OEHHA found mostly consistent evidence that PFOA and PFOS are associated with suppressed antibody response in humans, in particular, suppressed response to diphtheria, tetanus, and rubella vaccines. OEHHA also identified evidence that PFOA or PFOS can affect other immune outcomes such as asthma or certain infectious diseases or related symptoms, although this effect was not seen in all studies.

OEHHA evaluated the possibility that some of the results reported in the epidemiologic studies reviewed here may have been caused by confounding, outcome or exposure misclassification, selection bias, or some other error. Most of the epidemiologic studies reviewed here provided only a limited assessment of confounding. However, there was no convincing evidence

indicating that the associations reported for PFOA or PFOS and diminished antibody responses were caused by confounding. For example, age is a potential confounder since there is good evidence that it is associated with both PFAS levels and a number of different aspects of immune function. Importantly though, almost all of the studies OEHHA reviewed either limited their participants to a narrow range of ages or they adjusted for age in their statistical analyses. Other factors like body mass index (BMI), socioeconomic indicators, smoking, and breast-feeding may also be related to both PFAS exposure and immune function but most of the evidence OEHHA reviewed suggests these associations are not strong enough to cause many of the PFAS-immunotoxicity associations that were reported. For example, based on data from US NHANES, Calafat et al. (2007b) reported geometric mean serum PFOA concentrations of 5.0 (95% CI, 4.5-5.5), 5.4 (95% CI, 4.9-5.9), and 5.6 (95% CI, 5.1-6.1) ng/ml for people with less than a high school education, a high school education, and greater than a high school education (an indicator of socioeconomic status), respectively. They also reported PFOA levels in non-smokers, passive smokers, and smokers of 4.9 (95% CI, 4.5-5.4), 5.5 (95% CI, 4.9-6.1), and 5.4 (95% CI, 4.9-5.9) ng/ml, respectively. Overall, the small differences in PFOA levels across these different categories of education or smoking do not appear great enough to cause the 10-20% decreases in antibody response reported in the studies OEHHA reviewed (Axelson, 1978). For breast-feeding, while this factor was associated with higher PFAS exposures in the Faroe Islands cohorts, it was not strongly associated with antibody concentrations (Mogensen et al., 2015b; Budtz-Jorgensen and Grandjean, 2018) and thus unlikely to have caused major confounding. Finally, with regards to BMI, the Faroe Islands researchers wrote that statistical adjustment for this factor had "virtually no impact on the results" (Mogensen et al., 2015a).

Chemical exposures that are highly correlated with PFOA or PFOS and have significant immune effects could also cause confounding. In the 1997-2000 Faroe Islands cohort, correlations between serum levels of PFOA and PFOS with polychlorinated biphenyls (PCBs) in 5-year-old children were 0.00 and 0.08, respectively, which are likely too low to cause significant confounding (Grandjean et al., 2012). In fact, statistical adjustments for PCB levels had little effect on PFOA or PFOS results (Grandjean et al., 2017a; Grandjean et al., 2017b). Methylmercury may have some effects on the immune system, however the authors of the Faroe Islands studies wrote, "…exposures to methylmercury and polychlorinated biphenyls were only weakly correlated with serum concentrations of the PFAS, and confounding by these other exposures could therefore be ignored" (Budtz-Jorgensen and Grandjean, 2018). Correlations between PFOS and PFOA with most other organochlorine chemicals also appeared to be fairly low (data presented only in figure form) (Oulhote et al., 2017).

Many of the individual PFAS are correlated with each other, and these correlations can potentially make it difficult to determine the individual effects of any single compound. In some studies, these correlations are fairly strong. For example, in 1,562 children and adults ages 12 years and older in the 1999-2000 NHANES, Calafat et al. (2007b) reported a Pearson correlation coefficient of 0.64 ($p < 0.01$) between PFOA and PFOS serum concentrations. In 652 Danish men, the Spearman correlation coefficient between PFOA and PFOS serum concentrations was 0.71 ($p < 0.0001$) (Eriksen et al., 2011). Despite these high correlations, several pieces of evidence suggest the effects of PFOA and PFOS on antibody levels may be independent of each other. First, the correlations between PFOA and PFOS reported in the Faroe Islands cohorts are less strong than those reported in the Calafat et al. (2007b) and Eriksen et al. (2011) studies. For example, Pearson correlation coefficients between PFOA and PFOS serum concentrations measured at age 7 years in the 1997-2000 Faroe Islands cohort ranged from 0.29 to 0.50 (Table 5.1.7). Second, adjustment of PFOA- or PFOS-antibody response associations for other PFAS in the 1997-2000 Faroe Islands cohort generally resulted

in only small changes in effect sizes (Table 5.1.4) (Mogensen et al., 2015a; Budtz-Jorgensen and Grandjean, 2018). Third, animal studies provide clear evidence of an association between exposure to PFOA and PFOS and suppression of antibody response (NTP, 2016).

Table 5.1.7. Pearson correlation coefficients between PFOA, PFOS, and PFHxS at ages 5 and 7 years in the 1997-2000 Faroe Islands cohort (Mogensen et al., 2015a)

	Levels at age 7 years		
	PFOS	**PFOA**	**PFHxS**
Age 5 years			
PFOS	0.77	0.06	0.43
PFOA	0.34	0.61	0.39
PFHxS	0.40	0.27	0.85
Age 7 years			
PFOS	--		
PFOA	0.29	--	
PFHxS	0.50	0.34	--

OEHHA evaluated several other potential biases but did not find clear evidence that these were likely to be responsible for the PFAS-antibody response associations identified. For example, selection bias is a possibility, and a number of studies did not report accurate or thorough data on refusal or participation rates. Although not explicitly stated, most studies appear to have involved convenience samples. In some instances, the use of convenience sampling might lead to concerns about selection bias. However, in the studies OEHHA reviewed, participants did not appear to have been selected in a manner that would have been associated with both their PFAS levels and their immune function. As such, there is no obvious reason why convenience sampling would have introduced major bias.

Inaccuracies in the methods used to assess exposure and outcome can also introduce bias. Almost all of the studies OEHHA reviewed assessed exposure using serum levels of PFOA or PFOS, which is generally a valid and widely accepted method for assessing PFAS exposures (NTP, 2016). Very few of the studies mentioned whether or not research personnel were blinded when assessing exposure or outcome. However, although not explicitly stated, there is some indication that the laboratory personnel measuring the PFAS levels in the Faroe Islands studies were blinded to the outcome status of the participants (e.g., mention is made of “coded” laboratory samples, and PFAS and antibody levels were measured in different labs) (Grandjean et al., 2012; Grandjean et al., 2017b). In general, all of the studies OEHHA reviewed appeared to have assessed immune outcomes using the same methods in all participants, regardless of their PFAS exposure levels. This suggests that most bias that may have resulted from inaccuracies in outcome assessment was likely non-differential and therefore likely to have biased results towards the null, not towards the associations seen.

A number of the studies OEHHA reviewed used cross-sectional designs. Evidence for reverse causality has been seen in studies of PFOA or PFOS and outcomes such as renal function or age of menopause (Dhingra et al., 2017). Reverse causality might also be a source of error in cross-sectional studies of immune responses if the presence of an adverse immune outcome may lead people to use certain products or have other lifestyle changes that increase their PFAS exposure. While this may be possible, OEHHA was unable to find evidence to support it, at least to an extent that it would have caused major bias. Importantly, associations between PFOA or PFOS and decreased vaccine response have also been seen in several prospective analyses (Table 5.1.1), which are likely to be less susceptible to this bias.

Many of the results OEHHA reviewed involved only a single serum measurement of PFOA or PFOS. This could potentially lead to bias from exposure misclassification if the latency between exposure and the outcome is long or if the serum levels of these agents fluctuate significantly over time. However, the half-lives of PFOS and PFOA in humans appears to be long. In a study in retired fluorochemical production workers followed for up to 5 years, the half-lives of serum PFOA and PFOS were 3.5 and 4.8 years, respectively (Olsen et al., 2007). In addition, although serum PFOA and PFOS concentrations appear to be declining over time in most people, *relative* levels seem to remain fairly stable. For example, in the 1997-2000 Faroe Islands cohort, correlation coefficients between levels measured at age 5 and levels measured at age 7 were 0.61 and 0.77 for PFOA and PFOS, respectively (Table 5.1.7). Overall, the long half-lives of PFOA and PFOS and the fact that relative levels seem to remain stable over time suggests that a single measurement of PFOA or PFOS can be a good long-term marker of exposure in many people.

In addition to the human evidence, OEHHA also found consistent evidence that PFOA and PFOS are associated with suppressed antibody response in experimental animals. NTP (2016) identified six animal studies (including the DeWitt et al. (2016) study described in Table 5.1.5) reporting immunosuppression in mice following exposure to PFOA, determined by decreases in antigen-specific IgM and/or IgG antibody production in response to a challenge with T-cell specific antigens. Similarly, NTP identified seven studies in mice reporting suppressed antibody responses following exposure to PFOS. In OEHHA's review of animal immunotoxicity studies published from 2016 onward, one study reported decreased sheep red blood cell (SRBC)-specific and dinitrophenyl-ficoll (DNP)-specific IgM antibody responses in mice exposed to PFOA (DeWitt et al., 2016). OEHHA did not identify any experimental animal studies of PFOS and effects on antibody responses published from 2016 onward. Additionally, OEHHA identified one mouse study of PFOA and one mouse study of PFOS, which reported increases in IgE levels following multiple exposures to 150 mg/kg of the respective chemical (Lee et al., 2017; Lee et al., 2018). In these studies, IgE levels were increased in animals sensitized with ovalbumin, and in non-sensitized animals compared to their respective controls.

NTP (2016) noted that there was evidence from experimental animal studies indicating PFOA and PFOS affect multiple aspects of the immune system in addition to the suppression of antibody response. The recent animal immunotoxicity studies of PFOA identified by OEHHA primarily reported changes in spleen and thymus weight. NTP (2020) also reported lymphoid follicle atrophy in male rats, and pigment in the spleen in female rats. Similar to PFOA, the recent animal immunotoxicity studies of PFOS reported changes in spleen and thymus weight, in addition to changes in thymus lymphocyte populations and cytokine levels. NTP (2016) considered these endpoints as secondary outcomes, because these endpoints are "less indicative of overall immunotoxicity." Nonetheless, NTP (2016) reported that reductions in murine spleen and/or thymus weight at moderate to high doses (>10 mg/kg-day), and reductions in murine thymus cellularity at high doses (>20 mg/kg-day) were consistently observed across multiple studies for both PFOA and PFOS. For changes in cytokine levels, NTP noted the inherent difficulty in determining whether clear and consistent patterns exist, due to differences in study design and cells/tissues examined. Additionally, Zhong et al. (2016) reported that gestational exposure to PFOS induced decreased NK cell activity in pups, supporting previous findings reported in NTP (2016).

In summary, based on this review of the recent literature and the past evidence reviewed by NTP (2016), OEHHA identified consistent evidence in the human epidemiologic literature and from experimental animal studies that PFOA and PFOS are associated with decreased antibody

responses (Table 5.1.8). OEHHA's evaluations suggest that the associations observed in the human epidemiologic studies are not due to confounding, exposure or outcome misclassification, selection bias, or reverse causation. Positive findings were also seen in a number of studies involving other immune outcomes such as asthma, rhinitis, or lower respiratory tract infections, especially for PFOA, and these, along with the experimental animal data, provide further support that PFOA and PFOS are immunotoxic. Importantly, several of these associations have involved exposure levels that are close to those seen in the general US population.

Table 5.1.8. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and immunotoxicity

Outcome	PFOA	PFOS
Vaccine response in humans	Mostly consistent evidence for decreased vaccine response	Mostly consistent evidence for decreased vaccine response
Other immune-related outcomes in humans	-Some evidence for increased **asthma** and related outcomes but based mostly on cross-sectional data and not consistent across all studies -Some evidence for increases in **respiratory infections** in children but not consistent across all studies -Weak, mostly inconsistent, or no evidence for links to other outcomes	-Some evidence for increased **asthma** and related outcomes but based mostly on cross-sectional data and not consistent across all studies -Some evidence for increases in **respiratory infections** in children but not consistent across all studies -No associations or only weak evidence identified for other outcomes
Immunosuppression in animals	Consistent evidence for decreased antibody response	Consistent evidence for decreased antibody response
Effects on the spleen and/or thymus in animals	Consistent evidence for decreased spleen and/or thymus weight in mice	Consistent evidence for decreased spleen and/or thymus weight in mice
Other immune-related effects in animals	-Consistent evidence for decreased thymic cellularity -Few studies, consistent evidence for changes in NK cell activity	-Consistent evidence for decreased thymic cellularity -Few studies, consistent evidence for changes in NK cell activity

5.2. Liver Toxicity

US EPA has reviewed the human epidemiologic literature, published up to December 2015, on PFOA or PFOS and liver toxicity (US EPA, 2016b; US EPA, 2016d). In their reviews, US EPA identified a number of epidemiologic studies that investigated associations between PFOA and serum levels of liver enzymes such as alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT). All of these studies were primarily in adults. Although findings were not consistent across all studies, several reported statistically significant associations between increasing serum levels of PFOA and increasing blood levels of these enzymes. These findings were seen in both high exposure settings (e.g., occupational cohorts) and general population studies, and were seen both in studies using cross-sectional and prospective designs. Several of these studies controlled for factors that could potentially confound results, including BMI, alcohol intake, smoking, medication use, socioeconomic status,

and medical history. In addition, several studies involved scenarios where PFOA was the predominant PFAS to which participants were exposed (US EPA, 2016b; US EPA, 2016d). Based on this evidence, US EPA concluded, "Overall, an association of serum PFOA concentration with elevations in serum levels of ALT and GGT has been consistently observed in occupational, highly exposed residential communities, and the U.S. general population. The associations are not large in magnitude, but indicate the potential to affect liver function" (US EPA, 2016b).

US EPA (2016b) identified one epidemiologic study of PFOA and liver disease (e.g., hepatitis, cirrhosis, fatty liver disease). In this study, an OR of 2.02 (95% CI, 0.50-8.10) was reported for non-hepatitis liver disease among the most highly exposed workers at a chemical plant that used PFOA, although the number of cases was small (35 total cases) (Steenland et al., 2015). Findings for hepatitis were not reported.

For animal toxicity, a thorough examination of the literature of PFOA was previously conducted by other agencies (US EPA, 2016a; US EPA, 2016b; New Jersey DWQI, 2017; ATSDR, 2018a). US EPA (2016b) identified several hepatotoxic endpoints in animal studies that were commonly reported in the scientific literature, namely increases in serum levels of liver enzymes (e.g., ALT and/or AST), increased relative liver weight, hepatocellular hypertrophy, and hepatocellular necrosis.

For PFOS, US EPA identified two epidemiologic studies that investigated associations with liver toxicity. The first was a cross-sectional study of serum PFOS and liver enzymes in 2,216 adults over age 20 from the 1999–2000 and 2003–2004 US NHANES (Lin et al., 2010). In linear regression models adjusted for age, gender, race/ethnicity, smoking, drinking status, education, BMI, metabolic syndrome, iron saturation status, and insulin resistance, a borderline statistically significant positive association was found between serum PFOS concentrations and serum ALT (linear regression coefficient (β) = 1.01; p=0.066). Clear associations with GGT and total bilirubin were not seen. The second study was a cross-sectional analysis of serum PFOS and liver enzymes from the C8 Health Study. This study involved 47,092 residents of the Mid-Ohio Valley, West Virginia, who lived near a chemical plant known to have emitted PFOA into the surrounding environment (Gallo et al., 2012). In linear regression models adjusted for age, physical activity, BMI, average household income, education, race, alcohol consumption, and smoking, increasing serum concentrations of PFOS were associated with increasing serum levels of ALT (regression coefficient between lognormal (ln) PFOS and ln ALT = 0.020 (95% CI, 0.014-0.026). A positive association was also seen with direct bilirubin (β = 0.029 (95% CI, 0.024-0.034)). Clear associations with GGT were not seen. US EPA did not identify any studies of PFOS and liver diseases like fatty liver disease, cirrhosis or hepatitis. Overall, US EPA concluded that, "The epidemiological data supporting liver damage based on serum ALT and GGT as reported by Gallo et al. (2012) are not strong enough to support an association of serum PFOS alone with liver damage in humans, because in most of the epidemiology studies the serum contains a mixture of PFAS and possibly other exogenous chemicals" (US EPA, 2016d).

PFOS exposure has been consistently shown to induce liver toxicity in experimental animals. A thorough examination of this literature was previously conducted by other agencies (US EPA, 2016c; US EPA, 2016d; New Jersey DWQI, 2018). In general, increases in absolute and/or relative liver weight, increased liver histopathological effects (e.g., hypertrophy, necrosis, etc.), and increased biomarkers of liver damage were reported.

5.2.1. Recent Human Evidence

OEHHA identified nine studies of PFOA or PFOS and liver toxicity published since December 2015 or that were otherwise not included in the most recent US EPA (2016b) and US EPA (2016d) reviews (summarized in Appendix 7, Tables A7.5 and A7.6). In general, for PFOA, the more recent studies OEHHA identified support the conclusions of US EPA (2016b) and US EPA (2016d). All of the studies that examined PFOA and liver enzymes in adults published since the US EPA review reported statistically significant associations between increasing serum PFOA concentrations and increasing blood levels of ALT, AST, and/or GGT. This includes two prospective studies (Darrow et al., 2016; Salihovic et al., 2018). Both of these studies adjusted or otherwise controlled for a number of potential confounding factors including cholesterol levels, BMI, medication use, socioeconomic status, alcohol, or smoking.

Three of the more recent studies OEHHA identified were done in children, with only one reporting statistically significant associations between PFOA and increased liver enzymes. The positive study (Attanasio, 2019) differed from the two others in that it involved somewhat older children (ages 12-19 versus ages 6-11 (Mora et al., 2018) and ages 8-12 (Khalil et al., 2018)). In this study, associations were seen between increasing serum PFOA concentrations and ALT, AST, and GGT in girls but not in boys, and fairly large unexplained changes in results were seen after statistical adjustments. Overall, the reason why fairly consistent findings have been seen in adults while results in children are mixed is not known.

With regards to PFOS, the prospective cohort study by Salihovic et al. (2018) in 1,002 elderly adults in Sweden reported an association between increasing serum levels of PFOS and increasing ALT ($p < 0.001$). The other three recent studies OEHHA identified that examined PFOS and liver toxicity presented mixed results (Gleason et al., 2015; Jain and Ducatman, 2018b; Nian et al., 2019). Overall, two of the three negative studies were done in children. The other was based on 3,573 adults in the 2011-14 NHANES (Jain and Ducatman, 2018b). OEHHA could not identify major differences in study quality that might explain these inconsistent results.

5.2.2. Recent Animal Evidence

OEHHA's review of recent animal studies, published from 2016 onward, is summarized in Table 5.2.1. PFOA exposure has consistently been shown to induce liver toxicity in experimental animals. In general, increases in absolute and/or relative liver weight, increased liver histopathology, and increased biomarkers of liver damage were observed.

Table 5.2.1. Summary of recent animal toxicity studies of PFOA reporting effects on the liver

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male CD-1 mice (3-5/dose) Beggs et al. (2016)	0 or 3 mg/kg-day via gavage for 7 days	NA	↑ relative liver weight	NA[a]
Male Kunming mice (4/dose) Liu et al. (2016)	0 or 10 mg/kg-day via gavage for 14 days	NA	↑ serum ALT, AST, ALP, and LDH; vacuolar degeneration; necrosis; inflammatory cell infiltration; ↑ oxidative stress (↑ TNF-α and IL-6, ↑ MDA and H_2O_2, ↓ SOD and CAT activity); ↑ caspase-3 activity (apoptosis)	NA[a]
Male and female C57BL/6 mice (6/sex/dose) Rebholz et al. (2016)	0 or 3.5 mg/kg of feed (fat- and cholesterol-containing diet) (~0.55 mg/kg BW-day, according to authors) for six weeks	**Males:** 0.002 or 26.9 **Females:** 0.028 or 44.3 at six weeks	**Both sexes:** ↑ relative liver weight	NA[a]
Male and female BALB/c mice (6/sex/dose) Rebholz et al. (2016)	0 or 3.5 mg/kg of feed (fat- and cholesterol-containing diet) (~0.55 mg/kg BW-day, according to authors) for six weeks	**Males:** 0.005 or 28.2 **Females:** 0.086 or 35.6 at six weeks	**Both sexes:** ↑ relative liver weight	NA[a]

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Pregnant C57BL/6J mice (mated with FVB male mice) to produce hybrid offspring (6 for dams, 6-10/sex/dose for pups) van Esterik et al. (2016)	Dietary exposure to 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, and 3 mg/kg-day (targeted dose). Exposure started 2 weeks before mating and continued during mating (1 week), gestation (3 weeks), and lactation (3 weeks)	NA	**Both sexes:** nuclear dysmorphology (p=0.06) **Male pups:** ↑ absolute and relative liver weight; ↑ eosinophilic liver foci (p=0.07)	LOAEL: 0.003 mg/kg-day
Male BALB/c mice (5/dose) Yu et al. (2016)	0, 0.5, or 2.5 mg/kg-day via "oral infusion" for 28 days	0.011, 29.34, or 114.3 at 28 days	↑ absolute and relative liver weight; altered glucose metabolism	NOAEL: 0.5 mg/kg-day for ↑ liver weight
Male Sprague Dawley rats (5/dose) Cavallini et al. (2017)	Single dose of 0 or 150 mg/kg intragastrically, sacrifice after 48 or 96 hours	NA	↑ relative liver weight	NA[a]
Male SV129 WT and PPARα KO mice (4/dose) Das et al. (2017)	0 or 10 mg/kg-day via gavage for 7 days	NA	**WT and KO:** ↑ absolute and relative liver weight; hepatocellular hypertrophy; decreased DNA content	NA[a]
Male BALB/c mice (minimum of 8/dose)	0, 1, or 5 mg/kg-day orally[b] for 7 days	NA	↑ absolute liver weight; hepatocyte cytoplasmic vacuolization; ↑ serum ALT	LOAEL: 1 mg/kg-day for ↑ serum ALT levels

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Hui et al. (2017)				
Male Wistar rats (5-12/dose) Kawabata et al. (2017)	Single oral dose of 0 or 50 mg/kg via gavage, sacrifice after 9 days	33.3 at 9 days after exposure. Control level not determined	↑ absolute and relative liver weight	NA[a]
Male and female BALB/c mice (30/sex/dose) Li et al. (2017b)	0, 0.05, 0.5, or 2.5 mg/kg-day via gavage for 28 days	**Males:** 0, 1.2, 5.9 or 13.4 **Females:** 0, 0.97, 2.7 or 9.5 at 28 days	↑ absolute liver weight; hepatocellular hypertrophy and apoptosis; mitochondrial morphology changes; changes in mitochondrial membrane potential; oxidative DNA damage (ROS generation)	LOAEL: 0.05 mg/kg-day for hepatic mito-chondrial membrane potential changes, apoptosis, oxidative DNA damage
Male Kunming mice (number not specified) Wu et al. (2017)	Single oral dose of 0 or 5 mg/kg via gavage	NA	↑ hepatic cytoplasmic vesicles; ↑ inflammatory cells around the hepatic portal area	NA[a]
Male BALB/c mice (3/dose) Yan et al. (2017)	0, 0.08, 0.31, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	NA	hepatocyte swelling	Not provided[c]
Male BALB/c mice (20/dose) Zheng et al. (2017)	0 or 1.25 mg/kg-day orally[b] (for 28 days	0.04 or 55.5 at 28 days	↑ relative liver weight; altered glucose metabolism	NA[a]
Pregnant Kunming mice (8/dose) Qin et al. (2018)	0 or 5 mg/kg-day intragastricall y throughout gestation	NA	↑ ALT and AST in F1 pup serum on PND 21 (although the changes were not statistically significant)	NA[a]

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male Kunming mice (8/dose) Wu et al. (2018)	0, 1, or 5 mg/kg-day intragastrically for 21 days	NA	↑ absolute and relative liver weight; ↑ serum ALT and AST; ↑ hepatic vacuoles	NOAEL: 1 mg/kg-day for ↑ liver enzymes
Male C57BL/6 mice (6-8/dose) Crebelli et al. (2019)	0, 0.55, 5.5 and 28 mg/L in drinking water for 5 weeks (corresponding to 0, 0.1, 1, and 5 mg/kg-day)	0.0014, 3.094, 23.971, or 83.703 at 28 days	↑ absolute liver weight; cytoplasmic vacuolization; hepatocyte hypertrophy; irregular architecture of parenchyma; necrosis; ↑ serum ALT and AST	NOAEL: 1 mg/kg-day for liver histo-pathology, ↑ ALT and AST, and necrosis
Male miR-34A -/- KO and WT C57BL/6J mice[d] (15-18/dose) Cui et al. (2019)	0 or 5 mg/kg-day via gavage for 28 days	NA	**WT and KO:** ↑ relative liver weight; ↑ serum ALT, AST, and cholinesterase; ↓ total bile acid; swollen hepatocytes	NA[a]
Male BALB/c mice (12/dose) Guo et al. (2019)	0, 0.4, 2, or 10 mg/kg-day via gavage for 28 days	0.02, 13, 64, or 88	↑ absolute and relative liver weight; ↑ serum ALT and AST; hepatocellular hypertrophy; karyolysis; ↑ albumin; ↑ serum ammonia; ↓ serum BUN	LOAEL: 0.4 mg/kg-day based on ↑ liver weight, hepatocellular hypertrophy, and karyolysis
Pregnant Kunming mice (10/dose), female pups (5-10/dose) Li et al. (2019c)	0, 1, 2.5, 5, or 10 mg/kg-day via gavage from GD 1-17	NA	**Pups:** ↑ absolute and relative liver weight; swollen hepatocytes; vacuolar degeneration; dissolved nuclei; blurred liver architecture; ↑ serum ALT and AST; ↑ CAT, SOD, and 8-OHdG; ↓ histone acetylation	LOAEL: 1 mg/kg-day for ↑ serum ALT and AST

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male C57BL/6 mice (5/diet/treat-ment/time point) Li et al. (2019d)	0 or 1 mg/kg-day (in distilled water, presumably gavage) for 2, 8, or 16 weeks. Half of the animals were on low fat diet, other half on high fat diet	NA	↑ absolute and relative liver weight; hepatocellular hypertrophy and hyperplasia; hepatic lobular inflammation; ↓ in ALT; fibrosis induced by the high-fat diet	NA[a]
Male and female Sprague Dawley rats (10/sex/dose) NTP (2019a)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day (for males) and 0, 6.25, 12.5, 25, 50, or 100 mg/g-day (for females) via gavage for 28 days	**Males:** BD, 0.378, 0.503, 1.297, 3.34, or 10.9 **Females:** BD, 0.129, 0.292, 0.475, 1.67, or 6.71 at 28 days	hepatocyte hypertrophy; hepatocyte cytoplasmic alteration; ↑ absolute and relative liver weight; ↑ serum ALT and ALP **Males only:** ↑ serum AST and bilirubin	LOAEL: 0.625 mg/kg-day for hepatocyte cytoplasmic alteration and ↑ liver weight in males
Male APOE*3-Leiden.CETP transgenic mice[e] (8/dose) Pouwer et al. (2019)	0, 0.01, 0.291, or 30.2 mg/kg-day in diet for 6 weeks	0.005, 0.065, 1.524 or 144 at 6 weeks	↑ absolute and relative liver weight; ↑ ALT	NOAEL: 0.291 mg/kg-day for ↑ liver weight and ↑ ALT
Male APOE*3-Leiden.CETP transgenic mice (8/dose) Pouwer et al. (2019)	0, 0.01, 0.298, or 29.5 mg/kg-day in diet for 4 weeks	0, 0.051, 1.395, or 93.713 at 4 weeks	↑ absolute and relative liver weight; ↑ serum ALT; hepatic hypertrophy	NOAEL: 0.298 mg/kg-day for ↑ liver weight, ↑ ALT, hypertrophy and steatosis

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Pregnant Kunming mice (10/dose) Song et al. (2019)	0 or 20 mg/kg-day via gavage from GD 1-7	NA	**Dams:** ↑ relative liver weight; increased oxidative stress (↑ MDA, ↓ SOD and GSH-Px)	NA[a]
Male C57BL/6NCrl mice (4-6/dose) Wen et al. (2019c)	0, 1, or 3 mg/kg-day via gavage for 7 days	NA	↑ absolute liver weight; ↑ carboxylesterase protein levels and activity	LOAEL: 1 mg/kg-day for ↑ liver weight
Male C57BL/6NTac WT and PPARα KO mice (4-6/dose) Wen et al. (2019c)	0 or 3 mg/kg-day via gavage for 7 days	NA	**WT and KO:** ↑ absolute liver weight (effect more pronounced in WT animals); ↑ carboxylesterase protein levels and activity	NA[a]
Pregnant CD-1 mice (11-13 dams/dose) Blake et al. (2020)	0, 1, or 5 mg/kg-day via gavage from ED 1.5 to ED 11.5 or ED 17.5	ED11.5: BD, 25.4 or 117.3 ED17.5: 0.211, 18.7 or 95.1	**Dams:** ↑ absolute and relative liver weight; hepatic effects (cytoplasmic alteration, hypertrophy, ↓ glycogen, eosinophilic granular cytoplasm, ↓ hepatic mitosis, ↑ apoptosis, necrosis, abnormal ultrastructure, ↓ rough ER); ↓ albumin and total protein levels; ↑ serum AST and SDH **Pups:** no reported liver effects	LOAEL: 1 mg/kg-day for ↑ liver weight and liver effects
Female Sprague Dawley rats (10/dose) NTP (2020)	0, 300, or 1,000 ppm in feed (0, 29.6, or 98.6 mg/kg-day) for 16 weeks	BD, 20.4, or 72.3 at 16 weeks	↑ absolute and relative liver weight; hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; ↑ serum ALT and ALP	NOAEL: 300 ppm (29.6 mg/kg-day) for all liver endpoints
Male Sprague	0, 150, or 300 ppm in feed	BD, 193, or 243 at 16 weeks	↑ relative liver weight; liver necrosis; liver	LOAEL: 150 ppm

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Dawley rats (10/dose) NTP (2020)	(0, 15.6, or 31.7 mg/kg-day) for 16 weeks		pigment; hepatocyte hypertrophy; hepatocyte cytoplasmic alteration; hepatocyte single cell death; ↑ serum ALT and ALP; ↑ bile salts	(15.6 mg/kg-day) for all liver endpoints
Male Sprague Dawley rats (10/dose) NTP (2020)	0, 20, 40, or 80 ppm in feed (0, 1.9, 4.0, or 7.9 mg/kg-day) for 16 weeks	BD, 81.4, 130.8, or 159.6 at 16 weeks	↑ absolute and relative liver weight; liver necrosis; liver pigment; hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; hepatocyte single cell death; ↑ serum ALT and ALP	LOAEL: 20 ppm (1.9 mg/kg-day) for multiple liver endpoints
Female Sprague Dawley rats (50/dose) NTP (2020)	0, 300, or 1,000 ppm in feed (0, 18, or 63 mg/kg-day) for 107 weeks	BD, 20.4, or 72.3 at 16 weeks	liver necrosis; liver pigment; bile duct hyperplasia; hepatocyte cytoplasmic alteration; hepatocyte hypertrophy; hepatocyte single cell death; hepatocyte ↑ mitoses	LOAEL: 300 ppm (18 mg/kg-day) for hepatocyte cytoplasmic alteration and hepatocyte hypertrophy
Male Sprague Dawley rats (50/dose) NTP (2020)	0, 20, 40, or 80 ppm in feed (0, 1, 2.2, or 4.5 mg/kg-day) for 107 weeks	BD, 81.4, 130.8, or 159.6 at 16 weeks	liver cystic degeneration; liver eosinophilic and mixed cell focus; liver focal inflammation; liver necrosis; liver pigment; hepatocyte hypertrophy; hepatocyte cytoplasmic alteration; hepatocyte single cell death	LOAEL: 20 ppm (1 mg/kg-day) for liver necrosis, hepatocyte hypertrophy, hepatocyte cytoplasmic alteration

[a] LOAEL/NOAEL not applicable for single dose studies.
[b] The specific manner of oral administration was not stated in study.
[c] Histology data are presented in the supplementary materials, but specific doses at which hepatocyte swelling and lipid deposits become significant are not provided.
[d] miR-34A -/- KO mice do not produce the microRNA miR-34A. miR-34A functions as a tumor suppressor.
[e] APOE*3-Leiden.CETP transgenic mice are reported to have human-like lipoprotein metabolism.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BD, below limit of detection; BUN, blood urea nitrogen; CAT, catalase; ED, embryonic day; ER, endoplasmic reticulum; GD, gestation day; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein; H_2O_2, hydrogen peroxide; IL-6, interleukin 6; KO, knockout; LDH, lactate dehydrogenase; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; PND, postnatal day; PPARα, peroxisome proliferator-activated receptor alpha; SDH, sorbitol dehydrogenase; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha; WT, wild-type

Well-conducted studies demonstrating the lowest NOAELs/LOAELs are described here. Recently, NTP released toxicity data from subacute and chronic bioassays conducted in male and female Sprague Dawley rats. An additional cohort of animals was exposed to PFOA during gestation and lactation (perinatal exposure; 150 or 300 ppm PFOA in feed given to dams). An initial chronic study in male rats with concentrations of 0, 150, or 300 ppm in feed (0, 14.7, or 29.5 mg/kg-day) was ended at 21 weeks due to overt toxicity; however, it appears a subset of animals receiving these doses were examined at 16 weeks. The study was subsequently repeated with lower doses. Liver toxicity was observed in all of the studies, regardless of sex or duration. Common liver effects included increased relative liver weight, increased serum ALT and alkaline phosphatase (ALP), necrosis, increased liver pigment, hepatocyte cytoplasmic alteration and hypertrophy, and hepatocyte single cell death (NTP, 2019a; NTP, 2020). OEHHA identified lowest-observed-adverse-effect levels (LOAELs) of 0.625 mg/kg-day and 1 mg/kg-day for the 28-day and 107-week studies in male rats, respectively, for liver toxicity. This corresponds to plasma concentrations of 0.0507 and 0.0814 milligrams per milliliter (mg/ml), respectively (NTP, 2020). Plasma/serum concentration is the most appropriate dose metric for extrapolating toxicity data from rodent studies to humans because of the large difference in the chemical's biological half-life between rodents (1-3 weeks) and humans (2-3 years). This accounts for the accumulation of PFOA in humans due to the chemical's long half-life. Plasma concentration in the chronic male rat study was determined at 16 weeks, but because the plasma/serum half-life of PFOA is estimated to be 4-6 days in male rats (Lau et al., 2006; New Jersey DWQI, 2017), it is anticipated that by 16 weeks, a steady-state concentration would have been reached. Thus, the plasma concentration would remain relatively stable over the 107-week period of continuous dosing.

Li et al. (2017b) reported decreased body weight, increased absolute and relative liver weight, hepatocellular hypertrophy and apoptosis, lipid accumulation in hepatocyte cytoplasm, and other biomarkers of hepatotoxicity. Quantitative data for several endpoints and PFOA serum concentrations were presented as graphs. These graphical data were quantified using GetData Graph Digitizer software (version 2.26), and are presented in Table 5.2.2. Female mice were more sensitive to apoptosis than male mice. The administered dose of 0.05 mg/kg-day corresponds to a serum concentration of 0.97 microgram per milliliter (µg/ml) for females and 1.2 µg/ml for males, which was measured at the end of the exposure period. OEHHA identified a LOAEL of 0.05 mg/kg-day (serum concentration of 0.97 µg/ml) for changes in mitochondrial membrane potential (indicative of mitochondrial dysfunction), increases in biomarkers of apoptosis (caspase-9 and p53), and increased oxidative DNA damage (Li et al., 2017b).

Table 5.2.2. Dose metrics and endpoints in female BALB/c mice from Li et al. (2017b)

Administered dose (mg/kg-day)	Reported serum concentration (µg/ml)	Cells with mitochondrial membrane potential changes[a] (%)	Caspase-9 levels[a] (iU/g)	p53 levels[a] (iU/g)	8-OHdG[a] (ng/g)
0	0	1.2 ± 0.5	71.3 ± 4.2	28.9 ± 3.5	22.9 ± 7.3
0.05	0.97	12.3 ± 1.2**	130.2 ± 9.0**	46.8 ± 5.1**	68.6 ± 6.2**
0.5	2.7	17.6 ± 1.1**	157.9 ± 3.5**	58.3 ± 4.5**	87.9 ± 9.3**
2.5	9.5	39.3 ± 14.6**	220.9 ± 1.1**	69.0 ± 3.2**	96.8 ± 2.6**

[a] Mean ± standard deviation
8-OHdG, 8-hydroxydeoxyguanosine; iU/g, international units/gram

**p <0.01, statistical analysis (t-test) by OEHHA

van Esterik et al. (2016) reported non-statistically significant increases in liver histopathology (eosinophilic liver foci, nuclear dysmorphology, and lipid accumulation) in C57BL/6JxFVB mice exposed in utero and during lactation to PFOA. The authors also reported an increase in absolute liver weight in male F1 mice at 26 weeks of age. OEHHA obtained the individual animal liver weight data from the authors, and evaluated the absolute and relative liver weights for statistical and biological significance.

A thorough statistical analysis showed a significant increase in absolute and relative liver weight in male F1 mice at 26 weeks of age at all doses relative to control. Relative liver weights were obtained for F1 mice (sex unspecified), comprising 8 dose groups with approximately 10 animals from 2-5 litters in each group. Anova type II test with unbalanced design demonstrated statistical differences among groups (p=0.03) and lack of effect from specific litters. Levene's test for homogeneity of variance was not significant. However, normality of residuals was violated, as visually demonstrated by the lack of linearity in the normal Q-Q plot and significance in the Shapiro-Wilk normality test (p=0.0057). Thereafter, OEHHA applied a Kruskal-Wallis test (non-parametric analogue of Anova), which was significant (p=0.0047), indicating difference(s) among dose groups. Parametric and non-parametric t-tests demonstrated significant increases in all dose groups relative to the control group. Finally, rank-based trend tests (Spearman's rank correlation, Kendall's rank correlation) either for the full dataset or with exclusion of the control group were significant, indicating a positive trend in the dose-response data either in the presence ($p=2\times10^{-5}$ for both the Spearman's and Kendall's tests) or absence (p=0.007 and p=0.008, respectively) of the control group. Overall, the statistical analysis supports a dose-dependent increase for the relative liver weights in this experiment.

The relative liver weight values in the control group appear to be low in comparison to historical controls from the same research group and other sources, which may explain the observed increases in all treatment groups. OEHHA calculated a mean relative liver weight of 3.04% for control F1 animals, whereas mean relative liver weights following treatment ranged from 3.23-3.49% at 26 weeks (van Esterik et al., 2016). In a separate study from the same laboratory (van Esterik et al., 2014), the mean relative liver weight from control male mice was approximately 4.69% at 23 weeks. Additionally, reports from Jackson Laboratories state that the relative liver weight of the parent strain (male C57BL/6J mice) at 26 weeks is 4.30 ± 0.47%. This suggests that the control animals in (van Esterik et al., 2016) had unusually low liver weights that are not comparable to available historical values. However, Spearman's rank correlation and Kendall's rank correlation tests without the control group still showed a statistically significant trend (p <0.01 for both tests), indicating that the low control values were not solely responsible for the observed statistical significance.

Increased F1 liver weight appears to be a significant effect of PFOA exposure. However, there are several concerns that lower the overall confidence in this study. First, no details about the liver weight data are included in the publication. Second, the control liver weight values are below historical controls. Third, the authors reported that animals were fasted for 18 hours prior to sacrifice, which can lead to reductions in liver weight (Jensen et al., 2013).

Much like for PFOA, several animal studies published from 2016 onward reported various hepatotoxic endpoints following oral exposure to PFOS. Study details are summarized in Table 5.2.3.

Table 5.2.3. Summary of recent animal toxicity studies of PFOS reporting liver effects

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male CD-1 mice (3-5/dose) Beggs et al. (2016)	0 or 10 mg/kg-day via gavage for 7 days	NA	↑ relative liver weight	NA[a]
Pregnant mice (strain not specified) (3-5/dose) Mehri et al. (2016)	0, 1, 10, or 20 mg/kg-day orally[b] from GD 1-14	NA	fetal liver enlargement	doses that caused effect were not specified
Male Sprague Dawley rats (7/dose) Wan et al. (2016)	0, 1, or 10 mg/kg-day orally[b] for 4 weeks	NA	↑ absolute and relative liver weight; hepatocellular hypertrophy; cytoplasmic vacuolization; ↑ serum ALT and AST; inflammatory cellular infiltration; ↑ biomarkers of apoptosis	LOAEL: 1 mg/kg-day for ↑ serum ALT
Male C57BL/6 mice (10/dose) Xing et al. (2016)	0, 2.5, 5, or 10 mg/kg-day via gavage for 30 days	0.02, 70.2, 130.6, and 201.2 at 30 days	↑ relative liver weight; ↑ serum ALT, AST, ALP, and GGT; hepatocyte vacuolization and necrosis; hepatocyte hypertrophy; ↑ oxidative stress (↓ SOD, CAT, and GSH-Px); ↑ apoptosis	LOAEL: 2.5 mg/kg-day for ↑ relative liver weight and liver enzymes, oxidative stress, and apoptosis
Male C57BL/6 mice (5-6/dose) Zhang et al. (2016c)	0, 30, 60, or 120 mg/kg of feed (equivalent to 0, 3.48, 6.96, or 13.92 mg/kg BW-day)[c] for 21 or 23 days	0, 94.6, 176, and 392 at 21 days	↑ relative liver weight; ↑ ALT and bile acids; hepatocyte vacuolization and necrosis; ↑ MDA	LOAEL: 3.48 mg/kg-day for ↑ liver weight

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Pregnant C57BL/6 mice (12/dose) Zhong et al. (2016)	0, 0.1, 1, or 5 via gavage from GD 1-17	NA	**Pups:** ↑ absolute liver weight at 4 weeks (but not significant at 8 weeks)	NOAEL: 1 mg/kg-day for ↑ liver weight
Domestic cats (72 in clinics and shelters) Bost et al. (2016)	Case control study	Range: BD to 0.121 Geometric mean: 0.0089	Significant association with PFOS and liver disease in the highest quartile	NA[a]
Male and female Cynomolgus monkeys (6/sex/dose) Chang et al. (2017)	0 or 14 mg/kg via gavage on three separate occasions over 422 days; maximum PFOS serum concentrations of 165 µg/ml for females and 160.8 µg/ml for males on day 365	NA	no toxicologically significant effects reported	NOAEL: 165 µg/ml serum PFOS
Male and female Sprague Dawley rats (12/sex/dose) Bagley et al. (2017)	0 or 100 ppm in feed (equivalent to 6 mg/kg-day for males and 6.6 mg/kg-day for females)	NA	**Both sexes:** ↑ absolute and relative liver weight; mild necrosis; hepatocellular hypertrophy **Males:** cytoplasmic vacuolization	NA[a]
Male WT and ERβ KO mice (8/dose/group) Xu et al. (2017)	0 or 5 mg/kg-day via gavage for 28 days	NA	**WT:** hepatocyte degeneration and vacuolization; ↓ bile acids **KO:** effects reported in WT animals not observed	NA[a]

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male Sprague Dawley rats (6/dose) Han et al. (2018a)	0, 1, or 10 mg/kg-day via gavage for 4 weeks	NA	hepatocellular hypertrophy; cytoplasmic vacuolization; ↑ serum ALT, AST; ↑ oxidative stress and apoptosis	LOAEL: 1 mg/kg-day for ↑ liver enzymes, oxidative stress, and apoptosis
Male Sprague Dawley rats (6/dose) Han et al. (2018b)	0, 1, or 10 mg/kg-day via gavage for 4 weeks	NA	↑ absolute liver weight; hepatocyte degeneration; cytoplasmic vacuolization; ↑ serum ALT, AST	NOAEL: 1 mg/kg-day for ↑ liver enzymes
Male C57BL/6 mice (5/dose) Huck et al. (2018)	normal diet: 0 or 0.089 mg/kg-day high fat diet: 0 or 0.087 mg/kg-day for 28 days	NA	**Normal diet:** ↑ relative liver weight	NA[a]
Female CD-1 mice (≥4/dose) Lai et al. (2018)	0, 0.3, or 3 mg/kg-day via gavage for 7 weeks	0.024, 33.8, and 109.6 at 7 weeks	↑ absolute and relative liver weight; yellowish liver; altered glucose metabolism	NOAEL: 0.3 mg/kg-day for liver effects
Pregnant Kunming mice (5/dose) Liang et al. (2019)	0, 0.5 or 5 mg/kg-day intragastrically throughout gestation (20.5 days)	NA	**Dams:** ↑ absolute liver weight; cytoplasmic vacuolization; inflammatory cell infiltration and cellular deformation	NOAEL: 0.5 mg/kg-day for ↑ liver weight and histopathology in dams

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male mice (strain not specified) (4/dose) Lv et al. (2018)	0 or 10 mg/kg-day via gavage for 3 weeks	NA	↑ relative liver weight; ↑ ALT, AST and LDH; inflammatory cell infiltration; edema; cytoplasmic vacuolization; necrosis; architectural disorganization; ↑ oxidative stress (↑ MDA and H_2O_2, ↓ GSH); ↑ biomarkers of apoptosis	NA[a]
Male and female Sprague Dawley rats (10/sex/dose) NTP (2019b)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	**Males:** BD, 23.73, 51.56, 94.26, 173.7, or 318.2 **Females:** 0.0543, 30.53, 66.97, 135.1, 237.5, or 413.6 at 28 days	**Both sexes:** hepatocyte hypertrophy; ↑ absolute and relative liver weight; ↑ ALT, ALP, bile salt/acid, albumin, and direct bilirubin; **Males:** ↑ AST; ↓ globulin; hepatocyte cytoplasmic vacuolization; **Females:** hepatocyte cytoplasmic alteration; ↑ total bilirubin	LOAEL: 0.312 mg/kg-day for ↑ relative liver weight in males and females
Male ICR mice (10/dose) Su et al. (2019)	0 or 10 mg/kg-day for 21 days; administration method not explicitly stated, but presumably via gavage	NA	↑ absolute liver weight; disordered liver lobule; hepatocyte vacuolization; hydropic degeneration of hepatocytes; inflammatory cell infiltration; ↑ serum ALT and AST; ↑ hepatic TNF-α and IL-6	NA[a]

[a] LOAEL/NOAEL not applicable for single dose studies.
[b] The specific manner of oral administration was not stated in study.
[c] Calculated by OEHHA using a BW of 0.267 kg and a consumption rate of 0.023 kg/day (from US EPA, 1988).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BD, below limit of detection; BW, body weight; CAT, catalase; ERβ, estrogen receptor beta; GD, gestation day; GGT, gamma glutamyl transferase; GSH, glutathione; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein; H_2O_2, hydrogen peroxide; IL-6, interleukin-6; KO, knockout; LDH, lactate dehydrogenase; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; SOD, superoxide dismutase; TNF-α , tumor necrosis factor alpha; WT, wild-type

Zhang et al. (2016c) administered PFOS in the diet of male C57BL/6 mice. Where data were presented as graphs, values were determined using GetData Graph Digitizer, version 2.26. The authors reported increases in relative liver weight, increased ALT, liver histopathology (vacuolization and necrosis), and oxidative stress. In a parallel study, PFOS-induced hepatotoxicity was exacerbated in animals receiving a marginal methionine/choline deficient diet compared to animals on a control diet (Zhang et al., 2016c). OEHHA identified a LOAEL of 3.48 mg/kg-day (serum concentration of 94.6 µg/ml) for increased liver weight.

5.2.3. Recent Mechanistic Evidence

For PFOA, results from mechanistic studies are consistent with effects observed in the liver in animal studies, such as oxidative stress and liver injury. Increased reactive oxygen species (ROS) production resulting in cytotoxicity has been observed in mouse hepatocytes exposed to PFOA (Sun et al., 2019b; Xu et al., 2019b). Binding studies have shown that PFOA binds to the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) (Xu et al., 2018; Xu et al., 2019b). These studies also reported PFOA increased SOD activity; however, it had no effect on CAT activity. The PFOA-induced increase in SOD activity resulted in increased apoptosis in hepatocytes.

A study by Zhang et al. (2016a) showed evidence of cell proliferation of human hepatocytes at low concentrations (50-200 µM) and cytotoxicity at high concentrations (>400 µM) of PFOA. Orbach et al. (2018) reported that apoptotic cell death occurred in multi-cellular hepatic organotypic culture models (OCMs) of either primary human or rat cells exposed to PFOA. Decreased glutathione (GSH), interleukin-10 (IL-10), and mitochondrial activity were observed in both cell types. Increased apoptosis was also observed in mouse liver AML12 cells (Wu et al., 2017). Furthermore, PFOA induced autophagosome formation in HepG2 cells (Yan et al., 2017).

Gene expression studies with PFOA (both in vivo and in vitro) have found changes associated with a myriad of physiological functions, including liver necrosis, carcinogenesis, peroxisome proliferator-activated receptor (PPAR) signaling, fatty acid metabolism, regulation of cell proliferation, apoptosis regulation and immune response (Beggs et al., 2016; Liu et al., 2016; Rebholz et al., 2016; Song et al., 2016; Das et al., 2017; Hui et al., 2017; Li et al., 2017b; Zheng et al., 2017; Cui et al., 2019; Li et al., 2019c; Li et al., 2019d; Song et al., 2019; Wen et al., 2019c). Metabolomic analyses also revealed that PFOA affects multiple metabolic pathways, including amino acid, carbohydrate, and lipid metabolism (Yu et al., 2016).

PFOA can bind to nuclear receptors found in the liver, such as PPARs (α, γ and β/δ) (Dong et al., 2016a; Rose et al., 2016; Rosenmai et al., 2018; Li et al., 2019a). In HepG2 cells, PFOA can activate human pregnane X receptor (hPXR), a nuclear receptor involved in xenobiotic metabolism in the liver and intestine (Zhang et al., 2017). Furthermore, Dong et al. (2016a)

reported PPARα, constitutive androstane receptor (CAR), and PXR effects in the liver of rats exposed to PFOA orally for 28 days.

Guo et al. (2019) reported that PFOA causes changes in urea cycle protein levels in mice, which caused an increase in serum ammonia levels, leading to liver effects such as hypertrophy and karyolysis.

For PFOS, oxidative stress has been implicated in liver injury. Increases in ROS production and lipid oxidation have been measured in hepatocytes exposed to PFOS (Wan et al., 2016; Khansari et al., 2017; Selano et al., 2019). PFOS can bind to SOD and CAT, and alter their activities (Han et al., 2018a; Xu et al., 2018; Xu et al., 2019b). Rats exposed to PFOS showed increases in ROS and nitric oxide (NO), and Kupffer cell activation induced hepatocyte proliferation via the nuclear factor- κB/tumor necrosis factor-α (NF-κB/TNF-α) pathway (Han et al., 2018a; Han et al., 2018b). Choline supplementation reduced PFOS-induced hepatic oxidative stress and changes in lipid metabolism in male C57BL/6 mice (Zhang et al., 2016c), but had no impact on steatosis in Sprague Dawley rats (Bagley et al., 2017).

Liver gene expression studies with PFOS (both in vivo and in vitro) have found changes associated with a myriad of physiological functions, including liver necrosis, carcinogenesis, PPAR signaling, fatty acid metabolism, regulation of cell proliferation, apoptosis, and immune response (Beggs et al., 2016; Dong et al., 2016a; Song et al., 2016; Bagley et al., 2017; Xu et al., 2017; Han et al., 2018a; Han et al., 2018b; Huck et al., 2018; Lv et al., 2018; Liang et al., 2019). A study by Yao et al. (2016) demonstrated that PFOS induces apoptosis in HepG2 cells via mitophagy (removal of damaged mitochondria via autophagy), leading to hepatotoxicity.

PFOS has been linked to increases in blood glucose and insulin resistance in animal studies. A study by Qiu et al. (2016b) aimed to investigate the mechanism leading to insulin resistance. They found that in HepG2 cells, PFOS interfered with the phosphatidylinositol 3-kinase-serine/threonine protein kinase, specifically protein kinase B (PKB, also known as AKT), signaling pathway and increased the expression of genes related to gluconeogenesis, such as phosphoenolpyruvate carboxykinase (PEPCK). The authors concluded that the inactivation of AKT by PFOS increased gluconeogenesis and insulin resistance in the liver.

PFOS can bind to transporters such as fatty acid binding protein and the T4 serum carrier protein, transthyretin (TTR) (Cheng and Ng, 2018; Sheng et al., 2018; Selano et al., 2019), displacing endogenous ligands and disrupting essential processes in the liver. PFOS can also induce ERβ in mice (Xu et al., 2017). ERβ knockout mice did not display signs of hepatotoxicity observed in wild-type animals exposed to PFOS, such as hepatocyte degeneration and decreases in hepatic cholesterol and bile acids, indicating that ERβ may be a possible molecular target of PFOS induced liver toxicity.

PFOS can activate hPXR, a nuclear receptor involved in xenobiotic metabolism in the liver and intestine (Zhang et al., 2017). Activation of PXR has been associated with altered lipid metabolism, endocrine disruption and carcinogenesis. The authors postulate that the activation of hPXR may be one mechanism leading to toxicity observed in animals and humans exposed to PFOS, and provide a PPARα independent mechanism for liver toxicity. PPARs α, γ and β/δ are other nuclear receptors that PFOS can bind to in the liver (Rosenmai et al., 2018; Li et al., 2019a). Binding of PFOS to these receptors has been associated with effects seen in the liver, such as cell proliferation and differentiation and lipid metabolism.

Adding to the information about possible mechanisms, Xu et al. (2017) demonstrated that, ERβ (estrogen receptor beta) knockout mice did not show the hepatotoxic effects (hydropic degeneration and vacuolization of hepatocytes, decreased hepatic cholesterol and bile acid levels) that were present in wild-type (WT) mice (Xu et al., 2017).

5.2.4. Conclusions

Overall, the recent epidemiologic evidence OEHHA identified supports the conclusions reached by US EPA: the majority of studies available to date support an association between PFOA and increases in liver enzymes in adults. While similar evidence has been seen in some studies of PFOS, this evidence overall is not as clear or consistent. Associations between PFOA and increased liver enzymes were reported in a number of different study populations, across several different exposure scenarios (occupational, high environmental, and general population exposures), in studies using both cross-sectional and prospective designs, and in studies that adjusted for the common causes of liver disease such as alcohol use, BMI, and medication use. In several studies that provided both adjusted and unadjusted results, findings changed very little after adjusting for the major determinants of liver toxicity (Gallo et al., 2012; Salihovic et al., 2018). Almost all of these studies used commonly accepted methods for assessing both exposure (serum PFOA) and outcome (blood levels of liver enzymes). Although blinding of researchers was not mentioned in most publications, descriptions of the methods that were provided suggest that participants' exposure and outcome were evaluated independently in most, if not all, of these studies.

Most of the studies OEHHA reviewed assessed liver enzyme levels using only a single serum measurement. Reliance on a single measurement like this could lead to some misclassification since liver enzyme levels may fluctuate over time in some people. However, since exposure and outcome seem to have been evaluated independently of each other in these studies, this misclassification would most likely bias results to the null, not towards the positive associations reported in most studies.

None of the recent studies of PFOA and liver toxicity identified by OEHHA controlled for other PFAS. However, several of the studies identified by US EPA (2016b) that reported associations between PFOA and liver toxicity involved occupational cohorts where PFOA exposures seemed to have far outweighed those of PFOS and other PFAS. This, combined with the fact that the results of human studies of PFOS and liver toxicity have been less consistent than those of PFOA, suggests that the associations identified for PFOA were actually related to PFOA and not entirely due to correlations between PFOA and PFOS. Further information on the potential of PFOS and other PFAS to have confounded the relationships seen between PFOA and liver toxicity, specifically increases in ALT, is presented in Section 6.1.1.

As noted by US EPA, the magnitude of the associations between PFOA and increased liver enzymes appears to be fairly small. For example, in the analysis of PFOA and ALT by Gallo et al. (2012), the partial coefficient of determination (R^2) value for PFOA was only 0.2%. Relatively small effect sizes were also seen in the more recent studies OEHHA identified in this search. For example in Jain and Ducatman (2018b), a 10% increase in PFOA was associated with only a 0.68% increase in ALT, a 0.71% increase in GGT, and a 0.49% increase in AST. Based on a linear extrapolation, this represents about a 6.8% increase in ALT and a 4.9% increase in AST for a doubling of PFOA levels. These fairly small effect sizes raise concerns that they may be due to relatively small amounts of residual confounding or bias. Importantly though, the fact that associations were seen across a number of different study populations and the fact that results

changed only slightly after adjustments for potential confounders in several studies are evidence that this is not the case.

The relatively small effect sizes reported in most of the studies of PFOA and liver enzymes might also raise concerns about their clinical relevance. These effect sizes are generally lower than what might be seen with overt hepatitis or other severe liver disease. Regardless, as shown for lead and other prevalent exposures, small effect sizes like this, which might have only minor or unnoticeable effects in an otherwise healthy individual, can have very important impacts on a population basis (Miller et al., 2009), especially for very common exposures like PFOA.

In summary, the current epidemiologic literature provides consistent evidence that PFOA can cause hepatotoxicity in humans, and in particular, increases in liver enzyme levels (Table 5.2.4). Consistent evidence for overt liver diseases has not been seen. However most of these studies involve relatively small sample sizes or small numbers of cases, and as such may not have sufficient statistical power or sensitivity to detect these types of effects. Findings for PFOS and liver toxicity in human studies are overall less consistent than those seen for PFOA but a number of human studies do suggest that PFOS could be hepatotoxic in humans.

In addition to the human evidence, exposure to PFOA and PFOS has consistently been shown to induce liver toxicity in experimental animals. Toxicity responses in the liver of rats and mice exposed to PFOA were remarkably consistent across studies (Table 5.2.1). Increased absolute and/or relative liver weight were observed in nearly all of the toxicity studies that included an assessment of the liver. Increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), biomarkers of liver damage, were reported in several studies. Additionally, histopathological observations of the liver, including hypertrophy, cytoplasmic vacuolization, and oxidative stress were reported in multiple studies. Much like with PFOA, rats and mice exposed to PFOS have consistently displayed liver toxicities across studies, including increased absolute and/or relative liver weight, increased serum ALT and AST levels, histopathology including hypertrophy and vacuolization. Furthermore, the liver toxicity observed in rodent studies of PFOS is remarkably similar to toxicity observed in rodent studies of PFOA, suggesting that both chemicals are similar in their biological activities. The hepatotoxicity endpoints reported in recent animal studies (from 2016 onward) were quite consistent with data from earlier studies (prior to 2016).

Table 5.2.4. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and liver toxicity

Outcome	PFOA	PFOS
ALT in humans	Mostly consistent evidence for increases in ALT	A few findings of increases in ALT
Other liver enzymes and related biomarkers in humans	Some evidence for increases in AST, GGT and bilirubin	A few findings of increases in AST, GGT, and bilirubin but findings are mixed
Liver disease in humans	A few positive associations but small sample sizes	No data
Increased liver weight in animals	Consistent evidence	Consistent evidence
Liver histopathology in animals (hypertrophy, vacuolization, etc.)	Consistent evidence	Consistent evidence

Outcome	PFOA	PFOS
Biomarkers of liver toxicity in animals (ALT, AST, etc.)	Consistent evidence	Consistent evidence

In general, perturbations of lipid homeostasis induced by PFOA and PFOS in animals differ considerably from responses in humans. This may be due to activation of PPARα, which is known to impact lipid metabolic processes more prominently in rodents than in humans. An in-depth evaluation of lipid homeostasis perturbation by PFOA and PFOS in humans and laboratory animals is presented below.

5.3. Perturbation of Lipid Homeostasis

US EPA (2016b) concluded that the human epidemiology studies on PFOA and serum lipid levels "have generally found positive associations between serum PFOA concentration and total cholesterol (TC) in the PFOA-exposed workers and the high-exposure community (i.e., increasing lipid level with increasing PFOA); similar patterns are seen with low-density lipoproteins (LDLs) but not with high-density lipoproteins (HDLs)."

In its review, US EPA identified a number of studies showing associations between PFOA and increasing total cholesterol levels (US EPA, 2016b). This included seven studies performed in three separate occupational cohorts (3M, DuPont-Washington Works, and Italy) and five studies performed in the highly exposed community near the DuPont facility in West Virginia (e.g., the C8 Health Project studies). Evidence of a positive association between serum PFOA concentrations and increasing serum levels of total cholesterol were reported in both cross-sectional and longitudinal analyses and in analyses that adjusted or controlled for factors such as age, gender, BMI, alcohol, smoking, time of hire and the use of lipid lowering medications. Steenland et al. (2015) did not find an association between high occupational PFOA exposures and self-reported use of prescription medications for high cholesterol, but did identify associations between increasing serum concentrations of PFOA and increasing total cholesterol, LDL cholesterol, and triglyceride levels. No association was seen with HDL. Associations were also seen between serum PFOA and total cholesterol in two of the three large population based cross-sectional studies identified by US EPA. US EPA also identified a number of studies that reported associations between PFOA and LDL, HDL, and triglyceride levels, although these associations were much less consistent than those seen for total cholesterol.

The ATSDR (ATSDR, 2018a) identified several additional studies published prior to 2016 that were not included in the 2016 US EPA review. This included two population based studies in either pregnant women (N=854) (Skuladottir et al., 2015) or children (N=225) (Zeng et al., 2015) that reported associations between PFOA and total cholesterol. It also included a cross-sectional study of highly exposed workers (N=55) and nearby community residents (N=132) in China, which found an inverse association between PFOA and HDL cholesterol (Wang et al., 2012), and a lower exposure community study in the UK which reported an association between prenatal PFOA levels and increased total cholesterol and LDL in the offspring at ages 7 and 15 years, but only in analyses confined to the lowest tertile of PFOA exposure (Maisonet et al., 2015). Clear associations were not seen in the upper two tertiles.

In its 2016 review of PFOS, US EPA (2016d) concluded that, "Multiple epidemiologic studies have evaluated serum lipid status in association with PFOS concentration. These studies

provide support for an association between PFOS and small increases in total cholesterol." They also noted that, "Evidence for associations between other serum lipids and PFOS is mixed, with some studies showing an association with measurements of concurrent HDL and/or LDL and others failing to measure the serum lipoprotein complexes." In its review, US EPA identified only two higher exposure studies, both done in the 3M occupational cohort. In the first, a cross-sectional study involving approximately 500 workers, associations were identified between increasing serum PFOS concentrations and increasing cholesterol and triglycerides (Olsen et al., 2003a). In the second (published in the same paper), a longitudinal analysis involving 175 workers in the same facilities, clear associations were not seen (Olsen et al., 2003a). Serum levels of both PFOS and PFOA were high in these workers (geometric means of 0.44-0.91 µg/ml for PFOS and 0.33-1.13 µg/ml for PFOA). No community studies with high PFOS exposures were identified. US EPA noted a number of population-based studies that examined associations between PFOS and lipid levels, with the large majority finding at least some evidence of an association with increased total cholesterol, some finding associations with increased LDL, and mostly mixed or null results for HDL and triglycerides.

US EPA (2016b) also identified multiple studies in animals that report decreases in serum cholesterol and triglyceride levels in rodents following exposure to PFOA. However, US EPA noted that "decreases in triglycerides, cholesterol, and lipoprotein complexes are an expected consequence of PPARα activation in rodents," thus these changes would be expected considering that PFOA is a known PPARα activator.

US EPA (2016d) identified several studies reporting decreased cholesterol levels following PFOS administration in experimental animals (rats, mice, and monkeys). Additionally, PFOS induced differential gene expression in genes involved with lipid and cholesterol metabolism and transport.

5.3.1. Recent Human Evidence

OEHHA identified 28 human epidemiologic studies of PFOA and PFOS and lipid levels published since (or otherwise not included in) the 2016 US EPA reviews. These studies are summarized in Appendix 7, Tables A7.8 and A7.9. Overall, the findings from the studies OEHHA reviewed are mostly consistent with US EPA's 2016 review and conclusions. That is, a number of studies reported associations between increasing levels of PFOA and increasing total cholesterol and increasing LDL in adults. This included several studies with overall high quality ratings. Most of this evidence involved cross-sectional assessments, although positive associations between PFOA and total cholesterol, LDL, and triglycerides were also seen in the cohort study by (Lin et al., 2019). Positive associations were seen in several studies that OEHHA rated as having high quality. Several studies also identified associations between increasing PFOA and increasing triglyceride concentrations, but this was not as consistent as seen for total cholesterol or LDL. The findings for HDL in adults and findings in children appear to be less consistent than seen for total cholesterol and LDL in adults.

Findings for PFOS were mostly similar to those of PFOA. That is, several studies identified associations with PFOS and increasing total cholesterol and LDL, including several with reasonably high quality score ratings. Some studies did not identify these associations, although no study reported a clear association between increasing PFOS and decreases in total cholesterol or LDL concentrations.

5.3.2. Recent Animal Evidence

OEHHA's review of recent PFOA animal studies examining changes in lipid homeostasis, published from 2016 onward, is presented in Table 5.3.1.

Table 5.3.1. Summary of recent animal toxicity studies of PFOA reporting effects on lipid homeostasis

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male and female C57BL/6 mice (6/sex/dose) Rebholz et al. (2016)	0 or 3.5 mg/kg of feed (fat- and cholesterol-containing diet) (~0.55 mg/kg BW-day, according to authors) for six weeks	**Males:** 0.002 or 26.9 **Females:** 0.028 or 44.3 at six weeks	**Both sexes:** ↑ plasma cholesterol	NA[a]
Male and female BALB/c mice (6/sex/dose) Rebholz et al. (2016)	0 or 3.5 mg/kg of feed (fat- and cholesterol-containing diet) (~0.55 mg/kg BW-day, according to authors) for six weeks	**Males:** 0.005 or 28.2 **Females:** 0.086 or 35.6 at six weeks	**Both sexes:** ↓ hepatic cholesterol **Males:** ↑ plasma cholesterol	NA[a]
Male BALB/c mice (5/dose) Yu et al. (2016)	0, 0.5, or 2.5 mg/kg-day via "oral infusion" for 28 days	0.011, 29.34, or 114.3 at 28 days	changes in lipid metabolism; changes in fatty acid biosynthesis	LOAEL: 0.5 mg/kg-day
Male SV129 WT and PPARα KO mice (4/dose) Das et al. (2017)	0 or 10 mg/kg-day via gavage for 7 days	NA	**WT:** increased liver triglycerides (mild steatosis)	NA[a]
Male BALB/c mice (minimum of 8/dose) Hui et al. (2017)	0, 1, or 5 mg/kg-day orally[b] for 7 days	NA	↓ free fatty acids and serum triglycerides; ↑ liver triglycerides	LOAEL: 1 mg/kg-day for ↓ serum triglycerides and ↑ liver triglycerides

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male and female BALB/c mice (30/sex/dose) Li et al. (2017b)	0, 0.05, 0.5, or 2.5 mg/kg-day via gavage for 28 days	**Males:** 0, 1.2, 5.9 or 13.4 **Females:** 0, 0.97, 2.7 or 9.5 at 28 days	lipid accumulation in cytoplasm of hepatocytes	NOAEL: 0.05 mg/kg-day
Male Kunming mice (number not specified) Wu et al. (2017)	Single oral dose of 0 or 5 mg/kg via gavage	NA	↑ hepatic LDL cholesterol; ↓ hepatic HDL cholesterol	NA[a]
Male BALB/c mice (3/dose) Yan et al. (2017)	0, 0.08, 0.31, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	NA	lipid deposits	Not provided[b]
Pregnant Kunming mice (8/dose) Qin et al. (2018)	0 or 5 mg/kg-day intragastrically throughout gestation	NA	↑ triglycerides, and cholesterol in F1 pup serum on PND 21 (although the changes were not statistically significant)	NA[a]
Male Kunming mice (8/dose) Wu et al. (2018)	0, 1, or 5 mg/kg-day intragastrically for 21 days	NA	↓ serum triglycerides and HDL; ↑ triglycerides in liver; ↑ LDL in serum	NOAEL: 1 mg/kg-day for ↑ liver triglycerides
Male miR-34A -/- KO and WT C57BL/6J mice[c] (15-18/dose) Cui et al. (2019)	0 or 5 mg/kg-day via gavage for 28 days	NA	**WT and KO:** ↓ liver triglycerides	NA[a]

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Male BALB/c mice (12/dose) Guo et al. (2019)	0, 0.4, 2, or 10 mg/kg-day via gavage for 28 days	0.02, 13, 64, or 88	steatosis	NOAEL: 2 mg/kg-day
Male C57BL/6 mice (5/diet/treat-ment/time point) Li et al. (2019d)	0 or 1 mg/kg-day (in distilled water, presumably gavage) for 2, 8, or 16 weeks. Half of the animals were on low fat diet, other half on high fat diet	NA	↓ steatosis induced by the high-fat diet	NA[a]
Male and female Sprague Dawley rats (10/sex/dose) NTP (2019a)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day (for males) and 0, 6.25, 12.5, 25, 50, or 100 mg/g-day (for females) via gavage for 28 days	**Males:** BD, 0.378, 0.503, 1.297, 3.34, or 10.9 **Females:** BD, 0.129, 0.292, 0.475, 1.67, or 6.71 at 28 days	↓ in cholesterol and triglyceride levels in males, but ↑ in females	LOAEL: 0.625 mg/kg-day for ↓ cholesterol and triglycerides in males
Male APOE*3-Leiden.CETP transgenic mice[d] (8/dose) Pouwer et al. (2019)	0, 0.01, 0.291, or 30.2 mg/kg-day in diet for 6 weeks	0.005, 0.065, 1.524 or 144 at 6 weeks	↓ plasma triglycerides, total cholesterol, and non-HDL cholesterol; ↑ plasma HDL cholesterol	NOAEL: 0.291 mg/kg-day for changes in plasma lipid contents
Male APOE*3-Leiden.CETP transgenic mice[d] (8/dose) Pouwer et al. (2019)	0, 0.01, 0.298, or 29.5 mg/kg-day in diet for 4 weeks	0, 0.051, 1.395, or 93.713 at 4 weeks	microvesicular steatosis; ↓ plasma triglycerides and non-HDL cholesterol; ↑ plasma HDL cholesterol	NOAEL: 0.298 mg/kg-day for steatosis

Sex/Species/ Reference	Exposure	Serum/Plasma Concentrations (µg/ml)	Endpoints	NOAEL/LOAEL
Pregnant CD-1 mice (11-13 dams/dose) Blake et al. (2020)	0, 1, or 5 mg/kg-day via gavage from ED1.5 to ED11.5 or ED17.5	ED11.5: BD, 25.4 or 117.3 ED17.5: 0.211, 18.7 or 95.1	**Dams:** ↓ serum triglycerides	LOAEL: 1 mg/kg-day

[a] LOAEL/NOAEL not applicable for single dose studies.
[b] Histology data are presented in the supplementary materials, but specific doses at which hepatocyte swelling and lipid deposits become significant are not provided.
[c] miR-34A -/- KO mice do not produce the microRNA miR-34A. miR-34A functions as a tumor suppressor.
[d] APOE*3-Leiden.CETP transgenic mice are reported to have human-like lipoprotein metabolism.
Abbreviations: BD, below limit of detection; BW, body weight; ED, embryonic day; HDL, high density lipoprotein; KO, knockout; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PND, postnatal day; PPARα, peroxisome proliferator-activated receptor alpha; WT, wild-type

Pouwer et al. (2019) examined the hepatic effects of PFOA using a transgenic mouse model that possesses human-like lipid metabolism. In addition to microvesicular steatosis and hepatotoxicity, PFOA induced changes in plasma lipids, including decreased plasma triglycerides, decreased plasma non-HDL cholesterol, and increased HDL cholesterol. The authors suggested that this mouse model presents a suitable representation of lipid metabolism in humans, as these data supported a phase I clinical trial of PFOA in humans that showed an association between higher PFOA levels and lowered cholesterol. OEHHA identified a NOAEL of 0.29 mg/kg-day for steatosis and changes in plasma lipid contents.

Much like for PFOA, several animal studies published from 2016 onward reported changes in lipid homeostasis following oral exposure to PFOS. Study details are summarized in Table 5.3.2.

Table 5.3.2. Summary of recent animal toxicity studies of PFOS reporting lipid effects

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male C57BL/6 mice (5-6/dose) Zhang et al. (2016c)	0, 30, 60, or 120 mg/kg of feed (equivalent to 0, 3.48, 6.96, or 13.92 mg/kg BW-day)[a] for 21 or 23 days	0, 94.6, 176, and 392 at 21 days	↑ hepatic triglycerides and lipid content; altered lipid metabolism	LOAEL: 3.48 mg/kg-day
Male and female Sprague Dawley rats (12/sex/dose) Bagley et al. (2017)	0 or 100 ppm in feed (equivalent to 6 mg/kg-day for males and 6.6 mg/kg-day for females)	NA	**Males:** ↓ serum cholesterol and triglyceride levels; ↑ liver free fatty acids and triglycerides **Females:** ↓ liver free fatty acids and triglycerides	NA[b]
Male WT and ERβ KO mice (8/dose/group) Xu et al. (2017)	0 or 5 mg/kg-day via gavage for 28 days	NA	**WT:** ↓ hepatic cholesterol **KO:** effect reported in WT animals not observed	NA[b]
Male C57BL/6 mice (5/dose) Huck et al. (2018)	normal diet: 0 or 0.089 mg/kg-day high fat diet: 0 or 0.087 mg/kg-day for 28 days	NA	**Normal diet:** ↑ relative liver weight; steatosis **High fat diet:** ↓ steatosis	NA[b]
Female CD-1 mice (≥4/dose) Lai et al. (2018)	0, 0.3, or 3 mg/kg-day via gavage for 7 weeks	0.024, 33.8, and 109.6 at 7 weeks	↑ liver triglycerides; ↓ serum triglycerides	LOAEL: 0.3 mg/kg-day for ↑ liver triglycerides
Pregnant Kunming mice (5/dose) Liang et al. (2019)	0, 0.5 or 5 mg/kg-day intragastrically throughout gestation (20.5 days)	NA	**Dams:** ↑ liver triglycerides; ↑ liver lipid droplets; **Pups:** ↑ liver triglycerides, total cholesterol, and LDL; ↓ HDL	NOAEL: **Dams**: 0.5 mg/kg-day for ↑ liver triglycerides **Pups**: 0.5 mg/kg-day for changes in liver lipid contents

Sex/Species	Exposure	Serum/Plasma Concentration (µg/ml)	Endpoints	NOAEL/LOAEL
Male and female Sprague Dawley rats (10/sex/dose) NTP (2019b)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	**Males:** BD, 23.73, 51.56, 94.26, 173.7, or 318.2 **Females:** 0.0543, 30.53, 66.97, 135.1, 237.5, or 413.6 at 28 days	**Both sexes:** ↓ cholesterol and triglycerides	LOAEL: 0.312 mg/kg-day for ↓ cholesterol in males
Male ICR mice (10/dose) Su et al. (2019)	0 or 10 mg/kg-day for 21 days; administration method not explicitly stated, but presumably via gavage	NA	↑ triglycerides and total cholesterol	NA[b]

[a] Calculated by OEHHA using a BW of 0.267 kg and a consumption rate of 0.023 kg/day (from US EPA, 1988).
[b] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: BD, below limit of detection; BW, body weight; HDL, high density lipoprotein; KO, knockout; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; WT, wild-type

5.3.3. Recent Mechanistic Evidence

PFOA has been shown to disrupt lipid metabolism in the liver. One way PFOA does this is by changing the expression and activity of enzymes involved in fatty acid metabolism. Changes in fatty acid metabolism have been linked to liver disease. PFOA increases acyl-CoA oxidase activity in rat liver (Cavallini et al., 2017), and carboxylesterase mRNA and protein levels in male mice (Wen et al., 2019c). Carboxylesterases play a role in lipid metabolism and homeostasis (Lian et al., 2018). A proteomic study by Shao et al. (2018) showed that PFOA can bind to acetyl-CoA carboxylase a and b (Acaca and Acacb), enzymes involved in fatty acid metabolism. PFOA has also been shown to bind to human liver fatty acid-binding protein, which would displace uptake of fatty acids and thus potentially disrupt lipid regulation (Sheng et al., 2016; Sheng et al., 2018; Cheng et al., 2019). PFOA is also associated with changes in hepatic and serum cholesterol levels (Rebholz et al., 2016). Pouwer et al. (2019) showed that PFOA altered levels of cholesterol ester transfer protein, which subsequently changed cholesterol metabolism and homeostasis in male APOE*3 Leiden transgenic mice, which were made to have human-like lipoprotein metabolism.

For PFOS, liver gene expression studies (both in vivo and in vitro) have found changes associated with fatty acid metabolism (Beggs et al., 2016; Dong et al., 2016a; Song et al., 2016; Bagley et al., 2017; Xu et al., 2017; Han et al., 2018a; Han et al., 2018b; Huck et al., 2018; Lv et al., 2018; Liang et al., 2019).

Inflammation and endoplasmic reticulum stress have been associated with hepatic steatosis in mice exposed to PFOS (Su et al., 2019). Levels of TNF-α, a cell signaling protein associated with inflammatory stress, and endoplasmic reticulum stress proteins (activating transcription factor 6 [ATF6], Eukaryotic translation initiation factor 2 subunit 1 [eIF2α], 78kDa glucose-regulated protein [GRP78], X-box binding protein 1 [XBP1]) were increased in the liver in mice exposed to PFOS. ER stress pathways have been linked to hepatic steatosis (Zhang et al., 2014), thus perturbation to the normal processes may lead to liver toxicity.

5.3.4. Conclusions

As shown in Table A7.11 (Appendix 7), nine of the 16 studies in adults that reported on PFOA and total cholesterol (TC) or LDL identified some evidence of a positive association (marked as "+" or "(+)" in the table), two found no association (marked as "0"), one reported a negative association (increasing PFOA associated with a decrease in TC; marked as "-" or "(-)" in the table), and the others presented results that were difficult to interpret (marked as "U"). Of the seven studies of PFOA and TC or LDL that did not find positive associations, three reported positive associations with triglyceride levels. The average quality score in the studies that did and did not find some evidence of a positive association are 8.0 and 5.1, respectively. Only two of the nine of the studies of PFOA and TC or LDL in children reported some evidence of a positive association.

Eight of the 15 studies in adults that reported on PFOS and total cholesterol (TC) or LDL identified some evidence of a positive association, four found no association including the most recent NHANES study by (Liu et al., 2018b), one reported a negative association, and the others presented results that were difficult to interpret (marked as "U") (Appendix 7, Table A7.12). Only one of the PFOS studies that did not find evidence for a positive association with either TC or LDL reported an association for triglyceride levels. This was the relatively small cross-sectional study by (Yang et al., 2018) (N=145) and the major findings were not statistically significant. The average quality scores in the studies that did and did not report evidence of an association between PFOS and TC or LDL were 7.4 and 5.4, respectively. As with PFOA, the majority of results for PFOS in the recent studies in children were negative.

Several sources of possible bias in the studies reviewed were evaluated. First, OEHHA examined the possibility that confounding by diet or diet-related factors might have caused the associations between PFOA or PFOS and lipid levels reported in many of these studies. For example, if an increased consumption of certain foods (or greater exposure to food packaging) is associated with both increased PFOA exposure and with higher lipid levels, this could potentially confound any relationships between PFOA or PFOS and lipid levels. Since PFOA and PFOS have been found in a number of different foods (Appendix 7, Table A7.1), it is possible that increased dietary consumption overall (i.e., overall calorie intake) could be an important confounder. However, many of the studies OEHHA reviewed adjusted for BMI, waist circumference, or total calorie intake. Other studies adjusted for specific foods or dietary patterns (e.g., fat intake, Mediterranean diet) that might be related to total cholesterol levels. In a cross-sectional study of 965 Danish pregnant women, both mid-gestational serum PFOS and PFOA concentrations were found to be associated with increased meat or meat product intake and decreased vegetable intake (Skuladottir et al., 2015). However, the associations reported between PFOS and PFOA and total cholesterol changed very little with adjustments for fat, total calorie, or meat and vegetable intake (Table 5.3.3).

Table 5.3.3. Differences in mean total cholesterol levels (mmol/dl) between the fifth and first quintiles of serum PFOA or PFOS concentrations and p-trends across quintiles in Danish pregnant women (Skuladottir et al., 2015).

Adjustment	PFOA	PFOS
Age, parity, education, smoking and BMI	0.40 (p=0.008)	0.41 (p=0.007)
Plus fat intake and total calorie intake[1]	0.39 (p=0.01)	0.39 (p=0.01)
Plus meat, vegetable, and total calorie intake[1]	0.45 (p=0.003)	0.44 (p=0.004)

[1] Adjusted for these factors in addition to age, parity, education, smoking and BMI

Another factor suggesting that diet is not a major confounder in all of the studies reporting PFOA- or PFOS-lipid associations is that some of these associations have been seen in several high exposure studies. In occupational studies and community studies done in areas with known high drinking water contamination, most PFOA or PFOS intake is likely related to the environmental contamination or occupational exposure, and less is likely related to the dietary sources usually seen in the general population. As such, some of the usual diet-related sources of PFOA or PFOS are less likely to confound PFOS- or PFOA-lipid associations in these studies. For example, associations between PFOA and total cholesterol have been reported in several high exposure occupational studies where serum PFOA concentrations in the higher and lower PFOA exposure groups differ by 1,000 ng/ml or more (US EPA, 2016b). In contrast, most dietary studies suggest that dietary factors are usually only responsible for differences in serum PFOA of about 1-2 ng/ml or less (Jain, 2014; Skuladottir et al., 2015; Harris et al., 2017). While this might not apply to locally grown or produced foods, it would apply to other foods or food related items. Overall, the small contribution of many of the usual diet or diet-related sources to the overall exposure levels in the high exposure occupational or community studies suggest that confounding by these typical diet-related exposure sources is unlikely to be the cause of the associations identified in these studies.

Many of the studies identifying associations between PFOS or PFOA and lipid levels are cross-sectional studies. As mentioned above, studies of this design are frequently criticized based on their potential for reverse causation, or for the fact that they cannot assure that the most appropriate exposure window has been assessed (e.g., latency effects). With regards to the latter, the serum half-lives of both PFOA and PFOS are several years (Olsen et al., 2007), and studies have shown that serum concentrations measured in the same people over several years are generally very well correlated (Liu et al., 2018a). For example, Nøst et al. (2014), reported correlation coefficients between 0.60 and 0.84 for PFOA and PFOS in 53 Norwegian men in samples collected 6-8 years apart (Table 5.3.4). Overall, a single cross-sectional measurement of PFOA and PFOS concentrations in serum is likely to provide a fairly accurate picture of a person's exposure over a period of several years. Since OEHHA could not find convincing evidence that the latency between PFOA or PFOS exposure and adverse effects on lipid levels is longer than this, it seems that widespread major errors due to missing or inadequate exposure data is unlikely to have caused the associations reported in many of the studies reviewed.

Table 5.3.4. Spearman correlation coefficients for serum PFOA and PFOS concentrations measured in the same individuals over time (Nøst et al., 2014) (all p-values <0.05)

	Beginning and ending year of measurement			
Compound	**1979-1986**	**1986-1994**	**1994-2001**	**2001-2007**
PFOA	0.65	0.66	0.60	0.75
PFOS	0.84	0.65	0.62	0.81

Reverse casualty might be possible in some cross-sectional studies if having altered lipid levels or being diagnosed with a lipid related disease could lead to the use of certain medications or lead to changes in certain lifestyle factors that might affect one's PFOA or PFOS exposure. Again, this would seem less likely in the high exposure occupational or community studies, where these factors might not be the major determinants of exposure. In addition, several studies reported associations after excluding people taking lipid-lowering medications or after adjusting for use of these medications (Sakr et al., 2007; Steenland et al., 2009; Liu et al., 2018b; Jain and Ducatman, 2019; Lin et al., 2019). In addition, PFOA-lipid associations have been seen in a number of prospective studies (Olsen et al., 2003a; Sakr et al., 2007; Fitz-Simon et al., 2013), which might be less susceptible to reverse causality than some cross-sectional analyses.

Finally, few of the studies provided detailed information on participant selection or response/participation/refusal rates, and most appeared to involve convenience samples. Importantly though, none of the studies OEHHA reviewed appeared to have selected participants based on both PFOA and lipid levels, and thus widespread and important selection bias seems unlikely.

The evidence for PFOS also supports an association with increased total cholesterol and LDL levels in adults. Evidence based on high exposure scenarios appears to be limited to studies from a single occupational facility, where results have been mixed (Olsen et al., 2003a). Despite this, a number of large population based studies in adults with seemingly high quality have reported associations between PFOS and increases in total cholesterol (US EPA, 2016b; He et al., 2018; Dong et al., 2019). In most, if not all, of these studies serum PFOS levels are highly correlated with serum levels of PFOA or other PFAS. This raises the concern that some of the associations reported in these studies might be due to other PFAS. Because of these high correlations, appropriately adjusting for these other PFAS can be difficult in epidemiologic studies due to issues related to co-variance, and none of the PFOS-lipid studies OEHHA reviewed that identified positive associations with total cholesterol and LDL reported results with these adjustments. Some animal studies have identified PFOS related changes in lipid homeostasis in the liver, and this provides some biologic plausibility that PFOS can affect lipid metabolism in humans.

Using data from the 2003-14 NHANES on 8,948 adults, Dong et al. (2019) calculated benchmark doses (BMDs) using a hybrid approach. In Dong et al. (2019) this involved setting a cut-off point for elevated TC based on the distribution of TC values in the unexposed or lower exposed reference group. Here, this cut-off point was the upper 5th percentile of TC values in the lowest PFOA exposure group. The benchmark response was then defined as a 10% increase in values above this level. Reference doses (RfD) were then calculated using the following equation, where Vd is the volume of distribution (0.17 L/kg for PFOA and 0.23 L/kg for PFOS); $T_{1/2}$ is the half-life in serum (5.4 years for

PFOS and 2.3 years for PFOA); and BMDL is the lower 95% confidence interval of the BMD:

$$RfD = BMDL \times Vd \times Ln(2) \times 1{,}000/T_{1/2}$$

The results of these calculations are shown in Table 5.3.5. Based on data from the 2013-14 NHANES, the authors estimated that approximately 3.8% and 3.4% of the US population had PFOA or PFOS serum concentrations, respectively, above the BMDLs.

Table 5.3.5. Benchmark and reference doses for a 10% increase in serum total cholesterol based on 8,948 adults in NHANES 2003-14 (Dong et al., 2019)

Chemical	BMD (ng/ml)	BMDL (ng/ml)	RfD (ng/kg/day)
PFOA	10.5	5.6	0.8
PFOS	44.2	24.1	2.0

Abbreviations: BMD, benchmark dose; BMDL, lower 95% confidence interval of the BMD; NHANES, US National Health and Nutrition Examination Survey; RfD, reference dose

Although the hybrid approach has a number of advantages (see Crump (1995)), few details were provided in this paper on several important aspects of this approach or on other key issues, including the definition of the unexposed reference group, the distribution of PFOA or TC values in this group, model fit (e.g., the fit of linear versus non-linear models), the impact of potential confounders, or the role of reverse causality

For effects on lipid homeostasis in animals, several recent studies reported that PFOA decreased serum triglycerides and cholesterol in mice (Hui et al., 2017; Wu et al., 2018; Pouwer et al., 2019), which support the conclusions reached in other assessments. A study using APOE*3-Leiden.CETP transgenic mice, which are reported to have human-like lipoprotein metabolism, reported decreased plasma triglycerides and total cholesterol (Pouwer et al., 2019), which the authors suggest is predictive of human physiological responses to PFOA exposure. Conversely, one study reported that PFOA administered with a high-fat diet increased plasma cholesterol in mice (Rebholz et al., 2016). In rats, PFOA induced differential changes in triglyceride and cholesterol levels based on sex, where decreased levels were observed in males, and increased levels were observed in females (NTP, 2019a). Interestingly, several studies reported that levels of HDL and LDL cholesterol were differently impacted by PFOA exposure, although the results were inconsistent between studies (Wu et al., 2018; Pouwer et al., 2019).

Accumulation of triglycerides in the liver (steatosis) was also reported in several rodent studies (Das et al., 2017; Hui et al., 2017; Li et al., 2017b; Wu et al., 2018; Guo et al., 2019; Pouwer et al., 2019). Interestingly, this effect was not observed in PPARα knockout mice (Das et al., 2017), indicating that PPARα activation significantly contributes to the induction of hepatic steatosis. However, a few studies observed contradictory results in mice, where PFOA reduced liver triglycerides (Cui et al., 2019), and even reversed steatosis induced by a high-fat diet (Li et al., 2019d).

For PFOS, two recent studies in rats reported decreased serum cholesterol and triglycerides (Bagley et al., 2017; NTP, 2019b), although statistical significance was not reached for females in one study (Bagley et al., 2017). Conversely, one recent study in mice reported increased serum triglycerides and cholesterol (Su et al., 2019).

Much like PFOA, several studies reported increases in liver triglycerides in mice exposed to PFOS (Zhang et al., 2016c; Bagley et al., 2017; Lai et al., 2018; Liang et al., 2019), including in pups exposed gestationally (Liang et al., 2019). Gestational exposure also increased hepatic LDL levels, and decreased HDL levels (Liang et al., 2019). Huck et al. (2018) reported that PFOS induced steatosis in mice fed on a regular diet, but decreased steatosis in animals on a high-fat diet. In rats, levels of liver free fatty acids and triglycerides differed based on sex (increased in males, decreased in females) (Bagley et al., 2017). One study reported decreases in hepatic cholesterol in mice following PFOS exposure, but these effects were not observed in ERβ knockout animals (Xu et al., 2017).

In summary, the current epidemiologic literature provides evidence that PFOA and PFOS can cause increased total cholesterol in humans (Table 5.3.6). In contrast, some animal studies have shown decreased cholesterol with PFOA and PFOS exposure (Table 5.3.6). Different results in animals and humans may be explained by the stronger activity of PPARα in animals, which is involved in the metabolism of cholesterol and fatty acids.

Table 5.3.6. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and lipids

Outcome	PFOA	PFOS
Total cholesterol in humans	-Mostly consistent associations with increased total cholesterol in adults -Findings are less consistent in children	-Mostly consistent associations with increased total cholesterol in adults -Findings are less consistent in children
Other lipids in humans	Some associations identified for increased LDL and TGs, and for decreased HDL but fewer studies	Data on LDL, TGs, and HDL are inconclusive
Lipid effects in animals (TGs and cholesterol, steatosis)	Evidence of decreased serum TGs and cholesterol in several studies, and mixed results for increased steatosis	Inconsistent evidence for decreased serum and hepatic cholesterol and TGs, and steatosis

HDL, high density lipoprotein; LDL, low density lipoprotein; TGs, triglycerides

5.4. Thyroid Toxicity

The US EPA has reviewed the scientific literature on PFOA and PFOS and thyroid toxicity (US EPA, 2016b; US EPA, 2016d). This review included literature published up to December 2015. In their reviews, US EPA identified a number of epidemiologic studies that investigated associations between PFOA or PFOS and thyroid hormone levels or more overt thyroid diseases.

For PFOA, US EPA (2016b) identified three epidemiologic studies linking this agent to thyroid disease, primarily hypothyroidism. The first was a cross-sectional study involving US NHANES adult participants (Melzer et al., 2010). Here, the adjusted ORs for self-reported current thyroid disease were 2.24 (95% CI, 1.38–3.65) in women and 2.12 (95% CI, 0.93–4.82) in men, comparing those in the upper quartile to those in the lower two quartiles of serum PFOA. The second study investigated parent-reported thyroid disease in 10,725 children 1-17 years of age living near a Teflon™ manufacturing facility in the Mid-Ohio Valley (USA) (Lopez-Espinosa et al., 2012). In analyses adjusted for age and sex, the OR for hypothyroidism for an interquartile

contrast of 13 to 68 ng/ml in serum PFOA was 1.54 (95% CI, 1.00-2.37). Clear associations with thyroxine (T4) or thyroid stimulating hormone (TSH) were not seen. The third study was a large investigation that included adults in the same community (Winquist and Steenland, 2014). Here, an environmental fate and transport model was used to estimate yearly air and drinking water PFOA concentrations. These were then combined with information on residential history, drinking water consumption rates, and water sources to estimate each participant's yearly PFOA intake rate. These intake rates were then used in an absorption/distribution/metabolism/ excretion model to generate yearly estimates of serum PFOA concentrations for each participant. For participants who worked in the local chemical plant, where PFOA exposures were especially high, an occupational exposure model using work history and modeled job- and department-specific serum concentrations was used to generate yearly serum PFOA concentration estimates for years when the participant worked at the plant. Information on thyroid disease was initially based on self-reports then, confirmed using medical records. Hazard ratios (HRs) controlling for sex, race, education, smoking, and alcohol use for all thyroid diseases combined across cumulative PFOA exposure quintiles were 1.00, 1.24, 1.27, 1.36, and 1.37 in women (p-trend = 0.03) and 1.00, 1.12, 0.83, 1.01, and 1.05 among men (p-trend = 0.85).

With regards to PFOA and thyroid hormone levels, US EPA concluded, "Association between PFOA and TSH also was seen in pregnant females with anti-TPO antibodies (Webster et al., 2014). In contrast, generally null associations were found between PFOA and TSH or thyroid hormones (triiodothyronine (T3) or T4) in people who have not been diagnosed with thyroid disease" (Table 5.4.1).

For PFOS, US EPA (2016d) concluded that the epidemiologic studies they reviewed provided "limited support" for an association between PFOS and thyroid disease. This conclusion was primarily based on two large cross-sectional studies using participants of the US NHANES. The first involved data from NHANES 1999-2000, 2003-2004, and 2005-2006 and included 1,900 males and 2,066 females ages 20 and older (Melzer et al., 2010). In men, the OR adjusted for age, ethnicity, education, BMI, smoking status, and alcohol consumption for self-reported currently treated thyroid disease in those with serum PFOS concentrations ≥36.8 ng/ml (fourth quartile) compared to those with serum PFOS ≤25.5 ng/ml (first and second quartiles) was 2.68 (95% CI, 1.03–6.98). In women, this OR was 1.27 (95% CI, 0.82–1.97). The second study included 1,181 subjects >20 years of age from the 2007-2008 and 2009-2010 NHANES (Wen et al., 2013). In analyses adjusted for age, race, drinking, smoking, and urinary iodine levels, a 1-unit increase in ln-PFOS serum concentration was associated with subclinical hypothyroidism in both men (OR = 1.98; 95% CI, 1.19–3.28) and women (OR = 3.03; 95% CI, 1.14–8.07).

US EPA (2016d) also identified a number of epidemiologic studies that investigated associations between PFOS and levels of thyroid hormones, including T4, free thyroxine (fT4), T3, and TSH. Based on these studies, US EPA concluded that in most groups, including children, the evidence linking PFOS to these hormones was inconsistent. An exception was seen for pregnant women where the three studies in this group all identified associations between increasing serum concentrations of PFOS and increasing serum concentrations of TSH (Wang et al., 2013; Webster et al., 2014; Berg et al., 2015). In one of these, the association was only seen in women with elevated levels of anti-thyroid peroxidase (anti-TPO) antibodies (Webster et al., 2014).

Table 5.4.1. Summary of US EPA's conclusions on epidemiologic studies of PFOA or PFOS and thyroid toxicity (US EPA, 2016b; US EPA, 2016d)

Outcome	PFOA	PFOS
Thyroid hormone levels	No clear associations overall ↑TSH in anti-TPO positive (one study)	Inconsistent overall ↑TSH in pregnancy (three studies)
Thyroid disease	Two positive, one negative study	"Limited evidence" overall (two NHANES studies)

US EPA (2016b) identified two studies examining thyroid effects in experimental animals exposed to PFOA (Butenhoff et al., 2002; Martin et al., 2007, as cited by US EPA (2016b)) These studies reported decreases in total T3 and free T3 in monkeys, and decreases in total T4 and free T4 in male rats, both without significant changes in TSH. No thyroid hormone changes were observed in female rats, presumably due to the higher clearance rate of PFOA compared to male rats and other species.

Compared to PFOA, the thyroid toxicity database for PFOS in animals is more extensive. US EPA (2016d) identified several studies reporting thyroid effects in animals exposed to PFOS (Seacat et al., 2002; Lau et al., 2003; Thibodeaux et al., 2003; Luebker et al., 2005; Chang et al., 2007; Martin et al., 2007; Chang et al., 2008; Yu et al., 2011, as reported in US EPA (2016d)). In general, a reduction in fT4 and total T4 (tT4) levels, without significant changes in TSH, was consistently observed in rats exposed to PFOS. The evaluated studies included studies in pregnant rats, where PFOS reduced T4 and T3 levels in dams (Thibodeaux et al, 2003, as reported in US EPA (2016d)), and T4 in pups (Lau et al., 2003, as reported in US EPA (2016d)). In monkeys, PFOS induced a significant decrease in total T3 and tT4 levels in females only, with no changes in TSH (Seacat et al., 2002, as reported in US EPA (2016d)).

5.4.1. Recent Human Evidence

Overall, OEHHA found 25 publications, published since the 2016 US EPA review, that examined associations between PFOA or PFOS and thyroid hormone levels in humans (Appendix 7, Table A7.7).

PFOA and thyroid hormone levels: In non-pregnant adults, the findings for TSH overall are mixed although the majority of results suggest a positive association (10 of 19 results), with three studies reporting statistically significant positive associations and none reporting statistically significant negative associations (Table A7.7). Two of the three studies reporting statistically significant positive associations were in females (Heffernan et al., 2018; Zhang et al., 2018) while the other involved females and males combined (Blake et al., 2018). The results were mixed in the other four studies in non-pregnant females. Two studies in non-pregnant adults reported statistically significant inverse associations between PFOA and fT4 (Olsen et al., 2007; Zhang et al., 2018), although results from the other 13 studies on fT4, and the results for T4, were mixed. OEHHA did not identify clear differences in study quality between those studies that did and did not identify statistically significant associations.

In pregnant females, the majority of results (7 of 11) also showed positive associations between PFOA and TSH although none were statistically significant. A statistically significant inverse

association with fT4 was reported in one study (Preston et al., 2018), but clear trends between PFOA and fT4 or T4 in pregnant women were not seen across the different studies.

In newborns (samples typically collected in cord blood), consistent associations were not seen between PFOA and TSH, T4, or fT4. Serum PFOA levels can vary by age, with higher levels in children under 10 months of age (Mogensen et al., 2015a). A statistically significant positive association was seen between maternal PFOA concentrations and cord blood TSH in one study (Kim et al., 2011b), and statistically significant positive associations were seen between cord blood PFOA and infant T4 or fT4 in two others (de Cock et al., 2014; Aimuzi et al., 2019). However, two of these studies involved small sample sizes (N=29 and N=31 in Kim et al., 2011b and De Cock et al., 2014, respectively), these findings were not consistent with those reported in the other studies in newborns, and none of these studies appeared to be of markedly higher quality than the other newborn studies. Major differences in results were not seen between males and females amongst newborns.

In the 17 results OEHHA identified in children older than newborns, two cross-sectional studies reported statistically significant inverse associations between PFOA and TSH in female participants (Lopez-Espinosa et al., 2012; Lewis et al., 2015), and one reported a statistically significant positive association between modeled maternal PFOA during pregnancy and blood T4 concentrations in offspring 1-5 years of age (males and females combined) (Lopez-Espinosa et al., 2012). Findings from the other studies were mixed. Clear differences in study quality were not seen between those studies reporting statistically significant associations and those that did not. Overall, consistent trends were not seen in subgroups of either male or female children.

PFOS and thyroid hormone levels: In studies in adults, clear trends across the different studies were not seen between PFOS and TSH or T4. For fT4, the majority of results (10 of 18) were consistent with a positive association between PFOS and this hormone. However, among the studies reporting results that were statistically significant, four were consistent with a positive association and two were consistent with an inverse association. Among the latter two, one did not adjust for any potential confounders (Li et al., 2017d) and the other involved participants in a case-control study of premature ovarian insufficiency (Zhang et al., 2018). No other major differences in study quality were identified between those studies reporting positive and inverse associations.

In their review, US EPA (2016b) identified three studies in pregnant women that reported positive associations between PFOS and TSH (Wang et al., 2013; Webster et al., 2014; Berg et al., 2015). Another study published before 2016 that was not included in the US EPA review reported an inverse association between PFOS and TSH in pregnant women, although the result was not statistically significant (Wang et al., 2014). Since the 2016 US EPA review, six cross-sectional studies and one prospective study have been published and most have reported inverse associations between PFOS and TSH in pregnant women, with two of the results being statistically significant. Clear differences in study quality between those studies reporting positive associations and those reporting inverse associations were not seen. Overall, findings on TSH and PFOS to date are inconsistent.

In male newborns, two of the eight studies reported statistically significant inverse associations between PFOS concentrations and TSH in either cord blood (Tsai et al., 2017) or maternal blood during pregnancy (Preston et al., 2018). In contrast, two of the studies in newborn

females showed the opposite effect. Results in the other groups of children, and for T4 and FT4, have also been mixed.

Thyroid diseases: OEHHA identified four studies of PFOA or PFOS and thyroid disease published since the 2016 US EPA reviews (US EPA, 2016b; US EPA, 2016d). In the first, in analyses adjusted for age, BMI, and alcohol intake, ORs for an association between serum concentrations of PFOA and PFOS and self-reported thyroid disease were 1.60 (95% CI, 0.96-2.82) and 0.94 (95% CI, 0.84-1.01), respectively, in 154 adult fishermen (women were not included) from Wisconsin (Christensen et al., 2016). The thyroid outcomes in this study included benign thyroid tumors, Hashimoto's disease, Grave's disease, hypothyroidism, hyperthyroidism, goiter/enlarged thyroid gland, and "other thyroid or endocrine problem". The median serum concentration of PFOA in the men in this study was 2.5 ng/ml (IQR, 1.8-3.3 ng/ml).

In the second study, a case-control study of congenital hypothyroidism in South Korean infants, unadjusted mean serum PFOA concentrations were lower in controls (N=13) than cases (N=27) (means of 2.12 and 5.40 ng/ml, respectively; $p < 0.01$) (Kim et al., 2016a). Major differences between cases and controls were not seen for PFOS. Few details were provided on subject selection procedures or on the methods used to control for or evaluate potential confounding.

In the third study, Dufour et al. (2018) examined associations between PFOA and PFOS concentrations in cord blood from 221 mother-infant pairs and maternal hypothyroidism. Mothers were considered to be hypothyroid if levothyroxine use was recorded in their medical records. Mean PFOA and PFOS concentrations were 0.88 ng/ml (standard deviation (SD), 0.79) and 0.80 ng/ml (SD, 0.52), respectively. Odds ratios for the first through the fourth quartiles of PFOA concentrations were 1.00 (reference), 4.42 (95% CI, 1.23-21.14), 3.22 (95% CI, 0.88-15.38), and 5.62 (95% CI, 1.64-26.11). Odds ratios for the first through the fourth quartiles of PFOS concentrations were 1.00 (reference), 1.76 (95% CI, 1.23-21.14), 3.22 (95% CI, 1.08-10.92), and 2.95 (95% CI, 0.98-10.07). Odds ratios were adjusted for maternal age and tobacco use. There were 37 cases of hypothyroidism overall but the numbers in each quartile of PFOA or PFOS were not provided.

The fourth study involved an area in Ronneby, Sweden in which the local water supply was contaminated with PFAS from firefighting foams used at a nearby military air field practice site (Andersson et al., 2019). Serum levels of 257, 280 and 15 ng/ml were reported for PFHxS, PFOS and PFOA, respectively, in a biomonitoring study of local residents. Hyper- and hypothyroidism in the population was assessed by linking population registry information to registries on deaths, hospitalizations, clinic visits, and prescription medications. Overall, living at an address supplied by municipal water from the contaminated waterworks was not associated with clear effects on either outcome, in either men or women.

5.4.2. Recent Animal Evidence

Thyroid effects have been reported in animals environmentally exposed to PFAS. Levels of T3 were negatively associated with PFAS in polar bears and hooded seals (Bourgeon et al., 2017; Grønnestad et al., 2018) and PFOA was associated with hyperthyroidism in domestic cats (Bost et al., 2016).

NTP recently released subacute (28 days) and chronic (16 or 107 weeks) bioassays for PFOA conducted in male and female Sprague Dawley rats. Animals were given PFOA in feed (doses

are provided in Table 5.4.2). For the chronic studies, an additional group of animals was exposed to PFOA during gestation and lactation (perinatal exposure). Although the initial chronic study in male rats with concentrations of 0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed was ended at 21 weeks due to overt toxicity, it appears a subset of animals receiving those doses were examined at 16 weeks, and the study was repeated with lower doses. Results are summarized in Table 5.4.2. Thyroid follicular cell hypertrophy was observed in male and female rats in the 28-day studies, and in female rats in the 107-week studies. Thyroid toxicity was not observed in female rats in the 16-week studies and male rats in the 107-week studies (NTP, 2020). It should be noted, however, that male rats exposed perinatally in the 107-week studies had higher incidences of thyroid follicular cell hypertrophy, although statistical significance was not reached (p=0.087, Fisher's exact test, performed by NTP). OEHHA identified a LOAEL of 0.625 mg/kg-day for males and 6.25 mg/kg-day for females (corresponding to a plasma concentrations of 50.7 and 0.49 µg/ml, respectively) for changes in thyroid hormone levels in the 28-day studies, and a NOAEL of 14.7 mg/kg-day (plasma concentration of 193 µg/ml) for thyroid follicular cell hypertrophy and changes in thyroid weight in male rats in the chronic studies.

Table 5.4.2. Thyroid toxicity from subacute and chronic studies of PFOA in Sprague Dawley rats (NTP, 2019a; NTP, 2020)

Sex	Exposure	Endpoints	NOAEL/LOAEL
Male (10/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day via gavage for 28 days	Thyroid follicular cell hypertrophy (trend); ↑ relative thyroid weight; ↓ TSH, T3, fT4 and tT4	LOAEL: 0.625 mg/kg-day for changes in thyroid hormones
Female (10/dose)	0, 6.25, 12.5, 25, 50, or 100 mg/kg-day via gavage for 28 days	Thyroid follicular cell hypertrophy; ↑ TSH; ↓ fT4 and tT4	LOAEL: 6.25 mg/kg-day for increased TSH
Male (10/dose)	0, 150, or 300 ppm in feed (0, 14.7, or 29.5 mg/kg-day) for 16 weeks	↓ relative and ↑ absolute thyroid weight; thyroid follicular cell hypertrophy	NOAEL: 14.7 mg/kg-day for all thyroid endpoints
Male (10/dose)	0, 20, 40, or 80 ppm in feed (0, 1.8, 3.7, or 7.5 mg/kg-day) for 16 weeks	↓ absolute thyroid weight (not significant at the highest dose)	NOAEL: 1.8 mg/kg-day for
Female (50/dose)	0, 300, or 1,000 ppm in feed (0, 18, or 63 mg/kg-day) for 107 weeks	Thyroid follicular cell hypertrophy	NOAEL: 18 mg/kg-day

LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; TSH, thyroid stimulating hormone; T3, triiodothyronine; fT4, free thyroxine; tT4, total thyroxine

For PFOS, NTP (2019b) conducted subacute studies in male and female Sprague Dawley rats. Animals were given 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day PFOS via oral gavage for 28 days. Decreases in T3, fT4, and tT4 were observed in both sexes, while decreased absolute thyroid weight was reported in males only (NTP, 2019b). OEHHA identified a LOAEL of 0.312 mg/kg-day (corresponding to plasma concentrations of 23.7 and 30.5 µg/ml for males and females, respectively) based on decreases in fT4 and tT4 in both sexes.

A study in male and female cynomolgus monkeys given 14 mg/kg PFOS via oral gavage on three separate occasions over an observation period of 422 days showed a slight reduction in serum tT4 in both sexes (Chang et al., 2017). There were no significant changes in TSH or fT4. The authors did not consider the reduction in tT4 to be toxicologically relevant because a sufficient reservoir of inactive (bound to protein) T4 remained available to maintain thyroid hormone homeostasis.

5.4.3. Recent Mechanistic Evidence

Proper thyroid function is critical for appropriate metabolism and development. Evidence suggests that PFOA can impact thyroid gland function and perturb thyroid hormone homeostasis. Several recent mechanistic studies showed that PFOA can bind to TTR, a transport protein that carries T4 (Ren et al., 2016; Zhang et al., 2016b; Kar et al., 2017). PFOA effectively displaced T4 binding to TTR, which led to an increased amount of free T4 and an increased T4 uptake rate into rat hepatocytes (Selano et al., 2019). However, PFOA did not bind to thyroxine-binding globulin (TBG), another thyroid hormone transport protein (Ren et al., 2016).

PFOA did not inhibit cyclic adenosine monophosphate (cAMP) production induced by TSH in rat thyroid FRTL-5 cells (Croce et al., 2019), suggesting that PFOA did not interfere with TSH endocrine signaling. Furthermore, (Buckalew et al., 2020) report that PFOA inhibits iodide uptake in rat thyroid cells (FRTL-5), and in hNIS-HEK293T-EPA cells that express the human sodium-iodide symporter (hNIS) . NIS-mediated transport of iodide into the thyroid gland is critical for thyroid hormone synthesis, and disruption of this pathway can lead to thyroid dysfunction. In contrast, Wang et al. (2019a) did not observe any iodide uptake inhibition by PFOA in hNIS-HEK293T-EPA cells.

Like PFOA, PFOS can bind to TTR (Ren et al., 2016; Zhang et al., 2016b; Kar et al., 2017; Xin et al., 2018a), but not to TBG (Ren et al., 2016). However, PFOS inhibited iodide uptake more potently than PFOA in rat thyroid FRTL-5 cells and in hNIS-HEK293T-EPA cells (Wang et al., 2019a; Buckalew et al., 2020). PFOS also interacts with thyroid hormone receptors, and can stimulate cell proliferation in rat pituitary cancer (GH3) cells, a process which is regulated by thyroid hormone receptors (Xin et al., 2018a).

Similar to PFOA, PFOS did not inhibit cAMP production induced by TSH in rat thyroid FRTL-5 cells (Croce et al., 2019).

Dong et al. (2016a) reported expression changes in genes related to thyroid hormone homeostasis, including genes affected by constitutive androstane receptor/pregnane X receptor (CAR/PXR) activation.

5.4.4. Conclusions

Overall, for PFOA and thyroid hormone levels in humans, OEHHA did not find clear and consistent trends across the different epidemiologic studies reviewed. The majority of findings identified suggested that PFOA in adults (pregnant or not pregnant) might be associated with an increase in TSH. However, a number of studies reported the opposite effect and no obvious study design factor or other reason was found that explained this variability in results. OEHHA also did not see consistent associations between PFOS and thyroid hormone levels in humans. US EPA (2016d) identified three epidemiologic studies that reported positive associations

between PFOS and TSH in pregnant women. However, the four studies published since US EPA's review (US EPA, 2016b) (or otherwise not included in that review) reported essentially opposite findings.

The large majority of epidemiologic studies OEHHA reviewed on PFOA or PFOS and thyroid hormone levels were cross-sectional studies, where the temporal relationship between the exposure and the outcome can sometimes be difficult to discern. It is possible that people with altered thyroid hormone status may use certain products, change their diets, or otherwise have certain lifestyle changes that lead to increased PFOA or PFOS exposure ("reverse causation"). However, OEHHA found no evidence in the current literature to suggest that this is likely to be the case. In addition, the very large majority of people in the studies OEHHA reviewed had thyroid hormone levels that were within normal reference ranges, despite their PFOS or PFOA exposure. This suggests that the differences in thyroid hormone levels that were seen in people with different PFOA or PFOS levels in some studies were likely to have been too small to have caused major changes in lifestyle, diet, or product use that markedly affected their PFOA or PFOS intake. Overall, OEHHA found little evidence and little reason to believe the associations identified in some studies, or that the inconsistency overall, were a result of reverse causation.

Participation rates appeared to vary across studies although not all studies provided sufficient information to fully assess these rates or to assess any impacts of possible selection bias. However, OEHHA found little indication that selection bias was a major concern in these studies since most selected their participants prior to measuring PFAS or thyroid hormone levels. As such, participants' selection was most likely independent of their exposure or outcome status and therefore unlikely to have introduced major bias in most studies.

As seen in Table A7.7 (Appendix 7), the studies OEHHA reviewed on thyroid hormone levels were adjusted for a number of the more prevalent determinants of thyroid hormone levels or risk factors for thyroid disease including age, sex, smoking, race/ethnicity, and BMI. No major differences were found in the results from those studies that did or did not adjust for these factors. Only a few studies adjusted for other PFAS or for other chemical exposures (besides smoking) that might influence thyroid hormone levels (Shrestha et al., 2015; Byrne et al., 2018). Shrestha et al. (2015) assessed correlations between PFAS and PCBs and polybrominated diphenyl ethers (PBDEs), and between thyroid hormone levels and PCBs and polybrominated diphenyl ethers (PBDEs). Their results are shown in Table 5.4.3 and overall the correlations between PFOA or PFOS and these other chemical agents appear too low to have caused major confounding. Overall, OEHHA did not find clear evidence that confounding caused the inconsistency in the findings reviewed here.

Table 5.4.3 Pearson correlation coefficients between serum PCBs, PBDEs, PFOA, PFOS, and thyroid hormones (Shrestha et al., 2015)

	PFOS	PFOA	TSH	fT4	T4
Serum ∑ PCBs (ng/g serum total lipids)	0.30 (0.01)	0.16 (0.15)	0.04 (0.71)	0.08 (0.46)	0.09 (0.40)
Serum ∑ PBDEs (ng/g serum total lipids)	0.02 (0.86)	0.04 (0.68)	-0.02 (0.85)	-0.13 (0.23)	-0.10 (0.35)

The numbers in parentheses are p-values
Abbreviations: fT4, free thyroxine; ΣPBDEs, total polybrominated diphenyl ethers; ΣPCBs, total polychlorinated biphenyls; T4, total thyroxine; TSH, thyroid stimulating hormone

OEHHA identified only three epidemiologic studies of more overt thyroid conditions and PFOA or PFOS published since the 2016 US EPA reviews. Although these studies presented some suggestive evidence of an association between PFOS or PFOA and certain thyroid conditions,

small sample sizes, unusual dose-response patterns, lack of information on subject selection or potential confounders, or lack of replication limited the ability to make firm conclusions regarding these studies (Table 5.4.4).

The recent NTP studies in rats demonstrated that PFOA can adversely impact the thyroid. Decreases in T3 and T4 were observed in both male and female rats in the 28-day studies. This is somewhat consistent with previous results in rats, where T4 was significantly reduced, but only in male rats. The NTP studies also reported thyroid follicular cell atrophy in both male and female rats, regardless of duration (28 days to 107 weeks). These data suggest that PFOA can affect thyroid tissue directly, but more research is needed to determine the full impact of PFOA on thyroid function (Table 5.4.4).

The most recent PFOS studies from (NTP, 2019b) support previous reports in the literature that PFOS reduces T3 and T4 levels in rats. The overall body of evidence from the animal literature suggests that PFOS negatively impacts thyroid hormone levels (Table 5.4.4). Recent mechanistic studies suggest that PFOS may interact with thyroid hormone transporters and receptors in animals, which is similar to results reported in mechanistic studies with human thyroid hormone transporters and receptors (US EPA, 2016d).

Table 5.4.4. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and thyroid toxicity

Outcome	PFOA	PFOS
Thyroid hormone levels in humans	Many studies but highly inconsistent results	Many studies but highly inconsistent results
Thyroid disease in humans	Limited number of studies, no convincing evidence	Limited number of studies, no convincing evidence
Thyroid hormone levels in animals	Limited number of studies, but positive trends were reported	Positive evidence

5.5. Developmental and Reproductive Toxicity

In its finalized *Toxicological Profile for Perfluoroalkyls*, ATSDR noted there is some epidemiologic evidence that PFOA and PFOS are associated with impaired fertility (longer time to pregnancy and infertility) but the results are not consistent across studies and some studies have found associations between maternal PFOA or PFOS exposure and decreased birth weight, which was attenuated by about 50% after accounting for maternal glomerular filtration rates (ATSDR, 2021). ATSDR also noted inconsistent findings related to several other reproductive outcomes associated with exposure to PFOA and PFOS. NTP has not published reviews of the epidemiologic literature on developmental and reproductive toxicity (DART) of PFOA or PFOS.

In the Health Effects Support Documents for PFOA and PFOS, US EPA's review of the epidemiologic literature on DART effects of each of these chemicals included literature published from 2009 through 2015 (US EPA, 2016b; US EPA, 2016d). US EPA's review focused on the outcomes of pregnancy-related hypertension, preeclampsia, measures of fetal growth, and pubertal development. Related to these outcomes, US EPA reviewed epidemiologic studies examining gestational age, measures of fetal growth, miscarriage,

preterm birth, birth defects, postnatal growth and maturation (including neurodevelopment and pubertal development), risk of adult obesity following prenatal exposure, pregnancy-related hypertension, preeclampsia, fecundity, sperm count, and semen quality (US EPA, 2016b).

For PFOA, US EPA found that the data on DART were suggestive of an association with risk of pregnancy-induced hypertension or preeclampsia and the possibility of an effect of reduced birth weight. US EPA also noted there was conflicting evidence from two studies for altered puberty onset in females and there were limited data suggesting reduced fertility and fecundity in females, and an association with attention deficit hyperactivity disorder in children in studies of both a highly exposed community and the general population.

For PFOS, US EPA reviewed epidemiologic studies examining measures of developmental outcomes such as fetal growth restriction, puberty, as well as pregnancy-related hypertension, preeclampsia, gestational diabetes, and fertility (US EPA 2016d). In summarizing, US EPA noted that despite study limitations, including uncertainty concerning the possible effect of low glomerular filtration rate (GFR), "…the association between PFOS exposure and birth weight for the general population cannot be ruled out."

GFR is the flow rate of blood being filtered by the kidneys. It increases in the first half of pregnancy and declines slightly in the second half of pregnancy. Women whose GFR fails to increase sufficiently in pregnancy have been shown to have smaller babies (Verner et al., 2016). GFR may also influence urinary excretion of PFAS; people with lower GFR have higher blood levels of PFAS (Verner et al., 2016). Thus, GFR may confound the association between PFAS and some DART outcomes, such as lower birth weight, because changes in both may be due in some part to effects of low GFR (Savitz, 2014; Verner et al., 2016). In addition, maternal plasma volume expands in early pregnancy, diluting PFAS concentrations. Lower plasma volume expansion is also associated with reduced fetal growth and possibly also reduced GFR (Vesterinen et al., 2015) and may confound any association between PFAS exposure and fetal growth. However, the potential for confounding by GFR and plasma volume expansion appears greater when PFAS exposure is based on measurements in blood obtained in late pregnancy. Pregnancy hemodynamics appear less likely to substantially confound studies of effects of PFAS on fetal growth when serum or plasma is sampled early in pregnancy (Verner et al., 2016; Sagiv et al., 2018; Steenland et al., 2018).

US EPA also noted that a small set of studies reported associations with gestational diabetes, preeclampsia, and pregnancy-induced hypertension in populations with serum PFOS concentrations of 12-17 ng/ml (US EPA, 2016d).

Furthermore, US EPA found that the overall data suggest a consistent association between PFOS exposure and fertility and fecundity measures, despite concern over GFR and reverse causation. Studies examining semen quality were largely null (US EPA, 2016d).

Given US EPA's findings regarding reproductive and developmental effects of PFOA and PFOS, OEHHA evaluated the most recent literature, published since US EPA's reviews, for the following outcomes: pregnancy-related hypertension and preeclampsia, measures of fetal growth, pubertal development, and fertility and fecundity.

Developmental and reproductive effects of PFOA in animals were described in assessments by US EPA (2016b), New Jersey DWQI (2017) and ATSDR (2021). US EPA (2016b) reported numerous reproductive effects in animals, including reduced fertility in male mice, decreased

litter size, increased resorptions and stillbirths, and increased time to parturition. US EPA (2016b) also reported multiple developmental effects in rodents, including reduced postnatal growth, delays in developmental landmarks, reduced ossification, and delayed mammary gland development. Based on this evidence, PFOA was listed under Proposition 65 as a chemical known to the state of California to cause reproductive toxicity in 2017.

Developmental and reproductive effects of PFOS in animals were also described in assessments by US EPA (2016d), New Jersey DWQI (2018) and ATSDR (2021). US EPA (2016d) identified no studies reporting reduced fertility, but reported histopathological lesions in male rat testes, and increased pup mortality. It was hypothesized that neonatal mortality is caused by the interaction of PFOS with lung surfactant components, which alters lung morphology and pulmonary surfactant function in developing rodents. Adverse developmental effects include reduced pup body weight, developmental delays, and altered hormone and glucose regulation. As such, PFOS was listed under Proposition 65 as a chemical known to the state of California to cause reproductive toxicity in 2017.

5.5.1. Recent Human Evidence

Pregnancy-related hypertension and preeclampsia: OEHHA identified two studies of the association between PFOA or PFOS exposure and preeclampsia published since January 2016 (Huang et al., 2019b; Wikström et al., 2019), one of which also examined pregnancy-induced hypertension (Huang et al., 2019b). Two additional studies re-examined previously published data to determine whether uncertainties in exposure or geocoding of reconstructed PFOA exposures biased the epidemiologic findings regarding associations between PFOA and preeclampsia. The authors found that these sources of uncertainty did not have significant impacts on those findings (Avanasi et al., 2016a; Avanasi et al., 2016b); these studies will not be discussed further.

The preeclampsia studies are summarized in Appendix 7, Tables A7.13 and A7.14. Preeclampsia is a condition in which the pregnant woman is hypertensive because of reduced renal excretion associated with a decrease in GFR (US EPA, 2016b). Wikström et al. (2019) conducted a prospective cohort study in Sweden using early pregnancy (median 10 weeks) serum PFAS measurements and preeclampsia in the same pregnancy. The median serum PFOA concentration was 1.61 ng/ml and the median PFOS concentration was 5.39 ng/ml. A doubling in early pregnancy serum PFOS was associated with increased risk of preeclampsia for all women (OR = 1.53; 95% CI, 1.07-2.20) and nulliparous women OR = 2.02; 95% CI, 1.26-3.29). When the comparison was highest vs. lowest quartile of serum PFOS, the increase in risk was greater: OR = 2.68; 95% CI, 1.17-6.12). ORs were adjusted for parity (except when stratified by parity), age, weight, and smoking (based on serum cotinine). PFOA was also associated with increased risk of preeclampsia, but the ORs were somewhat smaller and not statistically significant. Wikström et al. (2019) did not adjust for the presence of other PFAS. A strength of this study is the use of early pregnancy PFAS measurements, which would avoid potential confounding by GFR. The correlations among some PFAS were relatively high: for PFOA and PFOS, r = 0.60; for PFOA and PFNA, r = 0.66; and for PFOS and PFNA, r = 0.55. PFNA was also associated with preeclampsia, with ORs that were somewhat higher than those for PFOA and statistically significant.

In the Huang et al. (2019b) cross-sectional study, cord blood samples were collected from 686 women shortly after birth and tested for PFAS. The median serum PFOA concentration was 6.98 ng/ml and the median PFOS concentration was 2.38 ng/ml. Risk of gestational

hypertension was 3.3% and risk of preeclampsia was 2.8%, and 91.5% of the women were nulliparous. PFOA was associated with non-statistically significant increases in risk of preeclampsia, while PFOS was associated with non-statistically significant reductions in risk. Although the authors note that PFOS levels in cord blood are highly correlated with PFOS in maternal blood, they also acknowledge that previous studies have reported that PFOA and PFOS decreased across pregnancy due to increased GFR and subsequent increased rate of elimination in urine. It is therefore possible that the measured PFAS levels do not represent levels at other points in the pregnancy that may have been more relevant to risks of hypertensive disorders of pregnancy (Huang et al., 2019b).

Huang et al. (2019b) also examined gestational hypertension. While ORs for the second tertile of cord serum PFOA and PFOS, compared to the first tertile, suggested protective effects, the associations were not statistically significant. The ORs for continuous exposure also suggested no associations (Huang et al., 2019b).

Measures of fetal growth – birth weight: The large number of studies examining PFOA and birth weight are summarized in Appendix 7, Table A7.15. Two recent cross-sectional studies reported statistically significant lower birth weight among children with higher PFOA concentrations in maternal or cord serum around the time of birth. Kwon et al. (2016) reported a change of −77.93 g (95% CI, −153.56 - −2.30) per unit change in log PFOA, and Li et al. (2017c) reported that each ln-unit change in total PFOA (i.e., linear and branched) was associated with a change of –112.7 g (95% CI, –171.9 - –53.5) and each ln-unit change in linear PFOA (n-PFOA) was associated with a change of –85.0 g (95% CI, –133.6 - –36.5) in birth weight. The findings from both of these studies are not adjusted for GFR or plasma volume expansion, which could confound the associations with samples in late pregnancy or around the time of birth (Verner et al., 2016; Govarts et al., 2018; Sagiv et al., 2018).

Six prospective cohort studies and a case-cohort study also reported statistically significant lower birth weight in association with serum or plasma PFOA concentrations during pregnancy. Two of these studies included samples with relatively high risk for lower birth weight. The largest change in birth weight was reported by Lauritzen et al. (2018) for the Swedish sub-sample (N=159, median 2nd trimester maternal serum PFOA concentration = 2.33 ng/ml): for male infants, β = –526 g (95% CI, –828 - –222) per ln-unit increase in PFOA. This case-cohort study included SGA cases, from Sweden and Norway, selected predominantly (82%) from parous participants who were considered high-risk for SGA; however, results for the Norwegian sub-sample were null (Lauritzen et al., 2018). Hjermitslev et al. (2020) studied PFOA exposure in Greenlandic Inuit women with high smoking rates and possibly high exposure to persistent organic pollutants through traditional diet, although the serum PFOA concentrations (sampled between 7 and 40 weeks gestation for one group and before the end of gestation week 13 for another group) were not high: median (range) = 1.06 (0.10-7.26) ng/ml. A 1 ng/ml increase in maternal serum PFOA was associated with a change in birth weight of –119 g (95% CI, –202 - –36.6) (adjusted for GA and other potential confounders) (Hjermitslev et al., 2020).

Meng et al. (2018) reported a more modest change in birth weight of –35.6 g (95% CI, –66.3 - –5.0) per doubling of PFOA measured in maternal plasma in the first two trimesters (92% in first trimester). Wikström et al. (2019) reported a ln-unit increase in maternal serum PFOA sampled at 10 weeks (median) was associated with a change in birth weight of –68 g (95% CI, –112 - –24) for all children, with a slightly stronger association in girls of –86 g (95% CI, –145 - –26). For both of these studies, the serum samples were generally taken before changes associated with GFR and increased blood volume would be expected to become important confounders.

For the remaining cohort studies that reported associations with lower birth weight, coefficients were β = –51.4 g (95% CI, –97.2 - –5.7) per ln-unit PFOA increase (Starling et al., 2019), β = –63.77 g (–122.83 - –4.71) per 2-SD increase in ln PFOA (Lenters et al., 2016), and β = –197 g (95% CI, –391 - –3) per log-unit PFOA increase (Minatoya et al., 2017). Blood samples were taken at various times or in late pregnancy in these studies, and no consideration of potential confounding by GFR was reported. Starling et al. (2019) adjusted for gestational weight gain and both Starling et al. (2019) and Minatoya et al. (2017) adjusted for gestational age at blood draw.

Twenty-three of the recent studies, including 11 prospective cohort, one retrospective cohort, and 11 cross-sectional studies, reported no statistically significant associations between prenatal PFOA and birth weight or birth weight *z*-score. Four large cohort studies with sample sizes ranging from N=1,202 to N=1,705 assessed PFOA exposure mainly in the first trimester of pregnancy, thereby minimizing concerns over confounding or reverse causation associated with GFR or weight gain, and reported no statistically significant associations between prenatal PFOA exposure and birth weight or birth weight *z*-score (Bach et al., 2016; Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017; Sagiv et al., 2018). The median PFOA concentrations in these studies were between 1.7 ng/ml and 5.8 ng/ml in maternal plasma (Ashley-Martin et al., 2017; Manzano-Salgado et al., 2017; Sagiv et al., 2018) and 2.0 ng/ml in maternal serum (Bach et al., 2016).

Some investigators examined possible sex differences in effects of prenatal PFOA exposure on birth weight. Of the six studies that reported possible stronger associations between PFOA and lower birth weight in girls, two reported statistically significant associations in girls but not boys: Hjermitslev et al. (2020) reported β = –161 g (95% CI, –283 - –40.1) for female infants, and β = –81.2 g (95% CI, –194 - 31.2) for male infants. Wikström et al. (2019) reported β = –86 g (95% CI, –145 - –26) for girls and β = –49 g (–113 - 15) for boys. Of four studies reporting a possible stronger association with lower birth weight in boys, Lauritzen et al. (2017) reported a statistically significant association, β = –526 g (95% CI, –828 - –222) in boys and β = –156 g (95% CI, –541 - 228) in girls.

Studies examining PFOS and birth weight are summarized in Appendix 7, Table A7.16. Three recent studies reported large, statistically significant associations between prenatal PFOS exposure and lower birth weight in small to modest samples: one cross-sectional study (N=98) reported that each log-unit increase in umbilical cord serum PFOS was associated with a change in birth weight of –417.3 g (95% CI, –742.1 - –92.4). The mean PFOS concentration was 4.07 ng/ml (Xu et al., 2019a). Another cross-sectional study (N=317) with a median cord serum PFOS concentration of 3.0 ng/ml reported that each ln-unit increase in PFOS was associated with a change in birth weight of –150.6 g (95% CI, –225.4 - –75.7) in male infants and –26.6 g (95% CI, –125.1 - 71.8) in females (Li et al., 2017c). Although the authors of these studies adjusted for some important potential confounders, the possibility of confounding or reverse causation associated with GFR due to exposure assessment at time of birth is of some concern, as are the substantial changes with adjustment for confounding. Lauritzen et al. (2017) conducted a case-cohort study with SGA cases selected predominantly from parous participants who were at high-risk for SGA in Sweden and Norway. In the Swedish sub-sample (N=159, median 2nd trimester maternal serum PFOS concentration = 16.4 ng/ml), the change in mean birth weight was –292 g (95% CI,–500 - –84) per ln-unit increase in PFOS. Results for the Norwegian sub-sample (N=265), with a median serum PFOS concentration of 9.74 ng/ml, were null (Lauritzen et al., 2017).

Three large prospective cohort studies, each of which included more than 1,500 participants, assessed PFOS exposure in early pregnancy and reported statistically significant associations with lower birth weight. Bach et al. (2016), with a median maternal serum PFOS concentration of 8.3 ng/ml, observed associations only for 2nd quartile compared to 1st quartile exposure, β = –86 g (95% CI, –159 - –13) for all births and β = –93 g (95% CI, –157 - –29) for term births. The association per IQR (4.8 ng/ml) of PFOS was null for all births, with contrasting but non-statistically significant associations in girls (β = –32 g (95% CI, –71 - 7)) and boys (β = 26 g (95% CI, –13 - 65)) (Bach et al., 2016). Wikstrom et al. (2019), with a median maternal serum PFOS concentration of 5.38 ng/ml, reported a change in birth weight of –46 g (95% CI, –88 - –3) per ln-unit increase in PFOS for all children, –85 g (95% CI, –145 - –25) for girls, and –13 g (95% CI, –73 - 47) for boys (Wikström et al., 2019). Meng et al. (2018) reported a change in birth weight of –45.2 g (95% CI, –76.8 - –13.6) per doubling of PFOS exposure in a population with a median maternal plasma PFOS concentration of 30.1 ng/ml (Meng et al., 2018). By assessing PFOS exposure early in pregnancy, these prospective studies reduce concerns about confounding and reverse causation associated with GFR and increased blood volume.

Two further prospective cohort studies with later pregnancy exposure assessment reported associations with reduced birth weight. In the study by Marks et al. (2019), each unit increase in PFOS concentration (median 13.8 ng/ml) was associated with a change in birth weight of −8.50 g (95% CI, −15.93 - −1.07). In a sensitivity analysis including only 1st trimester samples (N=115), associations were consistent with the entire study sample. Valvi et al. (2017) reported a change in birth weight of approximately −150 g ($p < 0.05$; presented graphically) for boys of the Faroe Islands with median maternal serum PFOS of 27.2 ng/ml.

One study (N=62) reported that prenatal PFOS exposure was associated with an increase in birth weight of 596 g (95% CI, 89 - 1,103) in girls. The median umbilical cord plasma PFOS concentration was 1.600 ng/ml and there was no association in boys (de Cock et al., 2016). No other studies reported statistically significant increases in birth weight associated with PFOS.

Twenty-two studies, including eight cross-sectional, one retrospective cohort, and 14 prospective cohort studies, reported no statistically significant associations between prenatal PFOS exposure and birth weight, term birth weight, birth weight *z*-score, or birth weight for GA *z*-score. Some of these studies included large samples, and PFOS concentrations ranged from less than 1 ng/ml in cord serum to more than 25 ng/ml in maternal plasma or maternal serum. For example, in a prospective cohort study (N=1,202), Manzano-Salgado et al. (2017) assessed PFOS in maternal plasma (median 6.05 ng/ml) in the first half of pregnancy and reported no association with birth weight. Sagiv et al. (2018) also assessed PFOS in early pregnancy and evaluated many potential confounders, including GFR and plasma albumin, and reported that maternal plasma PFOS (median 25.7 ng/ml) was not statistically significantly associated with term birth weight (β = −17.9 g (95% CI, −40.9 - 5.1)) in this cohort of 1,645 mother-infant pairs.

Measures of fetal growth – small for gestational age: Six studies, summarized in Appendix 7, Table A7.15, examined associations between exposure to PFOA and risk of SGA infants (Wang et al., 2016; Lauritzen et al., 2017; Manzano-Salgado et al., 2017; Govarts et al., 2018; Wikström et al., 2019; Xu et al., 2019a). Two of the smaller studies, one of which was a cross-sectional study in Hangzhou, China and had low exposure concentrations (median umbilical cord serum concentration=1.05 ng/ml) (Xu et al., 2019a), and one of which was a prospective cohort study in Taiwan with median 3rd trimester maternal serum PFOA concentrations of 2.34 ng/ml and 2.37 ng/ml for female and male infants, respectively (Wang et al., 2016), reported no associations with SGA.

The remaining four studies were conducted in Europe and had conflicting results within and between studies. The case-cohort study by Lauritzen et al. (2017) included parous participants from Sweden and Norway and sampled for women at high risk of SGA. In the sample of women from Norway (N=265), whose median PFOA concentration in second trimester maternal serum was 1.62 ng/ml, PFOA was associated, though not statistically significantly, with lower odds of SGA, OR = 0.66 (95% CI, 0.33-1.33). In Swedish women (N=159), with a median second trimester maternal serum PFOA concentration of 2.33 ng/ml, prenatal PFOA exposure was associated with a large and statistically significant increase in risk of SGA for all children (OR = 5.25; 95% CI, 1.68-16.4), and for boys (OR = 6.55; 95% CI, 1.14-37.5); but the increase was not statistically significant for girls (OR = 4.73; 95% CI, 0.79-28.3) (Lauritzen et al., 2017).

Govarts et al. (2018) analyzed pooled cross-sectional data for cohorts from Belgium, Norway, The Netherlands, and Slovakia (N=693), and reported evidence of an increase in risk for SGA overall, OR = 1.64 (95% CI, 0.97-2.76) per IQR cord serum PFOA. The association was statistically significant among mothers who smoked during pregnancy, OR = 2.18 (95% CI, 1.02-4.64), but not among nonsmokers, OR = 1.51 (0.87-2.63). Some cord serum PFOA levels were measured, but for two cohorts and part of a third cohort, cord serum PFOA levels were estimated using PFOA measured in breast milk. The median (IQR) cord serum PFOA concentration was 0.550 (0.299-1.200) ng/ml but varied by cohort. The magnitude, but not the direction, of the effect varied by cohort (data not reported). The authors did not address potential confounding by GFR (Govarts et al., 2018).

In a prospective cohort study with 1,202 participants in Spain, Manzano-Salgado et al. (2017) reported that first trimester maternal PFOA (median concentration 2.35 ng/ml) was associated with increased risk of SGA in male infants, OR = 1.18 (95% CI, 0.82-1.69) and decreased risk (protective effect) in female infants, OR = 0.72 (95% CI, 0.50-1.04), though neither was statistically significant. In another large prospective cohort study in Sweden (N=1,533), Wikström et al. (2019) reported increased risk of SGA birth associated with first trimester PFOA exposure (median serum concentration 1.61 ng/ml) for all children, OR = 1.43 (95% CI, 1.03-1.99), driven mainly by increased risk among girls, OR = 1.96 (95% CI, 1.18-3.28). The association was weaker and not statistically significant for boys, OR = 1.16 (95% CI, 0.75-1.78) (Wikström et al., 2019).

Five recent epidemiologic studies, summarized in Appendix 7, Table A7.16, examined the association between maternal PFOS exposure and SGA birth. Xu et al. (2019a) conducted a small (N=98) cross-sectional study using cord serum PFOS (median concentration of 4.07 ng/ml). The analysis was adjusted for important confounders and tap water consumption, but not GFR. (Xu et al., 2019a) reported a large association, with adjusted OR = 4.138 (95% CI, 1.07-15.98) per log-unit increase in PFOS; the unadjusted OR = 1.64. There was a large change in the OR after adjustment (Xu et al., 2019a).

Govarts et al. (2018) analyzed pooled cross-sectional data from four cohorts (N=693), and reported cord serum PFOS was associated with increased risk of SGA among mothers who smoked during pregnancy, OR = 1.63 (95% CI, 1.02-2.59). Among nonsmokers, PFOS was associated with decreased risk of SGA, OR = 0.66 (95% CI, 0.61-0.72). The median (IQR) cord serum PFOS concentration was 1.98 ng/ml but varied by cohort. Cord serum PFOS levels were either measured directly, or for most participants, PFOS levels were estimated based on breast milk PFOS levels. The magnitude, but not the direction, of the effect varied by cohort (data not reported). The authors did not address potential confounding by GFR (Govarts et al., 2018).

The case-cohort study by Lauritzen et al. (2017) included parous participants from Sweden and Norway and sampled for women at high risk of SGA. In the sample of women from Norway (N=265), with median second trimester PFOS concentration of 9.74 ng/ml, maternal PFOS exposure was not associated with SGA. In the Swedish cohort (N=159, median PFOS concentration = 16.4 ng/ml), a ln-unit increase in maternal PFOS was associated, though not statistically significantly, with SGA, OR = 2.51 (95% CI, 0.93-6.77) (Lauritzen et al., 2017).

In a prospective cohort study with 1,202 participants, Manzano-Salgado et al. (2017) reported that first trimester maternal PFOS (median concentration 6.05 ng/ml) was not associated with increased risk of SGA. In another large prospective cohort study (N=1,533), (Wikström et al., 2019) reported some non-statistically significant associations with first trimester PFOS exposure (median serum concentration 5.38 ng/ml). For all children, OR = 1.19 (95% CI, 0.87-1.64); girls, OR = 1.40 (95% CI, 0.83-2.35); and boys, OR = 1.08 (95% CI, 0.72-1.63) per ln-unit increase in prenatal serum PFOS (Wikström et al., 2019).

Pubertal development: OEHHA identified two recent retrospective cohort studies of PFOA and pubertal development (Appendix 7, Table A7.17), one of which also investigated the relationship between PFOS and pubertal development. Ernst et al. (2019) collected multiple puberty-related indicators at multiple times from most of their study participants beginning at age 11 years, and had PFOA measurements from the first trimester of the children's gestation. Plasma PFOA medians were reported separately for boys and girls in the two groups (samples) of study participants identified from within the cohort as a whole, and ranged between 4.1 and 5.1 ng/ml. No consistent patterns of associations between PFOA and pubertal indicators were evident in this cohort (Ernst et al., 2019).

Di Nisio et al. (2020), in contrast, assessed onset of menarche when participants were approximately 18 years old, and used lifelong residence in the area as a proxy for exposure. In a subset of participants with actual PFOA measurements, the median was 28.7 ng/ml in serum of exposed participants, and 2.6 ng/ml in "control" participants. The mean age at menarche was 164 days ($p < 0.001$) later in those who lived in the high PFOA area. PFOS concentrations were similar in the two groups (3.25 and 3.15 ng/ml) (Di Nisio et al., 2020).

Ernst et al. (2019) also examined PFOS in association with pubertal development and reported that in girls, early gestation PFOS exposure in the middle tertile (28.1-38.4 ng/ml and 23.3-31.5 ng/ml in the two study participant 'samples') was associated with earlier indicators of pubertal development compared to the lowest tertile (Appendix 7, Table A7.18). The highest exposure tertile was not consistently associated with pubertal development in girls. In boys, PFOS exposure above the lowest tertile appeared to be associated with earlier genital development and voice break, although most comparisons were not statistically significant. PFOS was not associated with other indicators of puberty in boys (Ernst et al., 2019).

Fertility and fecundity: Four recent studies examined possible associations between a woman's exposure to PFOA and fertility or fecundity (Appendix 7, Table A7.19). A cross-sectional study of 1,251 pregnant women examined early pregnancy plasma PFOA levels (median 5.03 ng/ml in nulliparous women and 3.43 ng/ml in parous women) and reported no association with time to pregnancy (TTP; an indicator of fecundability, which is defined as the probability of pregnancy per cycle) in nulliparous women. Although a statistically significant association with reduced fecundability in parous women was observed, the authors hypothesized that "the association in parous women was due to residual confounding" (Bach et al., 2018). A small (N=99) prospective cohort study also found no association between pre-

conception serum PFOA (geometric mean 2.79 ng/ml) and fecundability or ovarian reserve in women who had no history of fertility or fertility-related conditions (Crawford et al., 2017).

A very small prospective cohort study in a fertility clinic examined 34 women with a mean plasma PFOA concentration of 2.44 ng/g before in vitro fertilization (IVF). There were no statistically significant correlations between PFOA and blastocyst conversion, fertilization, ovarian function, or response to gonadotropin stimulation (McCoy et al., 2017). Another fertility clinic study was a case-control study with 157 cases of endometriosis-related infertility and 178 controls who had no reproductive endocrine disorders but were seeking infertility treatment for male reproductive dysfunction. Plasma PFOA (median 14.67 ng/ml among cases and 12.09 ng/ml among controls) was not associated with endometriosis-related infertility (Wang et al., 2017a)**.**

The four studies that examined the relationship between PFOA and fertility or fecundity also examined PFOS (Appendix 7, Table A7.20). In a cross-sectional study of 1,251 pregnant women, Bach et al. (2018) found no association between early pregnancy plasma PFOS (median concentration 30.2 ng/ml in nulliparous women and 26.0 ng/ml in parous women) and TTP in nulliparous women, but reduced fecundability in parous women. However, the authors attributed the association in parous women to confounding (Bach et al., 2018). A small (N=99) prospective cohort study of women who had no history of infertility or fertility-related conditions and were attempting to conceive found no association between pre-conception serum PFOS (geometric mean 9.29 ng/ml) and fecundability or ovarian reserve (Crawford et al., 2017).

McCoy et al. (2017) examined associations between plasma PFOS before IVF treatment (mean concentration 6.52 ng/g) and ovarian function, ovarian response, and fertilization among women undergoing IVF (N=34). Higher plasma PFOS levels were associated with lower plasma estradiol, r = –0.47 pg/ml ($p < 0.05$), but no other outcomes (McCoy et al., 2017).

A case-control study in a fertility clinic compared 157 cases of endometriosis-related infertility with 178 controls who had no reproductive endocrine disorders but were seeking infertility treatment for their male partners' reproductive dysfunction. The median plasma PFOS concentration was 6.40 ng/ml among cases and 6.60 ng/ml among controls. Women in the highest PFOS exposure tertile were less likely to have endometriosis-related infertility than women in the lowest tertile, OR = 0.66 (95% CI, 0.36 - 1.21), though the association was not statistically significant. The association was similar in an analysis that was restricted to women who had never been pregnant. In a sensitivity analysis, plasma PFOS was statistically significantly associated with lower odds of endometriosis-related infertility, OR = 0.47 (95% CI, 0.22 - 0.99), when restricted to women who had no other gynecologic pathology. This study also reported inverse or null associations between endometriosis-related infertility and other PFAS, with the exception of PFBS, which was associated with increased risk of endometriosis-related infertility (Wang et al., 2017a)**.**

5.5.2. Recent Animal Evidence

Studies of PFOA exposure reporting developmental and reproductive toxicity effects published from 2016 onward are summarized in Tables 5.5.1, 5.5.2, and 5.5.3.

Table 5.5.1. Summary of recent animal toxicity studies of PFOA reporting developmental toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant C57BL/6/Bkl mice (6/dose)	0 or 0.3 mg/kg-day in diet from GD 1-21	**Pups:** ↑ body weight; changes in femur and tibia bone morphometric properties; ↓ bone tissue mineral density	NA[a]	Koskela et al. (2016)
Pregnant C57BL/6J mice (6/dose for dams; 6-10/dose for pups)	Dietary exposure to 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, or 3 mg/kg-day (targeted dose to dams); exposure started 2 weeks before mating and continued during mating (1 week), gestation (3 weeks), and lactation (3 weeks)	**Dams:** ↓ litter size at two highest doses **Pups (both sexes):** ↓ body weight at PND 4; nuclear dysmorphology (p=0.06) **Male pups:** ↑ liver weight; ↑ eosinophilic liver foci (p=0.07)	**Dams:** NOAEL: 0.3 mg/kg-day for ↓ litter size **Pups:** NOAEL: 0.003 mg/kg-day for ↓ body weight in females on PND 4	van Esterik et al. (2016)
Pregnant CD-1 mice, male pups used for behavioral tests, 1 per litter per test. (4-17/dose, varied by behavioral test)	0, 0.1, 0.3, or 1 mg/kg-day via gavage from GD 1-17	**Pups:** ↑ ambulatory activity on PND 18; ↓ activity in animals injected with methamphetamine	NOAEL: 0.3 mg/kg-day based on ↑ ambulatory activity	Goulding et al. (2017)
Pregnant Kunming mice (10/dose), male offspring (7-10/dose) evaluated for effects on PND 21 and PND 70	0,1 ,2.5, or 5 mg/kg-day via gavage from GD 1-17	**Pups:** ↓ number of surviving mice at weaning; changes in absolute testis weight; ↓ serum testosterone levels (except in low dose animals on PND 70); ↓ Leydig cells; vacuolization of Sertoli cells; ↓ spermatozoa	LOAEL: 1 mg/kg-day for ↓ serum testosterone	Song et al. (2018)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant Kunming mice (10/dose), female pups (5-10/dose)	0, 1, 2.5, 5, or 10 mg/kg-day via gavage from GD 1-17	**Pups:** ↓ body weight; ↓ survival; ↑ absolute and relative liver weight; swollen hepatocytes; liver cell vacuolar degeneration and dissolved nuclei; blurred liver architecture; ↑ serum ALT and AST; ↑ CAT, SOD, and 8-OHdG; ↓ histone acetylation	LOAEL: 1 mg/kg-day for ↑ serum ALT and AST	Li et al. (2019c)

[a] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GD, gestation day; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PND, postnatal day; SOD, superoxide dismutase; 8-OHdG, 8-hydroxydeoxyguanosine

van Esterik et al. (2016) evaluated the developmental toxicity of PFOA in C57BL/6J mice. Toxicity in the F1 generation was monitored in 6-10 pups from 2-5 litters in each dose group. Decreased litter sizes were reported at the two highest doses. Additionally, several developmental effects were reported in pups, including the following: increased liver weight, and eosinophilic liver foci (p=0.07) in males; decreased femur length and femur weight, decreased quadriceps femoris muscle weight, decreased adipocyte cell size, and decreased serum triglycerides and cholesterol in females; and decreased body weight at PND 4, decreased tibia length, and hepatocellular anisokaryosis and karyomegaly in pups of both sexes (p=0.06). The decrease in body weight persisted until adulthood on a standard diet, but reverted to control levels once animals were placed on a high fat diet. Body weight data on PND 4 were digitized using GetData Graph Digitizer software (version 2.26), and evaluated for statistical significance ($p < 0.001$; student's T-test determined by OEHHA). OEHHA determined a NOAEL of 0.003 mg/kg-day based on decreased body weight in female pups on PND 4.

Table 5.5.2. Summary of recent animal toxicity studies of PFOA reporting reproductive toxicity in male animals

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male BALB/c mice (20/dose)	0, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	↓ fertility; ↓ litter weight after mating; disruption of blood-testis barrier; ↑ testicular IgG; ↑ TNF-α in testis	LOAEL: 1.25 mg/kg-day for disruption of blood-testis barrier	Lu et al. (2016a)
Male BALB/c mice (11/dose)	0, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	**Epididymis:** ↓ triglycerides and cholesterol; ↓ relative weight; ↑ MDA levels; ↓ GSH-Px levels	LOAEL: 1.25 mg/kg-day for ↓ in relative epididymis weight	Lu et al. (2016b)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male BALB/c mice (6/dose)	0, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	changes in levels of proteins involved with endocytosis and blood-testis barrier	NOAEL: 1.25 mg/kg-day	Lu et al. (2017)
Male Kunming mice (number not specified)	0 or 10 mg/kg-day intragastrically for 21 days	atrophy of seminiferous tubules; disorganization of seminiferous epithelium; absence of spermatozoa; depletion of spermatogonial cells; detachment of germ cells; ↓ absolute testis weight; ↓ epididymal sperm count; ↑ MDA; ↓ SOD and CAT activity	NA[a]	Yuan et al. (2017)
Male BALB/c mice (15/dose)	0, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	changes in CBG protein levels in testes; ↑ CBG and corticosterone levels in serum; ↓ adrenocorticotropic hormone levels in serum	LOAEL: 1.25 mg/kg-day for ↑ CBG levels in testis and serum	Sun et al. (2018b)
Male C57BL/6 mice (6-8/dose)	0, 0.55, 5.5, or 28 mg/L in drinking water for 5 weeks (0, 0.1, 1, and 5 mg/kg-day)	no observed DNA damage in testis; no change in testicular weight	NOAEL: 5 mg/kg-day	Crebelli et al. (2019)
Male Sprague Dawley rats (12/dose)	0, 25, or 50 mg/kg-day via gavage for 9 days; PFOA administration started 7 days after EDS treatment to kill Leydig cells	slowed recovery of serum testosterone; ↓ PCNA+ cells	LOAEL: 25 mg/kg-day for slowed recovery of serum testosterone	Lu et al. (2019)
Male and female Sprague Dawley rats (10/sex/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day for 28 days via gavage	**Males:** ↑ relative testis weight; ↓ absolute epididymis weight; ↓ cauda epididymis weight; ↓ cauda epididymis sperm count	NOAEL: 2.5 mg/kg-day for ↑ relative testis weight and ↓ cauda epididymis weight	NTP (2019a)
Male Sprague Dawley rats (10/dose)	0, 150, or 300 ppm in feed (0, 14.7, or 29.5 mg/kg-day) for 16 weeks	↓ absolute testis weight	NOAEL: 14.7 mg/kg-day	NTP (2020)

[a] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: CAT, catalase; CBG, corticosteroid binding globulin; EDS, ethane dimethyl sulfonate; GSH-Px, glutathione peroxidase; IgG, immunoglobulin G; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; PCNA, proliferating cell nuclear antigen; PFOA, perfluorooctanoic acid; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha

Table 5.5.3. Summary of recent animal toxicity studies of PFOA reporting reproductive toxicity in female animals

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male and female C57BL/6 mice (6/sex/dose)	0 or 3.5 mg/kg of feed (~0.55 mg/kg-day, according to authors)	↑ cholesterol in the ovary ($p=0.069$) and mammary glands ($p <0.05$)	NA[a]	Rebholz et al. (2016)
Pregnant Kunming mice (12/dose)	0, 2.5, 5, or 10 mg/kg-day via gavage from GD 1-7 or GD 1-13	**Dams:** ↑ number of resorbed embryos; ↑ serum estradiol; ↓ serum progesterone; ↓ number of corpora lutea; ↓ ratio of corpora lutea to ovarian areas; ↑ CAT and SOD activity, H_2O_2, and MDA levels in ovary; ↑ apoptosis protein markers (p53 and Bax) in ovary	LOAEL: 2.5 mg/kg-day for ↑ oxidative stress, ↑ apoptosis markers and ↓ in number of corpora lutea	Chen et al. (2017b)
Pregnant Kunming mice (10/dose)	0 or 20 mg/kg-day via gavage from GD 1-7	**Dams:** ↓ absolute and relative uterus weight; ↑ markers of uterine apoptosis	NA[a]	Song et al. (2019)
Female Sprague Dawley rats (10/dose)	0, 300, or 1,000 ppm in feed (0, 27.7, or 92.7 mg/kg-day) for 16 weeks	ovarian cysts	NOAEL: 27.7 mg/kg-day	NTP (2020)
Female Sprague Dawley rats (50/dose)	0, 300, or 1,000 ppm in feed (0, 18, or 63 mg/kg-day) for 107 weeks	squamous metaplasia in the endometrium	LOAEL: 18 mg/kg-day	NTP (2020)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant CD-1 mice (11-13 dams/dose)	0, 1, or 5 mg/kg-day via gavage from embryonic day 1.5 to embryonic day 11.5 or embryonic day 17.5	**Dams:** ↑ relative gestational weight gain; ↑ absolute, but ↓ relative placenta weight; placental lesions (labyrinth congestion, atrophy, fibrin clots, necrosis, and nodules) **Embryos:** ↓ viable weight	**Dams:** NOAEL: 1 mg/kg-day for placenta effects **Embryos:** NOAEL: 1 mg/kg-day	Blake et al. (2020)

[a] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: CAT, catalase; GD, gestation day; H_2O_2, hydrogen peroxide; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; SOD, superoxide dismutase

For PFOS, studies reporting developmental and reproductive toxicity effects published from 2016 onward are summarized in Tables 5.5.4, 5.5.5, and 5.5.6.

Table 5.5.4. Summary of recent animal toxicity studies of PFOS reporting developmental toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant Sprague Dawley rats (10/dose)	0, 5, or 20 mg/kg via gavage from GD 12-18	**Dams:** ↓ body weight; ↓ placenta weight; **Pups:** ↓ fetal body weight in males; ↑ serum corticosterone	LOAEL: 5 mg/kg-day for ↑ serum corticosterone in pups	Li et al. (2016a)
Pregnant mice (strain not specified, 3-5/dose)	0, 1, 10, or 20 mg/kg-day orally[a] from GD 1-14	**Fetus:** ↑ body weight; ↑ crown-rump length; brain necrosis; umbilical hernia; liver enlargement; brain anomaly	NOAEL: 1 mg/kg-day for ↑ crown-rump length	Mehri et al. (2016)
Pregnant C57BL/6 mice (10-12/dose), pups (12/dose)	0, 0.1, 1, or 5 mg/kg-day via gavage from GD 1-17	**Male pups:** ↓ serum testosterone; ↑ estradiol	NOAEL: 0.1 mg/kg-day for ↓ serum testosterone[b]	Zhong et al. (2016)
Pregnant CD-1 mice (6/dose)	0 or 0.3 mg/kg-day via gavage from GD 1-18.5	No apical toxicity endpoints observed	NA[c]	Lai et al. (2017a)
Pregnant CD-1 mice (6-8/dose), male pups (4/dose)	0, 0.3, or 3 mg/kg-day via gavage throughout gestation	**Male pups:** ↓ serum testosterone; ↓ epididymal sperm count; changes in polyunsaturated fatty acid levels	NOAEL: 0.3 mg/kg-day for ↓ serum testosterone and epididymal sperm count	Lai et al. (2017b)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Pregnant C57BL/6 mice (6/dose), pups (12/dose)	0 or 0.3 mg/kg-day via gavage throughout gestation; animals injected with 5 mg/kg DEN on PND 15	↑ ALT and AST in pups	NA[c]	Lai et al. (2017b)
Pregnant CD-1 mice (8/dose)	0, 0.5, 2.5, or 12.5 mg/kg-day via gavage from GD 1-17	↓ fetal weight; ↓ crown-rump length; ↓ placental weight and diameter; placental histopathology (reduced blood vessel branching, vascular collapse, atresia, basement membrane breakage)	LOAEL: 0.5 mg/kg-day for reduced placental weight on GD 18	Chen et al. (2018a)
Pregnant Kunming mice (5/dose)	0, 0.5, or 5 intragastrically throughout gestation (20.5 days)	**Pups:** ↑ liver triglycerides, total cholesterol, and LDL; ↓ HDL	NOAEL: 0.5 mg/kg-day	Liang et al. (2019)
Pregnant Sprague Dawley rats (4-5/dose for dams, 8 pups per litter tested)	0 or 1 mg/kg-day in gelatin to dams from GD 1 to PND 21	**Dams:** no observed adverse effects **Pups:** ↑ activity in open-field behavioral tests	NA[c]	Reardon et al. (2019)

[a] The specific manner of oral administration was not stated in study.
[b] Statistically significant effect at the mid-dose, but not at the high dose.
[c] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: DEN, diethylnitrosamine; GD, gestational day; HDL, high density lipoprotein; LDL, low density lipoprotein; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PND, postnatal day

Table 5.5.5. Summary of recent animal toxicity studies of PFOS reporting reproductive toxicity in male animals

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Male ICR mice (10/dose)	0, 0.5, 5, or 10 mg/kg-day via gavage for 4 weeks	↓ sperm count; ↓ blood-testis barrier integrity	NOAEL: 0.5 mg/kg-day	Qiu et al. (2016a)
Male C57 mice (12/dose)	0, 0.5, or 10 mg/kg-day via gavage for 5 weeks	↓ absolute and relative testis weight, sperm count, serum testosterone levels; vacuolization in spermatogonia, spermatocytes and Leydig cells; ↑ apoptotic cells in testes, apoptosis related proteins; ↑ ERα and ERβ protein expression; ↓ PCNA+ cells	LOAEL: 0.5 mg/kg-day for ↓ testis weight, ↑ testicular lesions, and ↑ apoptosis	Qu et al. (2016)
Male Sprague Dawley rats (5/dose)	0, 5, or 10 mg/kg-day via gavage for 21 days	↓ absolute testis and seminal vesicle weight; ↓ serum testosterone; ↓ sperm count; delayed Leydig cell differentiation	LOAEL: 5 mg/kg-day for ↓ sperm count and ↓ seminal vesicle weight	Li et al. (2018b)

Abbreviations: ERα,β, estrogen receptor alpha, beta; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PCNA, proliferating cell nuclear antigen

Table 5.5.6. Summary of recent animal toxicity studies of PFOS reporting reproductive toxicity in female animals

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Female ICR mice (20/dose)	0 or 10 mg/kg-day orally for 30 days	Prolongation of duration of diestrus; ↓ number of corpora lutea; ↓ serum levels of P4, LH and GnRH on day 7; ↓ serum levels of GnRH, E2, T4 and T3 on day 14; ↑ serum levels of CORT on day 14	NA[a]	Wang et al. (2018)
Male and female Sprague Dawley Rats (10/sex/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day for 28 days via gavage	**Females:** ↑ testosterone	NOAEL: 0.625 mg/kg-day for ↑ testosterone	NTP (2019b)

[a] LOAEL/NOAEL not applicable for single dose studies.
Abbreviations: CORT, corticosterone; E2, estradiol; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; P4, progesterone; T3, triiodothyronine; T4, thyroxine

5.5.3. Recent Mechanistic Evidence

Developmental toxicity

Animal studies have reported altered organ differentiation in offspring exposed to PFOA during gestation. To investigate mechanisms by which PFOA could affect organ development, a number of in vitro studies have looked at expression of key genes during the developmental stage. In a study comparing the effects of endocrine disrupting chemicals on rhesus monkey embryonic stem cells, PFOA was found to alter the expression of genes related to cellular infiltration by leukocytes, lung injury, liver necrosis, hypertrophy, and stress response (Midic et al., 2016). PFOA altered mRNA and protein expression in the testicular Dlk1-Dio3 imprinted gene cluster (important for the control of growth and development) in F1 rats exposed in utero (Song et al., 2018). Zhou et al. (2017) reported that PFOA induced changes in myocardial differentiation (measured by expression of the myosin heavy chain 6 (myh6)) gene in mouse embryonic R1 stem cells.

Stress response from PFOA exposure can alter organ development. PFOA induced an increase in ROS in human mesenchymal stem cells, and also altered expression of genes that encode surface marker proteins involved in cell-cell interactions, potentially affecting the cells' ability to both differentiate and self-renew (Liu et al., 2019a).

Changes in adiposity have been seen in animals and in humans from prenatal exposure to PFOA. These changes have been linked to activation of PPARγ. Liu et al. (2019a) observed upregulation of adipogenic markers, including PPARγ and fatty acid synthase, in mesenchymal stem cells. Gestational exposure to PFOA decreased expression of acetyl-histone H3, acetyl-histone H4 and increased expression of acyl-CoA thioesterase 1, long-chain acyl-CoA synthetase and palmitoyl-CoA oxidase 1 in the liver of F1 female mouse pups (Li et al., 2019b). Changes in expression of these genes can alter lipid metabolism and can lead to liver damage.

Similar to PFOA, PFOS can alter expression of genes involved in development. (Chen et al., 2018a) reported expression changes in genes related to angiogenesis in mouse placenta following exposure to PFOS. In J1 mouse embryonic stem cells, PFOS induced changes in expression of neural markers during global differentiation, suggesting the potential for developmental neurotoxicity (Yin et al., 2018). PFOS also inhibited placental 11-β hydroxysteroid dehydrogenase 2 in isolated rat microsomes, and altered expression of placental genes (Li et al., 2016b).

Changes in adiposity have been seen in animals and in humans because of prenatal exposure to PFOS. These changes have been linked to activation of PPARγ. Upregulation of adipogenic markers, which include PPARγ and fatty acid synthase, as well as promotion of adipogenic differentiation in mesenchymal stem cells were observed (Liu et al., 2019a; Liu et al., 2019b).

Cardiovascular effects from PFOS exposure have been seen in animals. To investigate the effect of PFOS on cardiac development, Zhang et al. (2016f) exposed D3 embryonic stem cells to PFOS and reported a significant decrease in embryoid body diameter, and a decrease in mRNA and protein levels of cardiac-specific transcription factors and mesodermal markers. As with PFOA, Zhou et al. (2017) reported that PFOS induced changes in myocardial differentiation in the mouse R1 embryonic stem cell line.

Changes in behavior were observed in pups exposed to PFOS from gestation through lactation, as noted in Table 5.5.4 (Reardon et al., 2019). The authors postulate that the changes in behavior are the result of altered metabolite profiles in the brain of F1 pups from PFOS exposure.

Reproductive toxicity

Effects on the male reproductive system have been observed in animal toxicity studies. Molecular studies show that PFOA can alter cell structure in male reproductive organs. In BALB/c mice, PFOA altered epididymal gene expression related to lipid metabolism, altered epididymal fatty acid composition, activated the protein kinase B/adenosine monophosphate protein kinase (AKT/AMPK) signaling pathway, and increased oxidative stress in sperm (Lu et al., 2016b). PFOA disrupted junctions between isolated mouse Sertoli cells, and altered the levels of proteins associated with endocytosis and the blood-testis barrier (Lu et al., 2016a; Lu et al., 2017).

PFOA has been shown to affect male hormone production. PFOA reduced testosterone levels in animals that were treated with ethane dimethyl sulfonate (EDS) to eliminate Leydig cells, and in isolated seminiferous tubules from EDS-injected animals (Lu et al., 2019). PFOA also altered gene and protein expression related to steroidogenesis in testis and seminiferous tubules in rats (Lu et al., 2019), and affected genes related to apoptosis in mouse testis (Yuan et al., 2017). In mouse Leydig tumor cells, PFOA reduced mitochondrial membrane potential, resulting in inhibition of steroidogenesis and reduced progesterone concentration in cells (Zhao et al., 2017). However, in another study, corticosteroid binding globulin (CBG) expression was increased and in turn, increased progesterone levels and steroidogenesis (Sun et al., 2018b).

Tian et al. (2019) reported that mouse Leydig tumor cells exposed to PFOA showed a reduction in androgen receptor (AR) gene expression and upregulation of pregnane X receptor (PXR). PFOA also decreased protein levels of Nrf2, and reduced antioxidant capacity in mouse testis (Yuan et al., 2017). In an in vitro model of human spermatogenesis using male human embryonic stems cells, PFOA decreased markers for primary spermatocytes and spermatogonia (Steves et al., 2018). However, in an in vitro study in human sperm cells, PFOA did not cause genotoxicity (Emerce and Cetin, 2018).

Female reproductive effects have also been observed in animal studies. Decreased oocyte viability and increases in ROS production in mouse ovaries were observed following exposure to PFOA (Lopez-Arellano et al., 2019). Studies have shown that disruption of gap junction intercellular communication because of PFOA exposure may result in a decrease in oocyte viability (Lopez-Arellano et al., 2019). Additionally, exposure to PFOA during gestation induced increased biomarkers of apoptosis in the uterus of pregnant mice (Song et al., 2019). Long-term exposure of trophoblast stem cells to PFOA affected genes related to cysteine metabolism and interleukin signaling, indicating suppression of viral response (Midic et al., 2016), thus potentially leaving the placenta and embryo more susceptible to viral infection.

PFOS administered to pregnant CD-1 mice altered testicular gene expression in F1 males (Lai et al., 2017a). PFOS also altered gene expression in the fetal liver of CD-1 mice, and activated the liver X receptor/ retinoid X receptor (LXR/RXR) pathway (Lai et al., 2017b). PFOS inhibited androgen production, increased apoptosis, and altered gene expression in rat Leydig cells (Li et al., 2018b). Qiu et al. (2016a) reported that PFOS altered expression of proteins related to the blood-testis barrier in mice in vivo and in isolated Sertoli cells.

Additionally, Qu et al. (2016) reported that PFOS altered expression levels of proteins related to apoptosis, and changed expression levels of estrogen receptors in the testis.

PFOS can interfere with actin microfilaments in Sertoli cells and can cause disruption in human Sertoli cell tight junction permeability (Li et al., 2016a; Chen et al., 2017a; Gao et al., 2017). Expression of HILI (piwi-like RNA-mediated gene silencing 2) in primary spermatocytes was decreased (Steves et al., 2018). Changes in expression of this gene can result in mutations in spermatids. In an in vitro study in human sperm cells, PFOA did not cause DNA damage (Emerce and Cetin, 2018).

5.5.4. Conclusions

Overall, the evidence for an association between prenatal PFOA exposure and lower birth weight remains mixed. A large association was seen in births to a relatively small group of women in Sweden, but not for the Norwegian group in the study with cases selected primarily from a high-risk population. Other associations reported from cross-sectional and other prospective cohort studies were more modest. The majority of studies reported no statistically significant associations with birth weight, despite some large samples and some PFOA concentrations >5 ng/ml (although most were much lower). A clear relationship between PFOA concentration and risk of decreased birth weight is not apparent.

Although a few studies reported large decreases in birth weight associated with prenatal PFOS exposure, larger prospective studies reported more modest associations, and the majority of recent studies, including large prospective studies with a range of PFOS concentrations, reported no statistically significant associations between prenatal exposure to PFOS and birth weight. The relationship between prenatal PFOS exposure and decreased birth weight remains unclear.

While half of the studies of SGA reported that prenatal PFOA exposure was statistically significantly associated with increased risk of SGA in one part of the sample, the results were inconsistent within and across studies. For women who were at elevated risk of delivering SGA infants, including those who smoked during pregnancy, PFOA exposure was associated with increased risk except among a Norwegian cohort (Lauritzen et al., 2017; Govarts et al., 2018). Other studies reported conflicting data on differences by sex, with one reporting a stronger association in female infants than in males (Wikström et al., 2019), while another reported a possible protective effect in females (Manzano-Salgado et al., 2017).

PFOS was associated with a statistically significant increased risk of SGA in two cross-sectional studies, with one study reporting increased risk among women who smoked during pregnancy, and decreased risk of SGA among non-smokers. The case-cohort and prospective cohort studies reported no associations and non-statistically significant associations between prenatal exposure to PFOS and risk of SGA birth.

OEHHA's review of the literature published since 2016 identified little new epidemiologic evidence supporting an association between higher PFOA levels in women and decreases in fertility or fecundity.

The few epidemiologic studies of PFOS exposure and fertility and fecundity published since 2016 provide little new epidemiologic evidence supporting an association between PFOS and

reduced fertility or fecundity, although a recent case-control study suggests a possible association between PFOS exposure and reduced risk of endometriosis-related infertility.

For PFOA, a number of studies in mice reported reproductive toxicity following exposure to PFOA for 1-4 weeks. In male mice, studies reported decreased testis and epididymis weights and sperm count, and effects on the blood-testis barrier. NTP (2019a) reported decreased absolute cauda epididymis weight and sperm count, and increased relative testis weight in male rats after 28 days of exposure via oral gavage. In a 16-week oral gavage study, decreased absolute testis weight was also observed in male rats (NTP, 2020). In female mice, studies reported decreases in litter size, changes in hormone levels, and effects on the ovary. Generally, these data support earlier findings reported by US EPA and others that PFOA is a developmental and reproductive toxicant (Table 5.5.7).

For PFOS, recent studies identified multiple adverse effects on reproductive health, such as decreases in testis and/or epididymis weights, decreases in sperm count, increases in apoptosis and apoptosis markers in the ovary or testis, changes in hormone levels, and changes in estrous cycle. Several studies also reported adverse effects in offspring exposed to PFOS during gestation, including reduced growth, changes in lipid and hormone homeostasis, and behavioral alterations. OEHHA did not identify any recent studies examining effects of PFOS on fetal lungs. Nonetheless, the recent data support earlier findings that PFOS adversely affects reproduction and development in animals (Table 5.5.7).

Table 5.5.7. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and developmental and reproductive toxicity

Outcome	PFOA	PFOS
Pregnancy-related hypertension and preeclampsia in humans	Data are suggestive of an association with risk of preeclampsia and pregnancy-related hypertension	Data are suggestive of an association with risk of preeclampsia and pregnancy-related hypertension
Measures of fetal growth in humans	Inconsistent evidence for decreased birth weight and risk of SGA	Inconsistent evidence for decreased birth weight and risk of SGA
Pubertal development	-Sparse, inconsistent evidence for changes in onset of puberty in prenatally exposed girls and boys -Sparse evidence for delayed puberty in girls and boys associated with postnatal exposure to high concentrations	-Sparse, inconsistent evidence for earlier puberty in prenatally exposed girls and boys -Sparse evidence for delayed puberty in girls and boys associated with postnatal exposure to high concentrations
Fertility and Fecundity in humans	Inconsistent evidence for reduced fertility or fecundity in women	Inconsistent evidence for reduced fertility or fecundity in women
Testicular/epididymal effects in animals	Positive evidence for decreased testicular and epididymal weight, decreased sperm count, and disruption of the blood-testis barrier	Positive evidence for decreased testicular and epididymal weight, and decreased sperm count

Outcome	PFOA	PFOS
Fertility/litter size effects in animals	Positive evidence for decreased litter sizes and pup survival	-No evidence for effects on fertility -Positive evidence of increased neonatal mortality
Adverse effects in animal offspring	Positive evidence for reduced fetal/birth weight, and increased liver effects in pups exposed during gestation	Positive evidence for decreased body weight and changes in hormone homeostasis in pups exposed during gestation

5.6. Neurotoxicity

In their review, US EPA (2016b) did not identify clear or consistent evidence from human epidemiologic studies linking PFOA or PFOS to neurotoxicity outcomes.

US EPA (2016b) identified two studies that reported behavioral effects in mice following exposure to PFOA. In one study, a single exposure to PFOA on postnatal day 10 induced changes in habituation and activity patterns months later, alongside protein expression changes of neuroactive proteins in the brain (Johansson et al., 2009, as reported by US EPA (2016b)). Additionally, gestational exposure induced sex-specific changes in exploratory behavior in pups (Onishchenko et al., 2011, as reported by US EPA (2016b)).

US EPA's literature review of the neurotoxic effects of PFOS in laboratory animals found inconsistent results for PFOS-induced learning and memory impairment, as determined by water maze testing (Luebker et al., 2005; Butenhoff et al., 2009; Long et al., 2013; Wang et al., 2015, as reported by US EPA (2016d)). Mechanistic studies revealed potential neurotoxic effects of PFOS, including effects on excitatory amino acids (Yang et al., 2009, as reported by US EPA (2016d)), changes in gene expression of neuroactive compounds and inflammatory markers (Wang et al., 2010; Zeng et al., 2011, as reported by US EPA (2016d)), and decreased neurite growth in cultured hippocampal cells (Liao et al., 2009, as reported by US EPA (2016d)).

5.6.1. Recent Animal Evidence

Guo et al. (2019) exposed male BALB/c mice (12/dose) to 0, 0.4, 2 or 10 mg/kg-day PFOA via oral gavage for 28 days and observed decreased glutamic acid content and increased glutamate synthetase in the mouse brain at the high dose. Additionally, in utero exposure to 5 mg/kg-day PFOA induced an increase, compared to controls, in cortical nerve cells and effectors of cell proliferation (including nerve growth factor) in Kunming mouse pups (Qin et al., 2018). However, an acute exposure in male rats (single oral dose of 50 mg/kg) had no effect on memory function (Kawabata et al., 2017).

Several studies reported neurotoxic effects following oral PFOS exposure. Changes in dopamine levels and dopaminergic gene expression in the hippocampus and cortex of rodents have been observed. Following a single PFOS exposure of 11.3 mg/kg on PND 10, (Hallgren and Viberg, 2016) observed changes in gene expression involved in dopaminergic signaling in mice at 2 months of age. In adult male rats, an exposure of 0.5 mg/kg-day was associated with an increase in dopamine receptor subtype 2 (D2) gene expression in the prefrontal cortex; other dopaminergic system changes were noted at doses of 1 mg/kg-day and higher (Salgado et al.,

2016). No deficits in tyrosine hydroxylase or dopamine aminotransferase were observed in mice (Patel et al., 2016).

Increased hallmarks of Alzheimer's disease were observed in adult rats exposed both pre- and perinatally to PFOS (Zhang et al., 2016e). Increased Tau mRNA and elevated protein levels, increased phosphorylation of Tau, and elevated β-amyloid aggregation were observed in the hippocampus of 90-day-old rats exposed pre- or postnatally to PFOS in drinking water (1.7-15 mg/L). A reduction in nuclei in the dentate gyrus of the hippocampus, an abnormality which the authors argue is consistent with memory dysfunction, was reported in mice exposed during gestation (20 mg/kg-day, administered to dams) (Mehri et al., 2016). PFOS exposure of Wistar dams from GD 11-20 at 1 or 2 mg/kg-day resulted in a reduction in glutamate receptor 2 (GluR2) expression to 82% of control at 1 mg/kg-d and 44% of control ($p < 0.01$) at 2 mg/kg-day in the cortex of the pups measured on PND 4 (Ishida et al., 2017). An increase in kainic acid-induced excitotoxicity was also observed in the rat pups exposed during gestation, likely due to the decrease in cortical GluR2 expression (Ishida et al., 2017). Excitotoxicity due to excess calcium influx results in neuronal cell damage and death, and is implicated in neurodegenerative disease (Dong et al., 2016b). Recently, Zhang et al. (2019a) reported changes in synaptic plasticity (decreased long-term potentiation, lower input/output and paired pulse facilitation curves, and decreased facilitated excitatory post-synaptic potentials in the hippocampus) in rats exposed both pre- and postnatally to PFOS. These results are similar to observed results from an earlier study that reported repression of long term potentiation in the hippocampus of Sprague Dawley rats exposed to PFOS via intracerebroventricular injection (Zhang et al., 2016d). Reardon et al. (2019) reported that rat pups exposed to 1 mg/kg-day PFOS from GD 1 to PND 21 (given to dams during gestation and lactation) had increased activity in open-field behavior tests, suggesting neurobehavioral changes due to chemical exposure.

5.6.2. Recent Mechanistic Evidence

PFOA increased nerve growth factor (NGF) in mouse neurons ex vivo (Qin et al., 2018), and altered neurotransmitter levels in mouse brain (Yu et al., 2016).

Much like in the animal toxicity literature, several in vitro studies have reported neurotoxic effects of PFOS exposure. (Dong et al., 2016b) exposed C17.2 neural stem cells to PFOS, and observed impaired cell cycle proliferation, and changes in protein and mRNA expression of components in the Wnt signaling pathway.

PFOS altered expression of dopaminergic genes in the cortex and hippocampus of male mouse pups (Hallgren and Viberg, 2016), and dopamine receptors in the amygdala, prefrontal cortex, and hippocampus of male rats (Salgado et al., 2016). PFOS reduced the number of dopaminergic neurons in a culture of primary mesencephalic neurons, and inhibited vesicular uptake of dopamine in HEK293 cells expressing the human vesicular monoamine transporter 2 (Patel et al., 2016).

Guo et al. (2017) exposed SK-N-SH neuroblastoma cells to PFOS, and observed changes in cellular morphology (shrunken and round cells), inhibition of cell growth, and changes in protein and mRNA levels of brain-derived neurotrophic factor (BDNF). The authors also reported increased methylation of the BDNF promoter, and changes in expression of genes for DNA methyltransferases, suggesting an epigenetic basis for the inhibition of cell growth. In a different human neuroblastoma cell line (SK-SY5Y), PFOS induced ROS, increased apoptotic markers, and changes in cellular morphology (smaller unattached cells, retraction of

pseudopods) (Sun et al., 2018a). A similar study in SK-SY5Y neuroblastoma cells reported increased oxidative stress, and markers of apoptosis, possibly mediated by the JNK signaling pathway (Sun et al., 2019a). Additionally, increased ROS and markers of apoptosis were observed in HAPI rat microglia following exposure to PFOS (Ge et al., 2016). PFOS induced cytotoxicity in SK-SY5Y cells, as indicated by lactose dehydrogenase release (Patel et al., 2016).

Oh et al. (2017) reported that PFOS causes endoplasmic reticulum stress in rat embryo primary cortical neurons. In neonatal rat primary hippocampal neurons and astrocytes, PFOS induced oxidative stress, changes in cellular morphology (shrinkage, rounding, detachment), autophagy, increased apoptosis, changes in glutamate/glutamine levels, and stunted neurite outgrowth (Li et al., 2017f). Additionally, when primary hippocampal neurons were exposed to the cellular medium used during PFOS experiments in astrocytes (termed astrocyte conditional media), increases in apoptosis and n-methyl d-aspartate (NMDA) receptor gene and protein expression were observed (Wang et al., 2019b). The authors proposed that increases in D-serine levels in the extracellular medium (released by astrocytes in response to PFOS exposure), led to neuronal toxicity mediated by NMDA receptor activation.

In neuronal differentiated PC12 (pheochromocytoma) cells, PFOS altered cell morphology (shrunken cells), increased ROS production, increased caspase-3 activity (marker of apoptosis), and induced autophagy (Li et al., 2017a).

PFOS altered expression of Tau mRNA and protein in the brain of F1 rats exposed pre- and postnatally. PFOS also induced accumulation of β-amyloid in the hippocampus, and changed expression levels of amyloidogenesis related genes (Zhang et al., 2016e).

Berntsen et al. (2018) reported that PFOS-induced cytotoxicity in cerebellar granular neurons in vitro can be modulated by the presence of NMDA receptor antagonists and calcium chelators. The authors suggest that PFOS induces excitotoxicity, and subsequent cell death, through activation of glutamate receptors and subsequent influx of extracellular calcium. PFOS exposure reduced glutamate receptor GluR2 expression (AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, receptor subunit) and increased excitotoxicity in primary cerebral cultures from rat fetuses on GD 18 (Ishida et al., 2017). Additionally, PFOS altered expression of AMPA-related genes in primary rat hippocampal neurons, and changed glutamate receptor levels in rats exposed both pre- and postnatally (Zhang et al., 2019a).

Taken together, there is a substantial body of in vivo and in vitro evidence that suggests that PFOS has neurotoxic potential. These in vitro studies support findings in animal toxicity studies (discussed above) that PFOS can have adverse effects on various parts of the central nervous system.

5.6.3. Conclusions

The evidence of PFOA-induced neurotoxicity in animals is very limited (Table 5.6.1). Changes in behavior, in addition to alterations in glutamatergic homeostasis and nerve cell proliferation suggest that PFOA may induce neurotoxic effects in animals, but more study is needed to draw any firm conclusions.

Recent animal and mechanistic studies of PFOS-induced neurotoxicity highlight effects that were not identified previously, including effects on dopaminergic signaling, excitotoxicity and

changes in glutamate signaling, and changes in synaptic plasticity. Additionally, hallmarks of Alzheimer's disease were observed in one study, which could potentially be associated with memory deficits observed in previous studies. In vitro studies in various cell lines also reported oxidative stress in response to PFOS exposure. As such, the evidence of PFOS-induced neurotoxicity is increasing, and the current body of evidence suggests PFOS can induce neurotoxicity via multiple modes of action (Table 5.6.1).

Table 5.6.1. Summary of OEHHA's conclusions regarding the experimental animal data on PFOA and PFOS and neurotoxicity

Outcome	PFOA	PFOS
Changes in glutamatergic signaling in animals	Positive evidence	Positive in vivo and in vitro mechanistic evidence
Behavioral alterations in animals	Positive evidence	Positive evidence
Learning and memory impairment in animals	No evidence	Inconsistent evidence, based on water maze results
Changes in dopaminergic signaling in animals	No evidence	Positive in vivo and in vitro mechanistic evidence

5.7. Cancer

US EPA reviewed the literature for PFOA and cancer published up to December 2015 and concluded that there was "suggestive evidence of carcinogenic potential" for PFOA and that, "Epidemiology studies demonstrate an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population" (US EPA, 2016b). In 2017 the International Agency for Research on Cancer (IARC) classified PFOA as possibly carcinogenic to humans (Group 2B) based on limited evidence in humans and in experimental animals (IARC, 2017a). With regards to the human evidence, IARC concluded that, "A positive association was observed for cancers of the testis and kidney."

With regards to PFOS and cancer, US EPA (2016b) concluded that, "A small number of epidemiology studies of PFOS exposure and cancer risk are available. While these studies do report elevated risk of bladder and prostate cancers, limitations in design and analysis preclude the ability to make definitive conclusions." In particular, US EPA noted the small numbers of cases in many of these studies and the fact that the results in several of the studies linking PFOS to increased risks of bladder or prostate cancer were not statistically significant. In addition, US EPA noted that, "…some [human epidemiologic studies of PFOS and cancer] are confounded by failure to adjust for smoking" but did not present clear evidence to support this assertion. IARC has not reviewed PFOS.

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; US EPA, 2016d), ATSDR (2018a), and others, OEHHA reviewed the epidemiologic literature on PFOA and PFOS and cancer, with a focus on cancer of the kidney, testis, bladder, breast, prostate, and pancreas. Detailed summaries of PFOA and cancer are shown in Appendix 7, Tables A7.21-27.

For animal studies, OEHHA reviewed all the available animal carcinogenicity data, including the recent NTP (2020) two-year bioassay of PFOA in male and female Sprague Dawley rats. Tumor incidence data and descriptions of rare tumor types are presented below.

5.7.1. Human Evidence

PFOA

Bladder cancer: For bladder cancer, epidemiologic studies have not found clear associations. The high exposure occupational study by Raleigh et al. (2014) identified an elevated relative risk in the most highly exposed workers (hazard ratio (HR) = 1.66; 95% CI, 0.86-3.18) but this was based on a relatively small number of cancer cases and involved limited or no information on potential confounders like smoking. In the other occupational cohort (Steenland and Woskie, 2012; Steenland et al., 2015), relative risk estimates were below 1.0 in the most highly exposed workers. Studies in highly exposed communities were also negative (Barry et al., 2013; Vieira et al., 2013; Mastrontonio et al., 2017).

Breast cancer: Some intriguing results have been reported for breast cancer, but because of the somewhat limited evidence and various weaknesses in these studies, firm conclusions cannot be made based solely on these studies. For example, Mastrontonio et al. (2017) found a statistically significant increase in breast cancer mortality in an area with relatively high drinking water contamination (relative risk (RR) = 1.11; 95% CI, 1.02-1.20), although the increase was small and this study was based on an ecologic design with very limited information on potential confounders. Bonefeld-Jorgensen et al. (2014) reported elevated RRs in women over 40 years old, but with an unusual dose-response pattern (highest RRs in the 2nd and 4th quintiles, all other RRs near 1.0). In a population based study, Mancini et al. (2020) identified elevated PFOA-related risks for estrogen receptor negative (OR = 7.73; 95% CI, 1.46–41.08) and progesterone receptor negative cases (OR = 3.44; 95% CI, 1.30–9.10) in women in the 2nd vs. 1st quartiles of serum PFOA, but ORs were lower and not statistically significant for the higher quartiles. The quartile cutoff points in these analyses appear to be based on all breast cancer cases, regardless of estrogen or progesterone receptor status. The total numbers of cases in the estrogen receptor negative and progesterone receptor negative analyses were 26 and 57, respectively. There were 194 controls. The numbers of cases and controls in each PFOA exposure category for these analyses were not provided.

Most of the studies OEHHA identified had only limited information on known breast cancer risk factors, and several studies assessed exposure using only a single or a small number of blood samples. The serum elimination half-life of PFOA appears to be fairly long (approximately 3-4 years) (Olsen et al., 2007). However, the latency period between PFOA exposure and breast cancer (if an association exists) is unknown, and assessing exposure based on a single serum sample or samples collected only during a single short window of time could miss or underestimate the impacts of exposure fluctuations or relevant exposure periods. These issues would most likely bias study results towards an underestimate of the true cancer risks.

Kidney cancer: OEHHA identified seven human studies of PFOA and kidney cancer (Table A7.23). Two of these studies are not informative, either because of the ecologic nature of the exposure data (Mastrontonio et al., 2017), or because of the very small number of cases (Girardi and Merler, 2019). Four of the remaining five studies reported statistically significant associations between PFOA and kidney cancer or renal cell carcinoma (RCC) incidence (Barry et al., 2013; Vieira et al., 2013; Shearer et al., 2021) or mortality (Steenland and Woskie, 2012). Evaluations of bias, confounding, and all other major aspects of causal inference for these studies all suggest that the results of these positive studies represent real effects. These evaluations are discussed in Chapter 6. One study, the occupational study by (Raleigh et al., 2014), did not find an association between PFOA and kidney cancer. A detailed analysis of the

potential reasons the results of this study differ from most of the other human studies of PFOA and kidney cancer is presented in Chapter 6. Overall, the findings from the human epidemiologic studies, combined with data from animal and mechanistic research, provide strong evidence that PFOA is a cause of kidney cancer.

Liver cancer: Liver cancer is relatively rare and the small numbers of liver cancer cases included in the studies OEHHA reviewed limited the ability of most of these studies to adequately investigate the relationship between PFOA and this cancer type. In the study that appears to have involved the highest PFOA exposure levels, Girardi and Merler (2019) reported SMRs of 1.02 (95% CI, 0.12-7.21; N=1 case), 2.72 (95% CI, 0.69-11.0; N=2 cases), and 3.07 (95% CI, 1.15-8.18; N=4 cases) for liver cancer by tertiles of estimated cumulative PFOA exposure, respectively, compared to regional rates. Compared to railroad workers, mortality rate ratios were even higher. Information on alcohol consumption or other potential confounders was not available, although it seems somewhat unlikely that confounding would cause relative risks this high. The liver cancer SMR was also elevated in workers who reportedly did not work directly with PFOA (SMR = 2.71; 95% CI, 1.02-7.22), although serum sampling results in a subset of these workers showed that they also had significant PFOA exposure (mean = 977 ng/ml). In another high exposure occupational study, Steenland and Woskie (2012) reported SMRs for liver cancer above 2.0 in PFOA exposed workers in the 1st and 3rd quartiles of cumulative PFOA exposure compared to workers at a nearby facility who were not exposed to PFOA. The number of cases was small (N=10 exposed cases overall) and SMRs in the other quartiles were near or below 1.0. In a study of tetrafluoroethylene (TFE) workers co-exposed to PFOA, Consonni et al. (2013) reported an SMR of 2.00 (95% CI, 0.54-5.12) for those in the highest PFOA category of cumulative exposure (not shown in Tables). However, every worker was also exposed to TFE, which has been linked to liver cancer in rodents (IARC, 2017b). In the 3M occupational cohort, SMRs for PFOA exposed workers were mostly near or below 1.0, although the number of cases was small (N=8 cases in all quartiles). Overall, the results of several high exposure occupational studies provide some evidence that PFOA is associated with liver cancer, although the small sample sizes of these studies limit the usefulness of these studies for dose-response analyses.

The lower exposure non-occupational studies all reported relative risks near 1.0, although the numbers of cases were small (e.g., the C8 studies), exposure ranges were limited (i.e., the population based study by Eriksen et al., 2009), or exposure assessment was ecological (i.e., Mastrontonio et al. (2017)). Because of these issues, these studies by themselves also cannot be used to make definitive conclusions regarding PFOA and liver cancer or to adequately assess dose-response relationships.

Pancreatic cancer: Pancreatic cancer is relatively rare. Because of this, cohort studies need very large sample sizes in order to have enough cases and sufficient statistical power to detect true associations in a convincing manner. Most of the studies OEHHA reviewed, including the high exposure occupational cohort studies, had relatively small sample sizes overall and therefore small numbers of pancreatic cancer cases, and clear associations have not been seen in these studies. By far the largest non-ecologic study OEHHA identified was the nested case-control study by Eriksen et al. (2009), which was based on a cohort of 57,053 people and had 128 cases of pancreatic cancer. In this population based study involving low exposure levels, relative risk estimates adjusted for smoking and diet generally seemed to increase with increasing quartiles of serum PFOA: 1.00 (reference), 0.88 (95% CI, 0.49-1.57), 1.33 (95% CI, 0.74-2.38), 1.55 (95% CI, 0.85-2.80), although the trend was not statistically significant (RR = 1.03 (95% CI, 0.98-1.10) for each 1 ng/ml increase in PFOA). Overall, while some limited

supporting evidence is present, firm conclusions regarding pancreatic cancer cannot be made based solely on the human epidemiologic literature published to date.

Prostate cancer: Several studies of prostate cancer, including the two high exposure occupational studies, have reported relative risk estimates above 1.0. Relative risk estimates in the most recent reports from these occupational studies range from 1.32 to 1.88 although none are statistically significant. Mortality HRs of 3.0 (95% CI, 0.9–9.7, N=10 cases) and 6.6 (95% CI, 1.1–37.7, N=2 cases) were reported for the two highest exposure groups in an earlier report from the 3M occupational cohort (Lundin et al., 2009). However, in a later report with six additional years of follow-up, relative risk estimates were much lower (HR = 1.32; 95% CI, 0.61-2.84) (Raleigh et al., 2014). The authors of the later report, which included additional "non-exposed" workers from a nearby 3M facility and more complex exposure assessment methologies, wrote that the earlier analyses "were limited by the qualitative nature of the exposure assessment and that the lowest exposed members of the population were more likely to be research and development professionals with lower overall baseline risks." A rationale for why research and development professionals would have 3-6 times lower risks than the other workers in the earlier study was not given. And, it would generally be expected that the potentially less accurate exposure assessment in the earlier study would bias results to the null, not towards the large SMRs that were reported. Given the fairly wide confidence intervals reported in each study, it is possible the inconsistency between the earlier and later reports from the 3M cohort is due to chance. Currently however, the reasons for these inconsistent results are unknown.

One factor to consider when looking at the data on prostate cancer as a whole is that none of the prostate cancer studies adjusted for prostate cancer screening. This could have potentially confounded some of the results of these studies, although OEHHA could not find any evidence that PFOA exposure is strongly related to cancer screening. Overall, the human evidence linking PFOA to prostate cancer has a number of limitations that prevent firm conclusions being made regarding PFOA and prostate cancer based on this evidence alone.

Testicular cancer: Testicular cancer is fairly rare, and too few cases of this cancer were identified in the occupational cohorts for the findings from these studies to be informative by themselves. However, elevated risks of testicular cancer were seen in two studies involving people living in areas with high drinking water contamination. In the retrospective cohort study of residents living near the DuPont facility in West Virginia, HRs of 1.00, 1.04, 1.91, and 3.17 were reported by quartile of exposure (p-trend = 0.04) (Barry et al., 2013). And, mostly similar findings were seen in the study by Vieira et al. (2013), which involved the same study area but used different methods for assessing exposure, different methods for ascertaining cancer cases, and different control groups. These findings are further supported by the elevated mortality relative risk of 1.86 (95% CI, 0.81-4.27) reported for residents of a highly contaminated area in Italy (Mastrontonio et al., 2017). The former is an ecologic study, but there is no reason to suspect that ecologic fallacy or exposure misclassification caused this elevation, and no major confounders are obvious. Research has also shown that risks of testicuilar cancer are elevated in firefighters, an occupation where PFOA exposures can be high (Soteriades et al., 2019). Overall, the epidemiologic literature to date suggests that PFOA is associated with testicular cancer.

PFOS

Fewer human epidemiologic data are available for PFOS and cancer so these studies are reviewed narratively here and summary tables are not provided.

Bladder cancer: Studies of highly exposed occupational cohorts are limited to a single facility, a chemical manufacturing plant in Decatur, Alabama, with geometric mean PFOS serum levels of about 1-2 ppm (or µg/ml) in the most highly exposed workers. PFOA was also present, with a mean serum level of 0.899 ppm reported (Olsen et al., 2003d). In a study of all workers for the period 1961 to 1997, bladder cancer mortality was elevated in the most highly exposed workers compared to Alabama state rates (SMR = 12.77; 95% CI, 2.63-37.35) although this involved only three exposed cases (Alexander et al., 2003). All of the bladder cancer deaths in this cohort were in the most highly exposed group. Information on potential confounders such as smoking were not available. In a follow-up study, current and past employees were sent a questionnaire in an attempt to identify incident cases of bladder cancer (Alexander and Olsen, 2007). The response rate was 74 percent, and 11 cases of bladder cancer were identified. The standardized incidence ratio (SIR) for the cohort overall compared to the US population was 1.28 (95% CI, 0.64-2.29) for men and women combined. The SIR was elevated in women (SIR = 6.42; 95% CI, 0.78-23.18; N=2 cases) but neither of these two cases worked in jobs with obvious high PFOS exposure.

Breast cancer: Two deaths from breast cancer were reported in the only high exposure occupational cohort study (SMR = 1.57; 95% CI, 0.19-5.66) (Alexander et al., 2003). No information on common breast cancer risk factors was available other than age, and findings for breast cancer incidence were not reported. Mastrontonio et al. (2017) found a slight increase in breast cancer mortality for a community with PFOS and PFOA drinking water contamination (SMR = 1.11; 95% CI, 1.02-1.20) although this study used an ecologic design and there was no individual information on exposure or most relevant confounders. All other studies of PFOS and breast cancer have involved relatively lower general population exposures. In the Danish National Cohort, clear associations with breast cancer incidence were not found overall (Bonefeld-Jorgensen et al., 2014), although some indication of an interaction between PFOS and the aromatase (CYP19) CC genotype was seen (p-interaction = 0.055, 36 cases in the CC genotype-higher PFOS exposure category) (Ghisari et al., 2017). In a case-control study nested in a large cohort of French women, breast cancer ORs adjusted for smoking, exercise, diet, and several reproductive and development factors were above 1.0 and statistically significant but without clear dose-response trends overall (Mancini et al., 2020). Dose-response trends were more consistent for estrogen receptor positive (p-trend = 0.04, 132 cases overall) and progesterone receptor positive tumors (p-trend = 0.02, 98 cases overall) but these findings have yet to be replicated in another study population. Replication (i.e., the Hill criterion of "consistency") is an important element in evaluating causality (Bradford Hill, 1965). However, the lack of replication does not mean that this finding is not real, and replication is not a required element of causal inference. Another general population study did not find evidence of increased breast cancer incidence with increasing perinatal serum levels of PFOS but did not examine breast cancer subtypes or genetic variants (Cohn et al., 2020). Several studies in which PFOS levels were measured after or near the time of cancer diagnosis have reported associations between PFOS and breast cancer (e.g., Tsai et al., 2020). Given the long half-life of PFOS in human blood, the exposure levels measured in these studies could represent exposures that occurred prior to cancer development. However, this is currently difficult to evaluate since data on the latency of PFOS-related cancer is not available. Overall, while a number of intriguing findings for PFOS and breast cancer have been reported, small sample

sizes, narrow exposure ranges, lack of data on potential confounders, latency issues, and lack of replication in particular subgroups have all limited the ability to make firm conclusions based on these results.

Liver cancer: The only high exposure occupational cohort study of liver cancer was too small to provide useful information regarding this cancer (only two liver cancer deaths total and only one in a high exposure job) (Alexander et al., 2003). A population based study involving mostly low exposures found no clear association between a single PFOS serum measurement and liver cancer (Eriksen et al., 2009). Overall, because of small sample sizes or limited exposure ranges the human epidemiologic literature on its own cannot be used to make conclusions regarding PFOS and liver cancer.

Prostate cancer: The results of the two studies that reported on PFOS exposure and prostate cancer were inconsistent. No significant associations were observed between prostate cancer and PFOS-exposed jobs within a PFOS manufacturing facility in Decatur, Alabama. The OR for those with high PFOS exposure for more than 1 year was 1.08 (95%CI, 0.44–2.69); the OR for the combined category of those with low or high PFOS exposure for 1 year or more was 1.36 (95%CI, 0.61–3.02) (Grice et al., 2007). In a case-cohort analysis within the Danish general population, an increase in prostate cancer was observed for the three upper quartiles of PFOS serum levels compared with the lowest quartile. For the lowest vs. the highest quartile, the incidence rate ratio (IRR) was 1.38 (95% CI, 0.99-1.93) (Eriksen et al., 2009). When PFOS was analyzed as a continuous variable, the IRR was 1.05 (95% CI, 0.97-1.14).

The inconsistent results may be partially explained by the differences in the method of exposure assessment between the two studies. In the occupational cohort, cumulative PFOS exposure was estimated based on a job-exposure matrix up to the year of the diagnosis. In the Danish cohort, serum samples were collected prospectively at the time of enrollment.

Other cancer types: Associations between PFOS and other cancer types have been examined, but to date clear and consistent associations with these other types have not been identified (Alexander et al., 2003; Olsen et al., 2004; Grice et al., 2007; Eriksen et al., 2009; Mastrontonio et al., 2017)

5.7.2. Animal Evidence

PFOA

Cancer bioassays in laboratory animals published prior to 2016 have been thoroughly described previously (US EPA, 2016b; New Jersey DWQI, 2017; IARC, 2017a). The studies published prior to 2016 are briefly described below, and significant tumor findings observed in these studies are presented in Table 5.7.1.

Table 5.7.1. Significant tumor incidences and cases of hyperplasia following exposure to PFOA (studies published prior to 2016)

Sex/Species	Exposure	Tumor type	Dose (mg/kg-day)	Incidence[a]	Reference
Male Sprague Dawley rats (50/dose)	Dietary for 106 weeks	Leydig cell adenoma	0, 1.3, or 14.2	0/33, 2/36, 7/44*	Butenhoff et al. (2012a), data from (Sibinsky, 1987)
		Pancreatic acinar cell hyperplasia		3/46, 1/46, 10/47	Caverly Rae et al. (2014)
Female Sprague Dawley rats (50/dose)		Ovarian tubular hyperplasia	0, 1.6, or 16.1	0/48, 7/50, 15/47	Butenhoff et al. (2012a)
Male Sprague Dawley rats (76-79/dose)	Dietary for 104 weeks	Hepatocellular adenoma	0[b] or 13.6	1/79, 10/76*	Biegel et al. (2001)
		Pancreatic acinar cell adenoma or carcinoma	0[b] or 13.6	1/79, 8/76*	
		Leydig cell adenoma	0[b] or 13.6	2/78, 8/76*	
Pregnant CD-1 mice (6-14 dams/dose or 21-37 female pups/dose)	Drinking water from GD 1-17, pups followed for 18 months	Hepatocellular adenoma	0, 0.01, 0.1, 0.3, 1, or 5	0/29, 1/29, 1/37, 4/26,* 0/31, 1/21	Filgo et al. (2015)
		Hepatic hemangio-sarcoma	0, 0.01, 0.1, 0.3, 1, or 5	0/29[c], 0/29 0/37, 1/26 0/31, 2/21	

[a] Incidence is number of animals with tumors/effective number of animals (animals that died prior to the first appearance of the tumor were excluded)
[b] Pair-fed control, fed same amount of food as treated group
[c] Significant trend ($p < 0.01$, determined by Filgo et al. (2015))
*$p < 0.05$, pairwise comparison with Fisher's exact test, statistical analysis by OEHHA
GD, gestation day

Sibinsky (1987), as reported by Butenhoff et al. (2012a), administered 0, 30, or 300 ppm PFOA to Sprague Dawley rats (0, 1.3, or 14.2 mg/kg-day for males; 0, 1.6 or 16.1 mg/kg-day for females) in the diet for 105-106 weeks. In male animals, a significant increase in Leydig cell adenomas was observed in the high dose group, and a positive trend with increasing dose was identified ($p=0.005$). An increase in pancreatic acinar cell hyperplasia was also observed in the high dose males following a re-evaluation of the Butenhoff et al. (2012a) data by Caverly Rae et al. (2014). In females, a significant increase in ovarian tubular hyperplasia was observed at the mid and high dose. However, a re-evaluation performed by Mann and Frame (2004) for E. I. du

Pont de Nemours and Company reclassified the ovarian tubular hyperplasia as gonadal stromal hyperplasia and found no significant increases in ovarian hyperplasia. Furthermore, an increase in mammary gland fibroadenoma in the high-dose group was initially reported in females, but a follow-up examination by a Pathology Working Group that included participants from former PFOA manufacturers (DuPont and 3M Company) found no significant increase over controls (Table 5.7.2 (Hardisty et al., 2010)).

Table 5.7.2. Mammary gland fibroadenoma data (single and multiple tumors) in female Sprague Dawley rats from Butenhoff et al. (2012a) and (Hardisty et al., 2010)

Tumor Type	0 ppm	30 ppm	300 ppm
Fibroadenomas	10/46	19/45	21/44*
Fibroadenomas re-evaluated by PWG	18/50	22/50	23/50

PWG, Pathology Working Group (Hardisty et al., 2010)
*p <0.05, reported by (Butenhoff et al., 2012a)

Biegel et al. (2001) administered 0 or 300 ppm (0 or 13.6 mg/kg-day) to male Sprague Dawley rats in the diet for 24 months. Statistically significant increases in tumors were reported at multiple sites; specifically liver (hepatocellular adenomas), pancreas (acinar cell adenomas or carcinomas), and testis (Leydig cell adenomas). Significant increases in pancreatic acinar cell hyperplasia and Leydig cell hyperplasia were also observed.

Filgo et al. (2015) exposed three different strains of pregnant mice (CD-1, 129/SV WT, and 129-SV PPARα KO) to doses of PFOA in drinking water ranging from 0 to 5 mg/kg-day from GD 1-17. Female offspring were observed for 18 months. A significant increase in hepatocellular adenomas, and a significant trend for hepatic hemangiosarcomas, were observed in the CD-1 F1 generation. Liver tumors were not observed in 129/SV WT mice. In the 129/SV PPARα KO mice, hepatocellular adenomas were observed at incidences of 0/6, 1/10, 1/10, 1/9, and 2/9 in the control, 0.1, 0.3, 1, and 3 mg/kg-day groups, respectively. It should be noted that the liver was the only organ evaluated in these studies and that exposures occurred only during the prenatal life stage.

Recently, NTP published the technical report on chronic cancer bioassays of PFOA administered in feed to male and female Sprague Dawley rats (NTP, 2020) (see Section 5.2.2). In these studies, in addition to groups exposed post-weaning via the diet for 107 weeks, NTP included groups that had perinatal (gestational and lactational) exposures plus post-weaning dietary exposures to PFOA.

In the male rat study, significant increases in hepatocellular adenomas and pancreatic acinar cell adenomas/adenocarcinomas were observed (Table 5.7.3). The incidence of hepatocellular adenoma was significantly increased at both 40 and 80 ppm by pairwise comparison with controls, with a significant dose-related trend ($p < 0.001$). In addition, four hepatocellular carcinomas were observed in the 300/80 ppm (perinatal/postweaning) group, but not in any other groups (Table 5.7.4). Although this increase was not statistically significant, hepatocellular carcinoma is a rare tumor in male rats with a historical control incidence of 0/340. The incidence of pancreatic acinar cell adenoma or adenocarcinoma combined (Table 5.7.3) was significantly increased in all three PFOA-treated groups by pairwise comparison with control, with a significant dose-related trend ($p < 0.001$). Pancreatic acinar cell adenocarcinoma

is a rare tumor type in male rats (historical control incidence of adenocarcinoma is 2/340), and was observed in all post-weaning treated groups with and without perinatal exposure, but not in controls (0/0 ppm) or the perinatal only exposure group (300/0 ppm) (Tables 5.7.3 and 5.7.4).

Table 5.7.3. Hepatocellular and pancreatic tumor incidences in male Sprague Dawley rats exposed to PFOA in the diet for 107 weeks (NTP, 2020)

Concen-tration in feed (ppm)	Dose (mg/kg-day)	Plasma concentration (mg/L)	Hepatocellular adenoma	Pancreatic acinar cell adenocarcinoma	Pancreatic acinar cell adenoma or adenocarcinoma
0	0	BD	0/36***	0/36	3/43***
20	1.0	81.4	0/42	3/42	29/49***
40	2.3	130.8	7/35**	1/36	26/41***
80	4.8	159.6	11/37***	3/38	32/40***

BD: below the limit of detection; values were considered zero for dose-response analysis.
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): **, p <0.01; ***, p <0.001.
Control group tumor incidences with asterisks indicate significant results from exact trend test (conducted by OEHHA): ***, p <0.001.

Table 5.7.4. Hepatocellular and pancreatic tumor incidences in male Sprague Dawley rats exposed to PFOA perinatally and in the diet for 107 weeks (NTP, 2020)

Perinatal/post-weaning concentration in feed (ppm)	Hepatocellular carcinoma	Hepatocellular adenoma or carcinoma	Pancreatic acinar cell adenocarcinoma	Pancreatic acinar cell adenoma or adenocarcinoma
0/0	0/36	0/36	0/36	3/38
300/0	0/35	0/35	0/35	7/39
300/20	0/38	1/38	2/38	20/42***
300/40	0/38	5/38*	1/38	30/43***
300/80	4/39	12/39***	3/39	30/41***

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): *, p <0.05; ***, p <0.001.

In the female rat study, pancreatic acinar cell adenoma/adenocarcinoma and uterine adenoma/adenocarcinoma were observed (Tables 5.7.5 and 5.7.6). Pancreatic acinar cell adenomas and adenocarcinomas are both rare in female rats (historical control incidence of 0/340 for adenoma and 0/340 for adenocarcinoma) and were observed in the PFOA-treated female rats. Specifically, there was one acinar cell adenoma and one adenocarcinoma in the 1,000 ppm group (Table 5.7.5), three acinar cell adenomas, one adenocarcinoma, and one ductal adenocarcinoma in the 300/1,000 ppm group (Table 5.7.6), and none in controls or any

other treated groups. NTP concluded that, "[T]here was some evidence of carcinogenic activity in female rats based on the increased incidence of pancreatic acinar cell adenoma or adenocarcinoma combined." There was a statistically significant increase of uterine adenocarcinoma at 1,000 ppm with a dose-related trend ($p < 0.05$). Besides uterine tumors, two types of rare tumors were observed and noted by NTP. Hepatocellular carcinomas were observed in the 1,000 ppm group (three carcinomas) as well as the 300/1,000 ppm group (four carcinomas), compared to one carcinoma in controls and none in any other treated groups (Table 5.7.6). NTP (2020) noted that hepatocellular carcinoma is a rare tumor in female rats (historical control incidence, 1/340) and the observed increase may be treatment-related.

Table 5.7.5. Pancreatic acinar cell and uterine tumor incidences in female Sprague Dawley rats exposed to PFOA in the diet for 107 weeks (NTP, 2020)

Concen-tration in feed (ppm)	Dose (mg/kg-day)	Plasma concentration (mg/L)	Pancreatic acinar cell adenoma or adenocarcinoma	Uterine adeno-carcinoma	Uterine adenoma or adenocarcinoma
0	0	BD[a]	0/24[b]	1/32*	2/32
300	18	20.4	0/30	5/39	5/39
1,000	63	72.3	2/27	8/35*	8/35

BD: below the limit of detection; values were considered zero for dose-response analysis.
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): *, $p < 0.05$.
Control group tumor incidences with asterisks indicate significant results from exact trend test (conducted by OEHHA): *, $p < 0.05$.

Table 5.7.6. Tumor incidences in female Sprague Dawley rats exposed to PFOA perinatally and in the diet for 107 weeks (NTP, 2020)

Perinatal/ post-weaning concen-tration in feed (ppm)	Pancreatic acinar cell adenoma or adeno-carcinoma	Pancreatic acinar cell adeno-carcinoma	Pancreatic ductal adeno-carcinoma	Hepato-cellular carcinoma	Uterine adeno-carcinoma	Uterine adenoma or adeno-carcinoma
0/0	0/30[a]	0/24	0/40	1/23	1/40	2/40
150/300	0/38	0/36	0/42	0/32	3/43	3/43
300/1,000	4/33	1/24	1/41	4/23	5/41	5/41

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
[a] This denominator is different than that from Table 5.7.5 because it was calculated using the day of first occurrence from the 300/1,000 ppm group (day 673).

NTP (2020) noted that, "The additional effect of perinatal exposure in combination with postnatal exposure was uncertain and limited to the observation of hepatocellular carcinomas" for the male rat study, and, "The combined perinatal and postweaning exposure was not observed to

change the neoplastic or nonneoplastic response compared to the postweaning exposure alone" for the female rat study.

PFOS

Summaries of the sole report of carcinogenicity bioassays (Butenhoff et al., 2012b) for PFOS have been previously published (US EPA, 2016d; New Jersey DWQI, 2018). The study design and significant results are briefly described below.

Butenhoff et al. (2012b) published a report of carcinogenicity studies from 2002 by 3M (Thomford, 2002), a former PFOS manufacturer. In these studies, male and female Sprague Dawley rats were administered 0, 0.5, 2, 5, or 20 ppm potassium PFOS (K^+PFOS; 0, 0.024, 0.098, 0.242, or 0.984 mg/kg-day for males; 0, 0.029, 0.120, 0.299, or 1.251 mg/kg-day for females) in the diet for two years. Due to mortality issues, female rats in the 2 ppm group were administered K^+PFOS in the diet for 103 weeks, instead of 105 weeks. An additional group, referred to as the "recovery group" here, was administered 20 ppm PFOS for one year, and then control diet for the next year (data not shown).

A statistically significant increase in hepatocellular adenoma incidence was observed in both male and female animals at the highest dose. Positive trends for hepatocellular adenomas were reported in both sexes. Hepatocellular carcinoma is a rare tumor in female Sprague Dawley rats, with a historical control incidence of 0/765 (Baldrick, 2005) and 3/1,314 (Charles River, 2004)[9]. One hepatocellular carcinoma was observed in female rats; thus combined hepatocellular adenoma/carcinoma incidence was also increased in female rats. An increase in pancreatic islet cell carcinoma (by trend) was also observed in male rats. Tumor incidence data for male and female rats are summarized in Tables 5.7.7 and 5.7.8 respectively. Additionally, in female rats, increases in rare thyroid follicular cell adenoma and carcinoma combined were observed (although not statistically significant) and increased incidence of mammary fibroadenoma was observed in the low-dose female rat group (Table 5.7.9). It should be noted that the relatively low effective number of female rats was not due to high levels of premature mortality (mortality in treated groups was comparable to controls), but due to the fact that the first incidence of some tumors appeared quite late in the bioassay (day 653 for hepatocellular carcinoma, day 666 for hepatocellular adenoma, day 671 for thyroid follicular cell adenoma, and day 731 for thyroid follicular cell carcinoma).

While no increase of thyroid follicular cell adenoma was observed in the groups of male rats fed PFOS in the diet for two years, a statistically significantly increased incidence of thyroid follicular cell adenoma was observed in the group of male rats fed 20 ppm PFOS in the diet for one year followed by control diet for another year, in comparison to the control group (control, 3/31; 20 ppm in diet for one year, 9/29; $p < 0.05$). In female rats, one rare thyroid follicular cell adenoma was observed in the group fed 20 ppm PFOS in the diet for one year followed by control diet for another year, and none was seen in the controls. No increases of other tumors were observed

[9] Charles River (2004) includes studies that were initiated or published between 1989 and 2002. In general, the more relevant historical control data are provided by studies conducted within 2-3 years of the Thomford (2002) study. The Thomford (2002) study started in 1998 and last two years. Therefore, a subset of studies from Charles River (2004), initiated or published between 1995 and 2002, were used in OEHHA's analysis.

in these groups of male or female rats fed PFOS in the diet for one year followed by control diet for another year.

Table 5.7.7. Hepatocellular and pancreatic tumor incidences in male Sprague Dawley rats exposed to K^+PFOS in the diet for 2 years (Thomford, 2002; Butenhoff et al., 2012b)

Concentration in feed (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L)[a]	Hepatocellular adenoma[b]	Pancreatic islet cell adenoma[c]	Pancreatic islet cell carcinoma[d]	Pancreatic islet cell adenoma or carcinoma[c]
0	0	0.014	0/41**	4/44	1/38*	5/44
0.5	0.024	2.64	3/42	3/44	2/41	5/44
2	0.098	12.1	3/47	4/48	2/44	6/48
5	0.242	32.3	1/44	4/46	5/44	8/46
20	0.984	121	7/43**	4/44	5/40	9/44

[a] Calculated by OEHHA
[b] First occurrence at day 512
[c] First occurrence at day 465
[d] First occurrence at day 542
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (calculated by OEHHA): **, $p < 0.01$.
Control group tumor incidences with asterisks indicate significant results from exact trend test (conducted by OEHHA): *, $p < 0.05$; **, $p < 0.01$.

Table 5.7.8. Hepatocellular tumor incidences in female Sprague Dawley rats exposed to K^+PFOS in the diet for 2 years (Thomford, 2002; Butenhoff et al., 2012b)

Concentration in feed (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L)[a]	Hepatocellular adenoma[b]	Hepatocellular carcinoma[c]	Hepatocellular adenoma or carcinoma[c]
0	0	0.841	0/28**	0/28	0/28**
0.5	0.029	5.49	1/26	0/29	1/29
2	0.120	23.0	1/15	0/16	1/16
5	0.299	66.4	1/28	0/31	1/31
20	1.251	215	5/31*	1/32	6/32*

[a] Calculated by OEHHA
[b] First occurrence at day 666
[c] First occurrence at day 653
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor.
Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (reported by study authors): *, $p < 0.05$.

Control group tumor incidences with asterisks indicate significant results from exact trend test (conducted by OEHHA): **, p <0.01.

Table 5.7.9. Thyroid and mammary gland tumor incidences in female Sprague Dawley rats exposed to PFOS in the diet for 2 years (Thomford, 2002; Butenhoff et al., 2012b)

Concen-tration in feed (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L)[#]	Thyroid follicular cell adenoma[a,b]	Thyroid follicular cell carcinoma[a]	Thyroid follicular cell adenoma or carcinoma[a,b]	Mammary gland fibroadenoma[c]
0	0	0.841	0/26	0/24	0/26	20/60
0.5	0.029	5.49	0/25	0/15	0/25	27/50*
2	0.120	23.0	0/14	0/9	0/14	20/48
5	0.299	66.4	2/26	1/15	3/26	24/49
20	1.251	215	1/30	0/25	1/30	11/60

[#] Calculated by OEHHA
[a] Thyroid follicular cell adenomas and carcinomas are both rare in female Crl:CD (SD) BR rats (adenoma: 0.55% and carcinoma: 0 (Baldrick, 2005); adenoma: 0.86%, carcinoma: 0.50%, based on studies initiated or published between 1995-2002 in Crl:CD (SD) BR rats and reported by Charles River (2004)). There was only one thyroid follicular cell carcinoma that was observed during terminal sacrifice on day 731. The authors conducted terminal sacrifices on a series of dates, as early as day 719. All terminally sacrificed animals with thyroid gland examined were included in the denominators for thyroid follicular cell carcinoma.
[b] First occurrence at day 671
[c] The first occurrence of mammary gland fibroadenoma happened within the first year on day 229, therefore, incidence of mammary gland fibroadenoma for the control and 20 ppm groups includes 10 animals each from the one-year interim sacrifice group. For the 0.5, 2, and 5 ppm groups, there were no interim sacrifice groups at one year.
Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (reported by study authors): *, p <0.05.

5.7.3. Mode of Action and Mechanistic Considerations

PPARα

PFOA and PFOS are known activators of PPARα, as several studies have reported activation of rodent and human PPARα in controlled in vitro systems (Takacs and Abbott, 2007; Wolf et al., 2008a; Wolf et al., 2012). PPARα is a nuclear receptor that regulates genes that modulate the transport and metabolism of many biomolecules, including lipids, lipoproteins, glucose/glycerol, cholesterol, bile acid, and amino acids (Rakhshandehroo et al., 2010). Activation of PPARα leads to an increase in peroxisome number and size, and changes in peroxisomal and microsomal fatty acid β-oxidation levels.

Previous assessments have examined the role of PPARα in PFOA-induced toxicity (US EPA, 2016b; US EPA, 2016d; New Jersey DWQI, 2017; IARC, 2017a; New Jersey DWQI, 2018). These reviews included analyses of PPARα-related endpoints in rodents, effects of PFOA on PPARα knockout mice, and in vitro studies with rodent and human PPARα in various cell

systems. US EPA (2016b) indicated that mechanistic data for PFOA supports the PPARα MOA for liver tumors in rats (Butenhoff et al., 2012a), and may play indirect roles in testicular tumor induction. Conversely, (US EPA, 2016d) indicated that the available evidence does not adequately support a PPARα MOA for PFOS. IARC (2017a) reported that there is moderate evidence for many potential mechanisms for PFOA-induced toxicity (including PPARα). OEHHA reviewed the recent literature exploring the role of PPARα in PFOA toxicity.

Many studies have demonstrated that PFOA can induce liver toxicity independent of PPARα activation. Filgo et al. (2015) administered 0, 0.1, 0.3, 1, or 3 mg/kg-day PFOA to pregnant 129/Sv WT and PPARα KO 129/Sv mice via oral gavage from GD 1-17, and followed the female offspring for 18 months. PPARα KO F1 females had a significant increase in non-neoplastic liver lesions, including hepatocyte hypertrophy, bile duct hyperplasia, and hematopoetic cell proliferation, and an increase in hepatocellular adenomas was seen although it did not reach statistical significance (see Section 5.7.2).

Wen et al. (2019c) administered 0 or 3 mg/kg-day PFOA to C57BL/6NTac WT and PPARα KO mice via oral gavage for 7 days. Both WT and KO animals had increased liver weights, although the effect was more pronounced in WT animals. Additionally, PFOA induced changes in carboxylesterase protein expression in WT versus KO animals. Estrogen receptor alpha (ERα) mRNA expression was increased in KO animals, and there was also suggestive evidence that KO animals had greater CAR and PXR activation than WT animals.

Das et al. (2017) administered 0 or 10 mg/kg-day PFOA to male SV129 WT and PPARα KO mice via oral gavage for seven days. Both WT and KO animals showed liver toxicity, including increased relative liver weight, hepatocellular hypertrophy, decreased hepatic DNA content, and increased hepatic triglycerides (significant in WT animals only). Wild-type animals also displayed mild steatosis that was absent in KO animals. A gene expression analysis indicated that PFOA altered expression of genes related to lipid catabolism, fatty acid synthesis, and triglyceride synthesis in both WT and KO mice, although the effect was more pronounced in WT animals.

Wolf et al. (2008b) administered 0, 1, 3, or 10 mg/kg-day PFOA via oral gavage to 129S1/SvlmJ WT and PPARα KO mice for 7 days. Both WT and KO mice showed liver toxicity, including increased relative and absolute liver weight, hepatocyte hypertrophy, and increased labeling index (only at the high dose in KO mice). However, PPARα KO mice had an increase in vacuole accumulation. Hepatic ultrastructural changes in treated PPARα KO mice (compared to PPARα KO control mice) included decreased cytoplasmic glycogen granules, less rough endoplasmic reticulum, and cytoplasmic rarefaction.

Gene expression studies in the liver of WT and PPARα KO mice revealed PPARα-independent alterations in response to administration of PFOA and PFOS. PFOA (0, 1, or 3 mg/kg-day orally for 7 days) administered to PPARα-KO mice induced changes in the expression genes related to fatty acid metabolism, inflammation, xenobiotic metabolism, and cell cycle control (Rosen et al., 2008b). PFOS (0, 3 or 10 mg/kg-day orally for 7 days) administered to PPARα-KO mice induced changes in the expression of genes related to cholesterol biosynthesis, oxidative phosphorylation, ribosome biogenesis, and bile acid/cholesterol homeostasis (Rosen et al., 2010). These results suggest that PFOA and PFOS can induce biological activity in a PPARα-independent manner, and that all physiological effects of PFOA and PFOS should not be attributed to activation of PPARα.

Rebholz et al. (2016) reported that 3.5 mg/kg-day PFOA in the diet for five weeks activated PPARα in male BALB/c mice, but not in female BALB/c mice or C57BL/6 mice of both sexes. Nonetheless, C57BL/6 mice exposed to PFOA showed increased relative liver weight and changes in plasma and hepatic cholesterol levels. Furthermore, the authors reported that BALB/c mice have more PPARα than C57BL/6 mice, and that treatment with PFOA increased PPARα mRNA expression in male BALB/c mice, but not in C57BL/6 mice of either sex. Increased PPARα mRNA is an indicator of PPARα receptor activation (Yaacob et al., 2001; Blanquart et al., 2004; Rebholz et al., 2016), suggesting that PPARα is more readily activated in BALB/c mice compared to C57BL/6 mice. Li et al. (2017b) observed that PFOA (0.05 mg/kg-day via oral gavage for 28 days) induced liver toxicity (changes in mitochondrial membrane potential, apoptosis, and oxidative DNA damage) in female BALB/c mice without PPARα activation.

In summary, many studies have shown that PFOA and PFOS 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. Many chemicals that increase peroxisome proliferation in rodents also induce liver tumors. However, there is substantial debate about whether hepatic effects of PPARα-activating compounds in rodents are relevant to humans due to interspecies differences in activation characteristics.

Klaunig et al. (2003) presents a list of key events in the hypothesized pathway leading from PPARα activator treatment to liver tumor development. The key events identified in the proposed tumor progression pathway are 1) activation of PPARα, 2) perturbation of cell proliferation and apoptosis, and 3) selective clonal expansion. Klaunig et al. (2003) noted that rats and mice are more responsive than humans to some effects of PPARα activators in the liver: peroxisome proliferation, induction of fatty acid β-oxidation metabolic pathways, minimum ligand concentration for receptor activation, maximum receptor activation. They suggested that the liver tumor induction observed from exposure to some PPARα activators in rats and mice is not relevant to human cancer risk assessment.

The PPARα rat/mouse liver tumor mode of action (MOA) hypothesis proposed by Klaunig et al. (2003) depended in part on studies by Peters et al. (1997) and Ward et al. (1998) using PPARα KO mice derived from WT SV129 mice. Peters et al. (1997) found that 11 months of treatment with the PPARα agonist Wy-14,643 did not induce liver tumors in nine PPARα KO mice but did induce multiple hepatocellular tumors in the corresponding WT mice. Diethyl hexyl phthalate (DEHP) also did not induce peroxisome proliferation in PPARα KO mice after 24 weeks of exposure (Ward et al., 1998). Klaunig et al. (2003) suggested that a long-term DEHP cancer bioassay in PPARα KO mice would also be negative.

A number of PPARα activator studies in rats and mice, which have generated data relevant to the proposed PPARα tumor MOA, have been performed since the publication of that MOA hypothesis. Much of those data have been reviewed by Guyton et al. (2009) and Rusyn and Corton (2012).

Recently, Corton et al. (2018) reviewed the evidence for a PPARα MOA for hepatic tumorigenesis in rodents. Similar to Klaunig et al. (2003), the authors identified key events associated with PPARα activators, including PPARα receptor activation, cell growth pathway

alteration, perturbations in hepatocyte growth and survival, and the selective clonal expansion of preneoplastic foci cells, and hypothesized additional MOAs for tumorigenesis when PPARα is not involved. For example, Ito et al. (2007) found that exposure of SV129-derived PPARα KO mice to 100 or 500 ppm DEHP in the diet for up to 22 months resulted in the induction of liver tumors in those mice, with a significant trend for dose-response. These data indicate that PPARα activators may induce liver tumors by a mechanism independent of PPARα activation. However, Corton et al. (2018) argue that the absence of significant increases in liver tumors in WT mice (of the same strain) at the same doses complicate the interpretation of these results. Furthermore, they proposed alternate MOAs for tumor induction in PPARα-null mice, including activation of CAR, and the involvement of steatosis and inflammation, none of which preclude PPARα activation in WT animals.

Yang et al. (2007) developed transgenic mice (from a WT SV129 mouse) that constitutively expressed activated PPARα in hepatocytes in a targeted manner. The authors then used these mice to study whether the activation of PPARα in hepatocytes only is sufficient to induce liver tumors. These transgenic mice demonstrated peroxisome proliferation (including increased PCoA activity) and hepatocyte proliferation at 8 to 10 weeks of age. However, no liver tumors were noted in more than 20 mice at 11 months of age. In contrast, WT mice fed 0.1% Wy-14,643 in the diet for 11 months developed hepatocellular carcinomas (exact number of tumor-bearing animals not provided by authors). These data indicate that PPARα activation by itself is not sufficient to induce hepatocarcinogenesis. However, Corton et al. (2018) argue that the VP16PPARα fusion protein does not function in the precise manner that endogenous PPARα does. The authors claim that differences in downstream protein-protein interactions and differences in global transcription profiles induced by VP16PPARα (compared to endogenous PPARα) explain why these transgenic mice do not develop liver tumors.

Recently, Filgo et al. (2015) reported liver tumors were observed in female offspring (at 18 months of age) of PPARα KO mice, but not WT mice following gestational PFOA exposure. This result supports the observations in the Ito et al. (2007) study, that a PPARα activator can induce tumors in PPARα KO animals, but not in WT animals. It is unclear why PPARα KO animals developed tumors, whereas their WT counterparts did not. However, if the PPARα carcinogenesis MOA proposed by Klaunig et al. (2003) and Corton et al. (2018) is correct, then one would expect the opposite result.

The development of tumors in PPARα KO mice indicates that there are additional MOAs that contribute to DEHP- and PFOA-induced tumorigenesis. Corton and colleagues suggested activation of CAR, or increased steatosis and inflammation as alternate MOAs for liver carcinogenesis in rodents. Recent studies have reported that PFOA interacts with CAR in PPARα KO mice (Wen et al., 2019c), and altered gene expression of transcriptional targets associated with CAR in wild type and PPARα KO mice (Rosen et al., 2008a; Rosen et al., 2008b; Rosen et al., 2017). Similarly, PFOS altered levels of CAR-related transcripts in rats (Dong et al., 2016a) and in mice (Rosen et al., 2017).Furthermore, oral administration of PFOA or PFOS in rats induced significant increases in *Cyp2b1* and *Cyp2b2* expression, a marker of increased CAR activity, compared to controls (Elcombe et al., 2010; Elcombe et al., 2012a; NTP, 2019a; NTP, 2019b), CAR activation by PFOA is greater in PPARα KO mice compared to WT mice (Wen et al., 2019c). Conversely, in vitro nuclear receptor assays with PFOS showed no activity for rat CAR (Bagley et al., 2017). Interaction with CAR may be one of many additional MOAs for PFOA and PFOS, and one should not exclude other mechanisms simply

because one MOA has been identified. Additional evidence is needed before CAR activation is accepted as an additional MOA for PFOA-induced carcinogenesis.

Alterations in lipid metabolism and hepatic inflammation are commonly observed following exposure to PFOA and PFOS. However, it is unclear if these effects directly contribute to carcinogenesis, as the majority of rodent studies evaluating lipid metabolism and liver inflammation are of acute or subacute durations, which are not suitable for determining carcinogenic potential. Additional data are needed to determine whether increased liver lipid content and inflammation are sufficient to induce carcinogenicity in rodents.

Corton et al. (2018) argue that the microenvironment following PPARα activation in transgenic mice with constitutive PPARα activity is different enough from WT mice to prevent the formation of liver tumors, despite major biomarkers of PPARα activation (peroxisome proliferation, hepatomegaly, and decreases in serum triglycerides and free fatty acids) being present in both WT (given a PPARα activator) and transgenic animals. This explanation suggests that the typical physiological responses following PPARα activation are inconsequential for hepatic tumorigenesis in rodents, and seems to undermine the importance of the hypothesized sequelae of events with regards to tumorigenesis.

In summary, these lines of evidence suggest that PFOA-induced hepatotoxicity, including carcinogenesis, in rodents is not solely the result of PPARα activation. Liver toxicity was observed in PPARα KO mice, and in studies where PPARα was not activated (Filgo et al., 2015; Rebholz et al., 2016; Das et al., 2017; Li et al., 2017b; Wen et al., 2019c). Furthermore, rodent carcinogenicity studies with other PPARα activators have demonstrated that PPARα activation is not required for liver tumor induction, and that constitutive activation of PPARα does not lead to tumorigenesis. Therefore, OEHHA considers rodent liver toxicity studies, including carcinogenicity studies, to be relevant to human health. This is consistent with the position outlined in (IARC, 2017a) for PFOA, where human relevance could not be excluded based on the available evidence.

Klaunig et al. (2003) proposed that Leydig cell tumors and pancreatic acinar cell tumors are also induced by activation of PPARα in rodents, and along with liver tumors comprise a PPARα mediated “tumor triad.” The proposed MOA for pancreatic acinar cell tumors in rats is that PPARα activation leads to altered bile acid composition, which causes an increase in cholecystokinin (CCK) levels, which is a growth factor for acinar cells. Binding to CCK1 receptors leads to acinar cell proliferation, and subsequently neoplasia (Caverly Rae et al., 2014). This MOA was proposed predominantly based on studies of the PPARα agonist Wy-14,643. The authors argue that since human acinar cells do not have functional CCK receptors, this MOA is of minimal relevance to humans, and chemicals that cause pancreatic acinar cell tumors in rats are unlikely to induce tumors in humans. The authors also posit that human pancreatic tumors are predominantly ductal, and typically do not originate from acinar cells, unlike rodents, where ductal tumors are rare and acinar cell tumors more common.

However, there is little experimental support for the PPARα MOA for these tumor types (Peraza et al., 2006). Biegel et al. (2001) reported that 300 ppm (13.6 mg/kg-day) PFOA in the diet for two years induced pancreatic acinar proliferation in male Sprague Dawley rats, but the PPARα model agonist Wy-14,643 did not, suggesting that PPARα is not involved in pancreatic tumor formation, and that a different MOA is responsible for tumor induction. Additionally, NTP (2020) reported a large increase in pancreatic acinar cell tumors in male rats, a modest increase in liver

tumors, and no significant increase in Leydig cell tumors. If the proposed PPARα MOA is dominant, then one would expect liver tumors to be the most prevalent tumor type.

Furthermore, Murphy et al. (2008) reported that CCK activates calcium signaling and enzyme secretion in human pancreatic acinar cells, indicating that this pathway does have direct human relevance. Additionally, site concordance is not necessary when determining human relevance of animal tumors, so the difference in pancreatic tumor types between humans and rodents is not considered. Without additional experimental evidence linking pancreatic and testicular tumors in rodents solely to PPARα activation, OEHHA concludes that there is no scientific basis to exclude these tumor types for evaluation of human cancer risk.

It is likely that carcinogenesis occurs through multiple modes of action. In the *Key Characteristics of Carcinogens* section below, possible carcinogenic mechanisms are organized by key characteristics and discussed in greater detail.

Key Characteristics of Carcinogens

A comprehensive review of the more than 100 agents known to cause cancer in humans identified 10 key characteristics (KCs) of carcinogens (Smith et al., 2016; IARC, 2020). As the name implies, KCs are characteristics of agents that cause cancer, in contrast to the hallmarks of cancer (Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011), which are properties of cancer cells and neoplasms, and also in contrast to modes of action, which are sequences of key events that transform normal cells into malignant tumors. Mode of action analysis depends on prior knowledge sufficient to hypothesize how an agent might cause cancer, knowledge that too often is incomplete. The KCs can encompass many types of mechanistic endpoints and are not constrained to previously formulated hypotheses, allowing a broader consideration of multiple mechanistic pathways and hypotheses. OEHHA uses this approach to systematically identify, organize, and summarize information on mechanisms of carcinogenesis.

For this assessment of PFOA and PFOS, OEHHA reviewed the evidence identified through literature searches on five of the KCs (Table 5.7.10).

Table 5.7.10. Evidence relevant to the key characteristics of carcinogens for PFOA and PFOS[1]

Key Characteristic	Example of Relevant Evidence	Relevant Evidence Reviewed for PFOA	Relevant Evidence Reviewed for PFOS
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts	NR	NR
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, UDS), intercalation, gene mutations, cytogenetic changes (e.g., CAs, MN)	✓	✓
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)	NR	NR

Key Characteristic	Example of Relevant Evidence	Relevant Evidence Reviewed for PFOA	Relevant Evidence Reviewed for PFOS
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression	NR	NR
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)	✓	✓
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production	NR	NR
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction	✓	✓
8. Modulates receptor-mediated effects	Receptor inactivation/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)	✓	✓
9. Causes immortalization	Inhibition of senescence, cell transformation	NR	NR
10. Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis	✓	✓

[1] This review was conducted based on information identified through a focused literature search conducted in March 2020. A checkmark (✓) means that evidence for that particular KC was reviewed, and NR stands for "not reviewed." Source of the KCs and examples: Smith et al. (2016); IARC (2019). Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would one KC alone.
Abbreviations: AhR, aryl hydrocarbon receptor; CA, chromosomal aberration; ER, estrogen receptor; MN, micronuclei; PPAR, peroxisome proliferator-activated receptor; UDS, unscheduled DNA synthesis.

The evidence relevant to these five KCs for PFOA and PFOS is briefly summarized below. More detailed discussions of the findings from studies of PFOA and PFOS relevant to these five KCs are provided in Appendix 8.

Overall, the evidence relevant to the KCs of carcinogens reviewed for PFOA is summarized as follows:

- KC2: PFOA has been tested in many genotoxicity test systems that have assessed numerous endpoints indicative of either mutagenicity, chromosomal effects, or DNA damage. Several studies provide evidence that PFOA causes DNA damage -- measured as increases in DNA strand breaks (in human cell lines and in non-mammalian species), γ-H2AX (in a human cell line), and 8-hydroxydeoxyguanosine (8-OHdG, a marker of oxidative DNA damage, in human cell lines and in rodent liver). Some studies provide evidence that PFOA may have chromosomal effects, while others do not, and several studies provide evidence that PFOA is not mutagenic.

- KC5: A number of studies indicate that PFOA may cause oxidative stress. Two studies in rodents and two studies in human cells have shown that PFOA led to increased 8-OHdG, while one study in human cells reported mixed results and two studies (one in exposed humans and one in a unicellular organism) found no effect. Several studies, including one in mice, four in human HepG2 cells, and one in a mouse cell line, have shown that PFOA increased intracellular production of reactive oxygen species (ROS), while a study in human-hamster hybrid cells showed increased intracellular production of both ROS and reactive nitrogen species (RNS). Increased lipid peroxidation was observed in mice, in human erythrocytes exposed in vitro, and in a rat cell line. PFOA also has been shown to alter total antioxidant capacity (TAC), antioxidant enzyme content or activity, and glutathione levels in mice, fish, and in in vitro studies of human erythrocytes and human HepG2 cells.
- KC7: Several animal studies have shown that PFOA suppresses IgM production as either a T cell dependent antibody response (TDAR) or T cell independent antibody response (TIAR), reduces cellularity and proliferation of T cells and B cells, and reduces the number of neutrophils. The preponderance of evidence shows that PFOA can suppress the immune system in ways that would allow neoplastic cells to escape immune surveillance, survive, and replicate to form tumors.
- KC8: Several animal studies have shown that PFOA alters gene expression in the liver, and that these effects are mediated through ERα, PPARα, PPARγ, PXR, and CAR. Evidence that PFOA can bind to or activate ERα, PPARα, and possibly PPARβ/δ comes from in silico modeling studies (human ERα), studies in human cells or cell lines (ERα, PPARα), and studies in animal tissue preparations or cell lines (ERα, PPARβ/δ). There is also evidence from studies in animals that PFOA can modulate levels of endogenous hormones, including estradiol, progesterone, testosterone and thyroid hormones, and possibly levels of growth factors in the testis and mammary gland.
- KC10: Several animal studies provide evidence that PFOA increases cell proliferation, based on respiratory tissue hyperplasia observed in rats, and stimulated mammary gland development in mice. In addition, PFOA increased levels of regulatory cell cycle proteins in a human breast epithelial cell line, and increased cell proliferation in multiple studies of human breast and ovarian cell lines.

Overall, the evidence relevant to the KCs of carcinogens reviewed for PFOS is summarized as follows:

- KC2: PFOS has been tested in many genotoxicity test systems that have assessed numerous endpoints indicative of either mutagenicity, chromosomal effects, or DNA damage. Some studies provide evidence that PFOS is mutagenic (in transgenic mice and fish and in transgenic mouse cells in vitro), several studies provide evidence of chromosomal effects (e.g., induction of micronuclei (MN) in rodents and zebrafish), and several studies provide evidence of DNA damage (e.g., induction of DNA strand breaks in rats, zebrafish, and other non-mammalian species).
- KC5: A number of studies indicate that PFOS may cause oxidative stress. Several studies, including one in rats, three in human HepG2 cells, two in rodent cells or cell lines, and one in *C. elegans*, have shown that PFOS increased intracellular production of ROS. Increased lipid peroxidation was observed in one study in rats and two studies in rat cells or cell lines. PFOS has also been shown to alter antioxidant enzyme activity and glutathione levels in one study in rats and two studies in human HepG2 cells.

- KC7: Several animal studies have shown that PFOS suppresses TDAR/TIAR IgM production and reduces cellularity of T cells and B cells. PFOS also suppresses NK cell activity, as shown in one study in cultured human blood cells and three studies in mice, although one other mouse study reported an increase in NK cell activity. The preponderance of evidence shows that PFOA and PFOS can suppress the immune system in ways that would allow neoplastic cells to escape immune surveillance, survive, and replicate to form tumors.
- KC8: Several animal studies have shown that PFOS alters the expression of genes that are regulated by ERα, PPARα, PPARγ, PXR, and CAR, and one reporter gene study shows PFOS activates murine PPARβ/δ in vitro. The evidence for the estrogenic effect of PFOS also comes from increased ER reporter activity in human cells in vitro, increased proliferation of estrogen-responsive human breast cancer cell lines in several studies, weak binding to ER in fish, and similar gene expression patterns between PFOS and E2 in fish. One reporter gene study indicates PFOS inhibited androgen receptor (AR) activation by dihydrotestosterone (DHT). There is also evidence from animal studies that PFOS can decrease thyroid hormone levels and increase estradiol levels.
- KC10: Two studies in rats provide evidence that PFOS increases cell proliferation and inhibits apoptosis in the liver, with the latter effect being long-lived. In multiple studies of human fetal liver, breast and ovarian cell lines, PFOS increased cell proliferation. In addition, PFOS altered the expression of proteins linked to cell proliferation, including increasing levels of regulatory cell cycle proteins and growth factors in a human fetal liver cell line, and increased cell proliferation in multiple studies of human breast, ovarian, and fetal liver cell lines.

5.7.4. Conclusions

Based on the evidence of cancer in human and animal studies (Table 5.7.11), OEHHA determined that PFOA and PFOS should be evaluated as carcinogens.

The association of PFOA and kidney cancer in humans provides strong evidence of carcinogenicity. Additionally, hepatic and pancreatic acinar tumors have been reported in animal carcinogenicity studies of PFOA, while hepatic and pancreatic islet tumors have been reported in animal carcinogenicity studies of PFOS. OEHHA determined that the cancer data derived from animal studies are relevant to human health, and support the positive cancer findings in the human epidemiology data. PFOA appears to act through multiple MOAs, and the PPARα MOA does not adequately explain the incidences of pancreatic and testicular tumors reported. Furthermore, there is considerable uncertainty whether hepatic tumors in rodents are solely induced by activation of PPARα, so the liver tumors in rodents induced by PFOA and PFOS should not be dismissed due to the assumption that it lacks human relevance.

Mechanistic studies of PFOA and PFOS have shown that these chemicals possess several of the key characteristics of carcinogens, including the ability to induce oxidative stress, inflammation, and modulate receptor-mediated effects. Additionally, there is suggestive evidence that PFOA and PFOS are genotoxic, thus a genotoxic MOA for cancer remains plausible. These data support the human and animal evidence that PFOA and PFOS present a carcinogenic hazard.

Table 5.7.11. Summary of OEHHA's conclusions regarding the human and experimental animal data on PFOA and PFOS and cancer

Outcome	PFOA	PFOS
Bladder cancer in humans	Mostly no association	Few studies, no clear or consistent associations
Breast cancer in humans	High relative risks in some subgroups but yet to be confirmed	High relative risks in some subgroups but yet to be confirmed
Kidney cancer in humans	Strong evidence, including a large multicentric study, mostly positive findings	Few studies, no clear or consistent associations
Liver cancer in humans	Some positive associations from occupational studies but small sample sizes	Few studies, small sample sizes
Pancreatic cancer in humans	Weak evidence, most studies with small sample sizes	Few studies, no clear or consistent associations
Prostate cancer in humans	Mostly no association	Few studies, no clear or consistent associations
Testicular cancer in humans	Some positive associations but small sample sizes	No studies
Liver tumors in animals	Positive evidence in both sexes of rats, identified by multiple laboratories, with one study showing evidence of transplacental carcinogenicity in mice	Positive evidence in both sexes of rats
Pancreatic tumors in animals	Positive evidence in male rats (multiple laboratories) and female rats	Positive evidence in male rats
Testicular tumors in animals	Positive evidence in multiple studies	Not reported
Uterine tumors in animals	Positive evidence in one study	Not reported

5.8. Other Toxic Effects

Effects of PFOA and PFOS on the immune system, liver, thyroid, reproduction and development, and the nervous system have been described in their respective sections above. Additional noncancer toxicities are reported in this section.

US EPA (2016b) reported that reduction in body weight was commonly observed in animal studies of PFOA. Additional toxicity endpoints in animal studies included increased absolute and/or relative kidney weight in rats (Butenhoff et al., 2004; Cui et al., 2009, as reported in US EPA, 2016b), effects on the lung, including pulmonary congestion, in rats (Cui et al., 2009, as reported in US EPA 2016b), effects on the adrenal cortex in male rats (Butenhoff et al., 2004, as reported in US EPA, 2016b), and increased serum leptin and insulin levels in mice (Hines et al., 2009, as reported in US EPA, 2016b).

Like PFOA, US EPA (2016d) reported that PFOS reduced body weight in rats and monkeys. In monkeys, oral administration of PFOS induced gastrointestinal toxicity (anorexia, emesis, black stool), lipid depletion in the adrenals, atrophy of pancreatic exocrine and serous alveolar cells, and pulmonary necrosis and inflammation (Goldenthal et al., 1978; Goldenthal et al., 1979; Seacat et al., 2002, as reported in US EPA, 2016d). Acute inhalation exposure in rats induced breathing disturbances and discoloration of the lung (Rusch et al., 1979, as reported in US EPA, 2016d).

5.8.1. Recent Animal Evidence

Animal studies of PFOA and PFOS, published from 2016 onward, reporting toxicity outcomes outside of the broad categories discussed in previous sections are described below.

Decreases in body weight gain were reported in a number of short-term studies of PFOA in rodents (Hui et al., 2017; Suo et al., 2017; Du et al., 2018; Crebelli et al., 2019; Cui et al., 2019; Song et al., 2019). However, one study did report an increase in body weight (Koskela et al., 2016).

Several PFOA studies reported adverse effects in adipose tissue in mice, including decreased adipose tissue mass (Li et al., 2019d; Pouwer et al., 2019) and increased adipocyte cell size (van Esterik et al., 2016). Du et al. (2018) reported adipose tissue histopathology (including atrophy, nucleolus deformation, cytoskeletal impairment, vacuolization, and reduced organelles), increased adipose cell death, and reduced leptin and adiponectin levels in the blood of mice. PFOA can activate uncoupling protein 1 (UCP1) in brown adipose tissue, which is normally activated by fatty acids (Shabalina et al., 2016). The result of activation of this protein leads to heat production and energy consumption in animals.

PFOA has been associated with adverse effects on bone health. Koskela et al. (2016) reported changes in bone density and morphometric properties in mice exposed to PFOA in utero. Additionally, in utero exposure to PFOA reduced tibia and femur length (along with a decrease in quadriceps femoris muscle weight) in mice (van Esterik et al., 2016).

Increased blood glucose levels (Zheng et al., 2017; Du et al., 2018) and increased insulin levels (Wu et al., 2017; Du et al., 2018; Wu et al., 2018) were reported in several mouse studies of PFOA. Furthermore, PFOA induced changes in pancreatic cytoarchitecture and serum trypsinase levels (Wu et al., 2017), and changes in glucose metabolism/homeostasis pathways (Wu et al., 2017; Zheng et al., 2017) in male mice.

Much like PFOA, PFOS has been reported to cause decreases in body weight gain in rodents (Li et al., 2016b; Qu et al., 2016; Wan et al., 2016; Xing et al., 2016; Bagley et al., 2017; Wang et al., 2018; Su et al., 2019). Other effects in rodents include decreased absolute kidney weight (Xing et al., 2016), sensitivity to light (Bagley et al., 2017), increased serum corticotropic releasing factor (CRF), adrenocorticotropic hormone (ACTH) and corticosterone (Salgado-Freiria et al., 2018), and changes in gut metabolism and gut bacteria (Xu et al., 2017; Lai et al., 2018; Salgado-Freiria et al., 2018). Acute exposure to PFOS increased mortality, rough hair, constipation, anorexia, asthenia, syncope, and reduced food consumption in mice (Xing et al., 2016).

PFOS has been associated with an early reduction in blood glucose following glucose or insulin administration in female mice (Lai et al., 2018), and increased insulin levels in the serum of male mice (Su et al., 2019).

5.8.2. Recent Mechanistic Evidence

Mechanistic studies of PFOA and PFOS, published from 2016 onward, that fall outside the broad toxicity categories above are described below.

In a metabolomics study using rat kidney mesangial cells, a model of diabetic kidney disease, PFOA altered amino acid biosynthesis and metabolism (Gong et al., 2019). Fibrosis and inflammation were also observed in PFOA exposed cells. In frog kidney cells, PFOA caused alterations in DNA/RNA (genotoxicity), secondary protein structure, lipids, and fatty acids in cells that formed monolayers and in cells differentiating into dome structures (Gorrochategui et al., 2016). Low doses of PFOA had a greater effect on cells forming monolayers while high doses had a greater effect on dome forming structures.

Animal studies show that PFOA can impact bone development, and this is supported by several in vitro studies. (Koskela et al., 2017) reported that PFOA induced an increase in osteoclasts derived from human peripheral blood, and an increase in osteoclast resorption area. In MC3T3-E1 osteoblast cells, PFOA increased levels of oxidative stress (increased ROS and mitochondrial superoxide), adversely impacted mitochrondrial function (increased membrane potential collapse, decreased adenosine triphosphate (ATP) production, increased cardiolipin peroxidation, and increased cytochrome c release), and osteoblast differentiation (decreased collagen synthesis, decreased alkaline phosphatase (ALP) activity, and decreased mineralization) (Choi et al., 2017). PFOA also altered mRNA and protein levels of transcription factors and the expression of other genes related to osteogenic differentiation in human mesenchymal stem cells (Liu et al., 2019a; Pan et al., 2019). In mouse osteoblast cells (MC3T3-E1), PFOA altered levels of osteoblast differentiation markers, increased the number of multinucleated osteoclasts, and altered bone resorption levels (Koskela et al., 2016).

Berntsen et al. (2017) exposed primary cerebellar granular neurons from rat pups to PFOA, and reported that PFOA accumulated diffusely in cell membranes. The authors also reported that vitamin E protected against cytotoxicity, suggesting that oxidative stress may be involved in PFOA-induced cytotoxicity, but there was no observed increase in ROS or lipid peroxidation.

PFOA induced markers of oxidative stress in human erythrocytes (Pan et al., 2018). PFOA also increased ROS levels and autophagy in the mitochondria and endoplasmic reticulum of A549 lung cancer cells via activation of the MAPK pathway and inhibition of the PI3K/AKT pathway (Xin et al., 2018b). Inhibition of autophagy increased PFOA toxicity, suggesting that autophagy has a protective function.

Studies have shown that PFOA can bind to steroid hormone receptors (Kang et al., 2016; Behr et al., 2018; Chaparro-Ortega et al., 2018; Qu et al., 2018; Cao et al., 2019; McComb et al., 2019; Xin et al., 2019). Interaction with steroid hormone receptors can alter normal hormone secretion. PFOA decreased adrenocorticotropic hormone, and increased corticosteroid binding globulin (CBG) and cortisone in mice (Sun et al., 2018b). Additionally, PFOA can bind to transporters such as fatty acid binding protein and T4 serum carrier-protein transthyretin (TTR) (Selano et al., 2019), displacing endogenous ligands and disrupting essential processes in the

liver. PFOA has also been shown to bind to pepsin, an enzyme in the stomach that breaks proteins down into polypeptides (Yue et al., 2016).

Associations between PFOA and changes in lipid metabolism and homeostasis have been reported in human epidemiology studies and animal toxicity studies, and these endpoints are supported by in vitro mechanistic studies. Adipocyte differentiation and lipid accumulation were observed in human and mouse adipocytes exposed to PFOA (Ma et al., 2018; Li et al., 2019b). In T3-L1 preadipocytes, PFOA caused a dose-dependent increase in adipocyte proliferation and increase in accumulation of lipids and triglycerides (Ma et al., 2018).

In a study comparing gene expression analyses of endocrine disrupting chemicals, PFOA altered genes related to cellular infiltration, necrosis and hypertrophy, and NRF2-mediated oxidative stress in rhesus monkey embryonic cells (Midic et al., 2016). In rhesus monkey trophoblast stem cells, PFOA induced gene expression changes in many different pathways, including cysteine metabolism, interleukin signaling, and PPAR, among others (Midic et al., 2018).

Recent in vitro studies indicate that PFOA can impact pancreatic cells. Liu et al. (2018d) showed that PFOA impaired specification of pancreatic progenitor cells from human embryonic cells. Additionally, PFOA decreased cell viability, increased apoptosis and oxidative stress and caused mitochondrial membrane potential collapse in rat pancreatic β-cell-derived RIN-m5F cells (Suh et al., 2017).

PFOA can activate the kallikrein-kinin system (KKS) in mice and in human retina endothelial cells. KKS is involved in regulating inflammation, blood pressure and vascular permeability (Liu et al., 2017a; Liu et al., 2018c).

PFOA induced decreased cell viability, cell morphology changes and increased intracellular calcium in chicken primary embryo cardiomyocytes (Lv et al., 2019). The observed toxicity was due to changes in protein expression of Wnt5a/Frizzled2, which regulates calcium homeostasis. Further, interaction of PFOA with PPARα was shown to play a role in regulating these proteins.

PFOA can partition into bacterial and model phospholipid bilayers, and affect lateral phospholipid interactions within the bilayer (Fitzgerald et al., 2018). While the significance of these findings to human health is unclear, alteration of the cellular membrane lipid bilayer could theoretically impair the function of many proteins embedded in the lipid bilayer.

In a metabolomics study of a diabetic kidney disease model of rat kidney mesangial cells, PFOS increased oxidative stress and changed amino acid biosynthesis and metabolism, which subsequently lead to increased fibrosis and inflammation (Gong et al., 2019). PFOS caused epithelial-mesenchymal transition (EMT)-associated renal fibrosis in rat tubular epithelial cells (Chou et al., 2017). Renal fibrosis was shown to be through Sirt1-mediated PPARγ deacetylation. PFOS induced apoptosis of rat renal tubular cells was also shown to be associated with Sirt1 and a PPARγ-dependent mechanism (Wen et al., 2016). Like PFOA, PFOS caused alteration in DNA/RNA, secondary protein structure, lipids, and fatty acids in frog kidney cells (Gorrochategui et al., 2016). The authors suggest that the nucleic acid alterations are indicative of genotoxicity.

PFOS suppressed osteogenic differentiation in human bone marrow derived mesenchymal stem cells via inhibition of calcium deposition and changes in mRNA levels of osteogenic markers

and transcription factors (Liu et al., 2019b). Liu et al. (2019b) also report changes in markers of bone turnover. Similarly, (Pan et al., 2019) reported changes in mRNA and protein levels of genes involved in osteogenic differentiation in human mesenchymal stem cells.

PFOS can activate UCP1 in brown adipose tissue, which is normally activated by fatty acids (Shabalina et al., 2016). Activation of this protein leads to heat production and energy consumption in animals. The authors suggest this may pose a risk of altered energy metabolism to newborns and infants, who normally have brown adipose tissue and can be exposed to PFOS through breastmilk.

Several in vitro and in silico studies demonstrate that PFOS affects adipogenesis and may be an environmental risk factor for obesity. PFOS induced adipogenesis in vitro in murine-derived 3T3-L1 preadipocytes and in vivo in mice through activation of Nrf2 signaling (Xu et al., 2016). In mice, PFOS also induced PPARγ and CCAAT-enhancer binding protein α expression, a transcription factor involved in adipogenesis and adipocyte differentiation. Molecular docking analyses show that PFOS can bind to PPARγ (as well as PPAR α and β), which are important regulatory pathways for adipogenesis (Li et al., 2018a). PFOS also increased lipid content in 3T3-L1 cells in a dose-dependent manner and enhanced expression of adipogenic related genes. However, PFOS decreased lipid accumulation in human differentiating adipocytes and affected DNA methylation of adipogenic genes, although gene expression was not affected, suggesting minimal impacts on adipogenic pathways (van den Dungen et al., 2017).

A binding study by Kumar et al. (2018) showed that PFOS binds to the alpha4-helical domain in proteins involved in cholesterol biotransformation and transport in mitochondria.

Chen et al. (2018c) and Chen et al. (2018d) reported increased markers of inflammation (glial fibrillary acidic protein and proliferating cell nuclear antigen (PCNA)), changes in cytokine expression (IL-1β, TNF-α), and modulation of signal transduction pathways (NF-κB, JAK2/STAT3) in C6 glioma cells (astrocytes) exposed to PFOS. Similarly, PFOS activated the JAK2/STAT3 pathway in astrocytes from newborn rat pups (Chen et al., 2018d). The authors propose that PFOS activates NF-κB and JAK2/STAT3 signaling, which subsequently activates astrocytes and leads to the secretion of pro-inflammatory cytokines. Chen et al. (2018d) also exposed SH-SY5Y human neuroblastoma cells to the cellular media used in the experiments with the C6 glioma cell line (containing cytokines secreted from astrocytes following exposure to PFOS). The neuroblastoma cells displayed elevated biomarkers of apoptosis (increased activation of caspase 3 and cleavage of poly (ADP-ribose) polymerase (PARP)), and the authors claim that TNF-α secreted from astrocytes in response to PFOS exposure is responsible.

PFOS altered gene and protein expression of neuroendocrine hormones (such as gonadotropin releasing hormone, luteinizing hormone, and follicle stimulating hormone) in the hypothalamic-pituitary-thyroid axis of rats (López-Doval et al., 2016). Additionally, PFOS induced changes in corticotropic releasing factor and glucocorticoid receptors in the brain of male rats (Salgado-Freiria et al., 2018), and reduced kisspeptin expression (which regulates gonadotropin releasing hormone in the hypothalamus) in female mice (Wang et al., 2018). PFOS can interact with steroid hormone receptors, which can result in subsequent changes to hormone secretion (Kang et al., 2016; Behr et al., 2018; Chaparro-Ortega et al., 2018; Qu et al., 2018; Cao et al., 2019; McComb et al., 2019). In mouse Leydig tumor cells, PFOS disrupted membrane potential, increased ROS production and disrupted progesterone production (Zhao et al., 2017).

Liu et al. (2018d) showed the PFOS impaired specification of pancreatic progenitor cells from human embryonic cells. Disrupted development of the pancreas can lead to metabolic diseases such as diabetes, which have been linked to PFOS exposure in people.

PFOS can activate the KKS in mice and in human retina endothelial cells. KKS is involved in regulating inflammation, blood pressure and vascular permeability (Liu et al., 2017a; Liu et al., 2018c).

A mechanism for bioaccumulative properties of PFOS may be through binding to phospholipids in lung cells and adipocytes (Sanchez Garcia et al., 2018). In a docking study, PFOS was shown to bind to human serum albumin (HSA) through electrostatic forces and hydrogen bonds (Liu et al., 2017b). This binding resulted in decreased esterase activity of HSA.

ToxCast High-Throughput Toxicity Screening

ToxCast™ (US EPA Toxicity Forecaster) is a chemical prioritization research program developed by the US EPA (Dix et al., 2007; Judson et al., 2010; Kavlock et al., 2012). ToxCast includes data generated by the Tox21 (Toxicity Testing in the 21st Century) program, which is a multi-agency collaboration between the National Institute of Environmental Health Sciences/National Toxicology Program, the National Center for Advancing Translational Sciences, the US Food and Drug Administration, and US EPA's National Center for Computational Toxicology. ToxCast utilizes various in vitro and zebrafish systems to identify chemical activity in a battery of high-throughput screening (HTS) assays. OEHHA explored ToxCast data on PFOA and PFOS using information that is publicly available on the Computational Toxicology (CompTox) Chemicals Dashboard.[10]

The ToxCast database on the CompTox Chemicals Dashboard reported that PFOA was active in 81 of the 1,073 assays in which it was tested, PFOA ammonium salt was active in 58 of the 860 assays in which it was tested, PFOS was active in 260 of the 1,165 assays in which it was tested, PFOS potassium salt was active in 179 of the 895 assays in which it was tested, and PFOS lithium salt was active in 26 of the 238 assays in which it was tested. ToxCast assays in which PFOA, PFOA ammonium salt, PFOS, PFOS potassium salt, and PFOS lithium salt were active are shown in Appendix 9, Tables A9.1-A9.5, respectively. Although there were several curve-fitting flags (flag details are not included in Appendix 9) associated with some ToxCast assays in which PFOA, PFOS, and their salts were active, OEHHA did not apply these flags as a cutoff for excluding assays, for the following reasons. First, these curve-fitting flags are subject to change as the ToxCast data analysis pipeline evolves (Thomas et al., 2019). Second, completely filtering out all active assay calls that have curve-fitting flags is not recommended because potential biological signals could be omitted (Judson et al., 2016).

There are several limitations that exist in the PFOA and PFOS ToxCast datasets. First, the purity grades of PFOA, PFOA ammonium salt, PFOS, PFOS potassium salt, and PFOS lithium used in the ToxCast assays were not reported, while the purity grades of the test substances used in the Tox21 assays are reported as "unknown/inconclusive," based on the Tox21 quality control analyses.[11] Also, PFOA and PFOS and their salts are surfactants, and the disposition of these chemicals in ToxCast in vitro assay systems (other than the zebra fish assays) remain uncertain. It has been acknowledged that chemical disposition and partitioning can greatly

[10] https://comptox.epa.gov/dashboard, accessed on May 3, 2021

[11] https://tripod.nih.gov/tox21/samples, accessed on May 3, 2021

affect the accuracy of predictions from in vitro test systems.[12] Additionally, the surfactant properties of PFOA and PFOS may cause cell lysis and cytotoxicity at high concentrations in cell-based assays, e.g., in human bronchial epithelial cells (Sorli et al., 2020). Due to these limitations, the ToxCast data on PFOA and PFOS and their salts are not considered to contribute much to the overall body of mechanistic evidence for these chemicals.

5.8.3. Conclusions

PFOA induces multiple toxicity endpoints in experimental animals, including reduction in body weight, adverse effects on adipose tissue, negative impacts on bone health, and perturbations of glucose homeostasis. In vitro mechanistic studies of PFOA on adipose tissue and osteoclasts/osteoblasts support the in vivo evidence. Other mechanistic studies reveal additional potential MOAs of PFOA, including inducing oxidative stress, interaction with steroid hormone receptors, perturbation of metabolism, disruption of normal macromolecule structure, and inducing fibrosis and inflammation in rat kidney cells.

Much like PFOA, PFOS commonly induces reductions in body weight in animals. Other endpoints have been reported in the literature, but consistent observations across studies are not observed. Mechanistically, PFOS behaves similarly to PFOA in many of the evaluated studies, including effects on bone and adipose tissue, generation of ROS, interactions with steroid hormone receptors, and induction of fibrosis and inflammation in rat kidney cells.

[12] Tox21 Cross-Partner Projects. 4. *In vitro* Chemical Disposition. Available: https://tox21.gov/projects/. Crizer D, Sipes N, Waidyananthaet S et al (2020): *In Vitro* Disposition of Tox21 Chemicals: Initial Results and Next Steps. Available: https://www.epa.gov/sites/production/files/2020-10/documents/7_david_crizer_epa_nams_conference_2020_508c.pdf

6. DOSE-RESPONSE ASSESSMENT

6.1. Noncancer Dose-Response Analyses and Acceptable Daily Dose Derivation

6.1.1. Perfluorooctanoic Acid

Human Studies

Based on its review of the currently available human studies, OEHHA has determined that the most sensitive noncancer endpoints for PFOA are immunotoxicity, liver toxicity, and alterations in lipid metabolism or production. A number of studies have also linked PFOA to adverse effects related to the thyroid gland and alterations of thyroid hormone levels; however, these findings were too inconsistent for OEHHA to make firm conclusions regarding these outcomes. Associations with several reproductive and developmental outcomes have also been reported. While the positive associations reported in some of these studies are cause for concern, overall, the findings from study to study were also somewhat inconsistent, and OEHHA was unable to identify studies that could be used to accurately evaluate the dose-response patterns for these outcomes.

In the following sections, OEHHA presents the human studies it considered as candidate studies for its POD determinations.

Immunotoxicity

A number of animal and human studies have provided evidence that PFOA can increase the risks of immune-related diseases or otherwise adversely affect the immune system. With regards to human studies, increased serum levels of PFOA have been linked to diminished antibody levels in response to vaccinations for diphtheria, tetanus, rubella, mumps, and influenza (Hib) (Granum et al., 2013; Looker et al., 2014; Stein et al., 2016b; Grandjean et al., 2017a; Grandjean et al., 2017b; Pilkerton et al., 2018; Abraham et al., 2020). These findings have been seen in both adults and children, although the greatest effect sizes have been reported in children. For example, decreases in tetanus and diphtheria antibody concentrations of 25% or more have been reported for each doubling of PFOA serum concentration in children from the Faroe Islands (Grandjean et al., 2017a) (Table 5.1.1). A number of other studies have identified associations between PFOA and immune-related diseases such as asthma or respiratory tract infections (Qin et al., 2017; Timmermann et al., 2017; Impinen et al., 2018; Averina et al., 2019; Kvalem et al., 2020), although the evidence on these outcomes is less consistent than that seen for diminished vaccine response.

The most sensitive endpoints for vaccine response appear to be response to childhood influenza, tetanus, and diphtheria vaccinations. The human studies that considered these endpoints include research from two Faroe Islands cohorts and the study in healthy one-year old children in Germany by Abraham et al. (2020). These studies, and the PODs that OEHHA derived from them, are discussed below.

Abraham et al. (2020):

NOAEC/LOAEC method: Details of the Abraham et al. (2020) study are provided in Chapter 5. The results from this study for influenza (Hib), tetanus, and diphtheria antibody levels are shown in Table 6.1.1. The NOAECs are highlighted in green in this table. These are the mean PFOA serum concentrations in the highest quintile of PFOA that did not show a statistically significant difference in antibody levels, which in all three cases is the 4th quintile, compared to the reference category (the lowest quintile of PFOA). The p-values in this table were calculated by the study authors using linear regression models adjusted for the number of vaccinations the child had received and for the time since the last vaccination. As shown, the NOAECs ranged from 18.9 to 19.4 ng/ml. The authors also presented data by PFOA deciles, which involved smaller sample sizes and less statistical power but showed similar NOAECs (20.5 to 22.4 ng/ml).

Table 6.1.1. Mean serum antibody concentrations by quintiles of serum PFOA (ng/ml) from Abraham et al. (2020). The NOAECs are highlighted in green.

Anti-Hib (mg/dl)					
Quintile	**PFOA[1]**	**N**	**Mean[2]**	**SD**	**p-value**
1	3.4	20	1.84	0.68	Ref
2	8.5	20	1.84	0.71	0.98
3	14.8	20	1.84	0.84	0.98
4	19.4	20	1.50	0.55	0.09
5	25.7	18	1.19	0.6	0.003

Anti-tetanus (mg/L)					
Quintile	**PFOA[1]**	**N**	**Mean[2]**	**SD**	**p-value**
1	3.4	20	1.07	0.31	Ref
2	8.5	20	1.04	0.44	0.77
3	14.5	20	1.02	0.39	0.67
4	18.9	20	1.02	0.35	0.61
5	25.3	20	0.74	0.36	0.003

Anti-diphtheria (IU/ml)					
Quintile	**PFOA[1]**	**N**	**Mean[2]**	**SD**	**p-value**
1	3.4	20	0.50	0.41	Ref
2	8.5	20	0.57	0.29	0.56
3	14.5	20	0.64	0.36	0.26
4	18.9	20	0.40	0.45	0.49
5	25.3	20	0.13	0.53	0.02

Abbreviations: Ref, reference category; SD, standard deviation
[1] Mean PFOA serum concentrations (ng/ml) in each quintile
[2] Log_{10} values of the adjusted antibody levels

Knee function method: The authors of Abraham et al. (2020) also estimated NOAECs using the knee function method. Here, they used the breakpoint in a piecewise linear regression model with two segments to estimate the 'knee' function, the point at which the association between increasing PFOA and decreasing antibody levels begins to occur. An informative visual display of these results can be seen in Figure 3 of the Abraham et al. (2020) publication. Based on this method, the study authors reported NOAECs of 12.2, 16.9, and 16.2 ng/ml for Hib, tetanus, and diphtheria, respectively.

BMD method: The means of the log10 transformed adjusted antibody concentrations at each PFOA quintile are shown in Table 6.1..1. OEHHA performed BMD modeling using a 10% decrease in vaccine response as the BMR. A BMR of 10% is within the lower range of the response levels seen in this study, which ranged from 0 to 86%. A one SD change from the control mean (quantile 1) was not used because it was generally outside of the observed range of responses. BMD modeling based on clinical reference levels for long- or short-term immunity were also not performed because mean antibody levels in all PFOA quintiles appeared to fall above clinical reference levels (Plotkin, 2010).

Because a 10% decrease in the mean of the log10 values is not the same as a 10% decrease on the absolute (non-logged) scale, the BMR was estimated after back-transforming the log10 values shown in Table 6.1.1 out of the log10 scale (see model run outputs in Appendix 10). This should be considered a somewhat conservative estimate (i.e., slightly less than 10%) since the mean of the log10 transformed antibody levels is typically lower than the log10 of the mean of the absolute (non-logged) levels. Using this BMR, the Hill model gave a BMD_{10} of 16.8 ng/ml and a $BMDL_{10}$ of 2.8 ng/ml (p-value for model fit=0.99) for Hib. This BMD:BMDL ratio is fairly large (e.g., >5) highlighting the uncertainty in this particular dataset. The corresponding BMD_{10} and $BMDL_{10}$ values for tetanus antibody levels were 20.0 and 6.5 ng/ml (p-value for model fit=0.68). For diphtheria antibody levels, the corresponding values were 17.0 and 11.4 ng/ml (p-value for model fit=0.35). Overall, the effect of a 10% decrease in antibody response is likely to be minor in most children. However, the impacts of these small decreases could be much more important in children who already have compromised or borderline-compromised immune systems for other reasons. As such, these small effects could have important implications for the population as a whole, especially given the very widespread nature of PFOA exposure.

Faroe Islands studies:

NOAEC/LOAEC method: Associations between serum PFOA concentrations and serum antibody levels in response to diphtheria and tetanus vaccinations in children were assessed in two Faroe Islands cohorts (Grandjean et al., 2017a). Details of these studies are provided in Appendix 7, Table A7.3. Categorical data, which can be used to develop NOAECs, were not available from the peer-reviewed publications but results for diphtheria have been published in a preliminary report from the Europen Food Safety Authority (EFSA, 2020). These are shown in Table 6.1.2. As seen, the NOAEC is a serum level of 4.75 ng/ml. Categorical data for tetanus were not available.

Table 6.1.2. Mean serum diphtheria antibody concentrations at age 7 by quintiles of serum PFOA (ng/ml) at age 5 from the Faroe Islands cohort (EFSA, 2020). The NOAEC is highlighted in green.

Quintile	PFOA[1]	N	Mean[2]	SD	p-value
1	2.75	86	0.15	1.60	Ref
2	3.45	86	-0.23	1.97	0.22
3	4.05	86	-0.28	1.89	0.11
4	4.75	86	0.13	2.09	0.94
5	6.10	86	-0.60	1.90	0.007

Abbreviations: Ref, reference category; SD, standard deviation
[1] Mean PFOA serum concentrations (ng/ml) in each quintile
[2] Log2 values of mean serum antibody concentrations

BMD method: As reviewed in Chapter 5 and shown in Table 5.1.4, BMD calculations have been performed by two of the Faroe Islands study investigators (Budtz-Jorgensen and Grandjean, 2018). Using a piecewise model with a 5% decrease in antibody levels as the BMR, the authors reported a BMD_{05} and $BMDL_{05}$ of 0.67 and 0.17 ng/ml, respectively, for tetanus antibody, and a BMD_{05} and $BMDL_{05}$ of 1.06 and 0.20 ng/ml, respectively, for diphtheria antibody. Both of these BMDLs are well below the lowest PFOA serum concentration observed in this study. OEHHA performed its own BMD modeling and obtained mostly similar results, with large BMD:BMDL ratios (e.g., >5) and BMDLs that were also well below the range of observed values. The most likely reason the BMD:BMDL ratios were so large was the high degree of variability (i.e., the very large standard deviations) in antibody levels seen in each PFOA exposure category.

Liver Toxicity

As reviewed above, animal and human studies have shown that PFOA can cause liver toxicity. The most consistent liver toxicity-related endpoint seen in the human epidemiologic data are increases in the liver enzyme, ALT. By far the two largest studies of PFOA and ALT in humans are those of Gallo et al. (2012) and Darrow et al. (2016), both performed in the C8 study area (mid-Ohio River Valley, West Virginia and Ohio). Both of these studies were cross-sectional and both involved over 30,000 adults. Details on the Darrow et al. (2016) study can be found in Appendix 7, Table A7.5. The Gallo et al. (2012) study was reviewed by US EPA (2016b). The Gallo et al. (2012) and Darrow et al. (2016) studies were similar in all major aspects of study design except that while Gallo et al. (2012) based PFOA exposure on measured PFOA serum concentrations, the Darrow et al. (2016) study based PFOA exposure on modeled PFOA serum levels. These modeled levels were developed using the participant's residences, PFOA drinking water concentrations, emissions data, environmental fate and transport models, and other factors. Further details on this exposure model can be found in Shin et al. (2011).

Both the Darrow et al. (2016) and Gallo et al. (2012) studies evaluated the relationship between PFOA and ALT using two general types of analyses. In the first, subjects were divided into quantiles of PFOA exposure (quintiles in Darrow et al. (2016) and deciles in Gallo et al. (2012)), and linear regression models were used to compare mean ALT levels in each non-reference quantile to the mean ALT level in the lowest (reference) quantile. In the second type of analysis, a cut-off point was used to define high or elevated ALT, and ORs for having an ALT level above this cutoff point were calculated for each non-reference quantile compared to the lowest (reference) quantile. The cutoff values used to define elevated ALT levels in both studies were 45 IU/L for men and 34 IU/L for women. These are clinically based reference levels used

by the International Federation of Clinical Chemistry and Laboratory Medicine (Schumann et al., 2002), and were approximately the 90th percentile of all ALT values in these studies.

ALT means:

NOAEC/LOAEC method: OEHHA selected the Darrow et al. (2016) study involving modeled PFOA exposure levels for its dose-response analysis of mean ALT concentrations. Gallo et al. (2012) presented similar data for measured serum PFOA concentrations in figure form and these also showed a dose-response relationship between increasing PFOA and increasing ALT. A validation study has shown a good correlation between measured and modeled PFOA serum levels in the C8 cohort (Shin et al., 2011). The results from Darrow et al. (2016) are shown in Table 6.1.3. Sample sizes in each PFOA quintile were not provided, so they were estimated by dividing the total sample size by five. The mean PFOA concentrations in each quintile were also not provided, so OEHHA used the category midpoints. An exception to this was quintile 5. Here a value of 507 ng/ml was used since another publication involving a subsample of the C8 cohort (Vieira et al., 2013) showed that this was likely to be the most common exposure level, or close to the most common exposure level, in participants with modeled exposures above 81 ng/ml, the lower cut-off point of the 5th quintile in Darrow et al. (2016). This value had no impact on the LOAEC or NOAEC derived from this study, and the effect of selecting this value on BMD calculations was evaluated in sensitivity analyses. The Darrow et al. (2016) study authors used a linear regression analysis to calculate the difference in adjusted mean ALT levels between PFOA quintiles 2-5 and quintile 1. Because ALT levels were lognormal transformed for these analyses, the regression coefficients (b) can be used to calculate the percent differences in ALT values between these quintiles (on an absolute, non-logged scale) using the equation: percent difference = [exp(b) − 1] × 100%. As seen in Table 6.1.3, the NOAEC is 8.6 ng/ml.

Table 6.1.3. Regression coefficients for the mean differences in serum ALT by quintile of modeled serum PFOA (ng/ml) from Darrow et al. (2016). The NOAEC is highlighted in green.

Quintile	N	PFOA[1]	b	95% CI
1	6,145	4.2	0	Ref
2	6,145	8.6	0.001	-0.016-0.018
3	6,145	19.0	0.023	0.007-0.040
4	6,145	54.1	0.036	0.019-0.053
5	6,145	507	0.048	0.031-0.066

Abbreviations: b, regression coefficient; CI, confidence interval; Ref, reference category
[1] Category midpoints for PFOA serum concentrations (ng/ml) except highest quintile based on data presented in Vieira et al. (2013)

BMD method: OEHHA performed a BMD analysis of the Darrow et al. (2016) data shown in Table 6.1.3, but when data from all five quintiles were used, these calculations resulted in either non-convergence, wide BMD:BMDL ratios (e.g., >5), or BMDLs that were far below the range of observed PFOA values. As such, these results were not used to derive a POD. Similar results were obtained when other estimates of the mean or median PFOA concentration in quintile 5 were used (e.g., the estimated quintile 5 mean of 235 ng/ml based on Gallo et al. (2012)). When the highest PFOA quintile was excluded, the Hill model for a BMR_{01} (a 1% increase in

mean ALT from the reference category) gave a BMD_{01} and $BMDL_{01}$ of 14.1 and 8.0 respectively. The regression coefficient in quintile 5 corresponds to a difference of 4.9%, so a BMR_{05} was outside the range of observed responses. A BMR involving a one standard deviation change in ALT was also above the range of observed responses (i.e., >4.9% increase in mean ALT from the reference category).

ORs for high ALT:

NOAEC/LOAEC method: OEHHA selected the Gallo et al. (2012) study for its analyses of ORs for high ALT in the C8 cohort because the results of this study showed a more consistent and clear dose-response pattern than seen in Darrow et al. (2016). The ORs from the Gallo et al. (2012) study are shown in Table 6.1.4. Mean PFOA concentrations in each decile were not provided with the OR results, so they were based on digitized data from Figure 1 of the Gallo et al. (2012) publication. As seen in Table 6.1.4, the NOAEC is 9.8 ng/ml. The OR at the LOAEC (decile 3; OR = 1.19) represents about a 1.5% increase in the prevalence of having a high ALT compared to the prevalence in lowest decile (i.e., estimated prevalences of 10.4% vs. 8.9% in deciles 3 and 1, respectively).

BMD method: OEHHA used two general analytic approaches to calculate a BMD for the Gallo et al. (2012) ORs: the Generalized Least Squares for Trend Estimation (glst) method (Greenland and Longnecker, 1992) performed in Stata version 15, and analyses using the US EPA BMDS. Because a BMR of 5% was above the range of observed effect sizes in this study, a BMR of 1% (that is, a 1% increase in the prevalence of having elevated ALT compared to the lowest decile) was selected. This BMR is consistent with the guidance provided by US EPA (US EPA, 2012) in that it is in the lower range of the observed effect sizes, and it likely represents an important adverse effect (discussed in further detail below). BMD analyses using all ten deciles resulted in relatively poor model fit and BMDs that were not well correlated with the observed data. BMD analyses using only deciles 1-5 resulted in much improved model fit and, on visual inspection, resulted in BMDs that correlated well with the observed data. Using deciles 1-5, the BMD_{01} and $BMDL_{01}$ based on the glst method (p-value for model fit=0.96) were 12.4 and 10.6 ng/ml, respectively. The corresponding values using BMDS (Hill model; p-value for model fit=0.96) were 11.8 and 10.6 ng/ml, respectively.

Table 6.1.4. Odds ratios for elevated ALT by deciles of measured PFOA serum concentrations (ng/ml) from Gallo et al. (2012). The NOAEC is highlighted in green.

Decile	PFOA[1]	N	OR	95% CI
1	5.8	4,645	1.00	Ref
2	9.8	4,645	1.09	0.94-1.26
3	13.5	4,645	1.19	1.03-1.37
4	18.0	4,645	1.26	1.09-1.45
5	24.2	4,645	1.40	1.22-1.62
6	32.7	4,645	1.39	1.21-1.60
7	47.1	4,645	1.31	1.14-1.52
8	70.9	4,645	1.42	1.23-1.64
9	117.9	4,645	1.40	1.21-1.62
10	353.1	4,645	1.54	1.33-1.78

Abbreviations: CI, confidence interval; OR, odds ratio;
Ref, reference category
[1] Mean PFOA concentrations (ng/ml) in each decile based on digitized data from Figure 1 in Gallo et al. (2012)

Serum Lipid Concentrations

A number of human studies have identified associations between increasing serum concentrations of PFOA and increasing serum concentrations of total cholesterol (TC). OEHHA considered three epidemiologic studies for dose-response analyses of PFOA and TC: Dong et al. (2019), Lin et al. (2019), and Steenland et al. (2009). Associations between PFOA and TC were more consistent in studies of adults than in studies of children, and these three studies were the largest studies in adults that identified associations between PFOA and TC. In addition, each of these three studies presented results that were adjusted for several potentially important confounders (e.g., age, gender, and BMI); each used established methods to assess PFOA exposure and lipid levels; and each presented data in a format that could be used for dose-response analyses (e.g., mean differences or ORs).

Lin et al. (2019):

The Lin et al. (2019) study included subjects who were participants of a clinical trial of the effect of lifestyle modifications on pre-diabetes. This study included 888 pre-diabetic adults who were recruited from 27 medical centers in the US during the years 1996-1999. The study involved both cross-sectional and prospective components, with the results of both components providing evidence of an association between PFOA and increased TC. For its POD calculations, OEHHA used the results from the cross-sectional component because they were presented in a format that was more amendable to dose-response analysis and there was no convincing evidence that reverse causality was responsible for the effects identified (reverse causality is discussed in greater detail in the summary of PFOS PODs below).

NOAEC/LOAEC method: The results of Lin et al. (2019) are shown in Table 6.1.5. As shown, the NOAEC for TC was 4.2 ng/ml. Results for LDL and TG are shown for comparison purposes, and are similar to those for TC. The mean TC concentration in each PFOA quartile was not provided by the study authors; only mean differences were given. However, the authors did report that the mean TC level in all subjects combined was 204 (SD, ±35.4) mg/dl. Using this value, and the sample sizes and mean differences, the mean TC level in the lowest quartile can be estimated to be about 195 mg/dl. Given this, the mean difference of 10.13 mg/dl at the LOAEC represents about a 5% increase in TC compared to the lowest PFOA exposure group.

Table 6.1.5. Mean differences in serum TC, TG, and LDL by quartiles of serum PFOA (ng/ml) from Lin et al. (2019). The NOAEC is highlighted in green.

Quartile	N	PFOA[1]	TC[2]	TG[2]	LDL[2]
1	221	2.6 (2.0-3.0)	Ref	Ref	Ref
2	222	4.2 (3.8-4.5)	2.00 (-4.64-8.65)	15.92 (-2.15-34.00)	-0.30 (-6.38-5.77)
3	227	5.6 (5.3-6.2)	10.13 (3.56-16.70)	14.03 (-3.83-31.89)	7.88 (1.87-13.88)
4	228	8.4 (7.4-10.3)	13.36 (6.63-20.10)	36.80 (18.49-55.10)	6.70 (0.55-12.86)

Abbreviations: LDL, low-density lipoprotein; Ref, reference category; TC, total cholesterol; TG, triglycerides

[1] Median PFOA (IQR) in each quartile
[2] Mean differences in lipid levels compared to quartile 1; data in parentheses are 95% confidence intervals

BMD method: OEHHA performed a BMD analysis of the Lin et al. (2019) data for TC shown in Table 7.5 using the Hill model and a BMR of 5%. Standard deviations for the mean differences ($SD_{mean\ differences}$) were derived from the confidence intervals using the following equations:

> Standard error (SE) = (upper 95% confidence interval – lower 95% confidence interval) ÷ 3.92
>
> $SD_{mean\ differences} = SE \div \sqrt{([1 \div N_{ref}] + [1 \div N_{exp}])}$, where N_{ref} is the sample size in quartile 1 and N_{exp} is the sample size in quartiles 2-4 (Cochrane Collaboration, 2011). The SD in the reference group (quartile 1) was estimated by using the average of the SDs in quartiles 2-4.

The BMD_{05} and $BMDL_{05}$ were 5.5 and 4.6 ng/ml, respectively. The BMDS was unable to calculate model fit parameters but the visual display showed an excellent model fit.

Dong et al. (2019):

Using data from US NHANES for the years 2003-2014 on 8,948 adults, Dong et al. (2019) calculated a BMD for PFOA and TC using a hybrid approach (Crump, 1995). Here, the cut-off point for elevated TC was set at the upper 5th percentile of TC values in the lowest PFOA exposure group (the actual TC value at this cutoff point was not provided), and the BMR was defined as a 10% increase in the number of people with TC values above this level. Further details on this analysis are provided in Section 5.3.4. Using this method, Dong et al. (2019) reported a BMD_{10} and $BMDL_{10}$ of 10.5 and 5.6 ng/ml, respectively (Table 5.3.5). Key variables or other key results such as the cut-off point used to define elevated TC or model fit parameters were not provided.

Steenland et al. (2009):

Steenland et al. (2009) performed a cross-sectional investigation of the relationship between serum PFOA levels and serum lipid levels in 46,294 adults from the C8 cohort. Serum samples and data on potentially important confounders were collected in the years 2005-2006. In their statistical analyses, the authors analyzed serum TC levels as both a continuous and a categorical variable. In the latter, ORs for having an elevated TC concentration were calculated using a value of ≥240 mg/dl to define elevated TC. This value has been a commonly used guideline for defining high TC (US DHHS, 2005). Fifteen percent of the study population had TC concentrations above this level. Subjects taking cholesterol-lowering medications were excluded, and results were adjusted for age, gender, BMI, education, smoking, exercise, and current alcohol consumption. Results were similar in people who fasted prior to serum collection compared to those who did not. Associations with TC were also seen for PFOS but the correlation between PFOA and PFOS was only moderate (R=0.32), the mean serum concentrations of PFOA were markedly higher than those of PFOS (80.3 vs. 22.4 ng/ml, respectively), and adjustment of the PFOA results for PFOS had only relatively small effects.

TC means

NOAEC/LOAEC method: Regression coefficients for the mean differences in TC concentrations between deciles 2-10 and decile 1 are shown in Table 6.1.6. These coefficients were

developed by the study authors using TC values that were lognormal transformed. As such, the regression coefficients can be used to calculate the percent difference in TC values in deciles 2-10 compared to decile 1 using the equation: percent difference = [exp(b) − 1] × 100%. The SEs and p-values for the regression coefficients in Table 7.6 were provided by the study authors and were based on their linear regression analyses. The mean PFOA concentrations in each PFOA decile were not provided so they were estimated using deciles from another C8 study (Gallo et al., 2012). The sample sizes in each decile were also not provided so they were estimated using the total sample size divided by 10. SDs were estimated from the SEs using the equations provided above for mean differences. As shown in Table 6.1.6, the increase in TC in decile 2 compared to decile 1 was statistically significant. Thus, no NOAEC can be derived from these data, and the LOAEC is 9.8 ng/ml.

Table 6.1.6. Regression coefficients for the mean differences in serum TC and related results by deciles of serum PFOA (ng/ml) from Steenland et al. (2009). The LOAEC is highlighted in blue.

Decile	PFOA	N	Regression results for lnTC						Estimated percent change in TC		
			b	SE	p	CI_L	CI_U	SD	Change[1]	CI_L	CI_U
1	5.8	4,629	0	Ref				0.192	Ref		
2	9.8	4,629	0.01	0.004	0.0026	0.002	0.018	0.192	1.01%	0.22%	1.80%
3	13.5	4,629	0.02	0.004	<0.0001	0.012	0.028	0.192	2.02%	1.22%	2.82%
4	18.0	4,629	0.03	0.004	<0.0001	0.022	0.038	0.192	3.05%	2.24%	3.86%
5	24.2	4,629	0.04	0.004	<0.0001	0.032	0.048	0.192	4.08%	3.27%	4.90%
6	32.7	4,629	0.03	0.004	<0.0001	0.022	0.038	0.192	3.05%	2.24%	3.86%
7	47.1	4,629	0.04	0.004	<0.0001	0.032	0.048	0.192	4.08%	3.27%	4.90%
8	70.9	4,629	0.04	0.004	<0.0001	0.032	0.048	0.192	4.08%	3.27%	4.90%
9	117.9	4,629	0.04	0.004	<0.0001	0.032	0.048	0.192	4.08%	3.27%	4.90%
10	353.1	4,629	0.05	0.004	<0.0001	0.042	0.058	0.192	5.13%	4.31%	5.95%

Abbreviations: b, regression coefficient; CI_L, lower 95% confidence interval; CI_U, upper 95% confidence interval; ln, lognormal; Ref, reference; SD, standard deviation; SE, standard error; TC, total cholesterol
[1] The percent increase in TC values compared to decile 1

BMD method: Using data on all PFOA deciles and a BMR_{01} gave a poor model fit for all models using BMDS. Removing the highest dose group gives a BMD_{01} and $BMDL_{01}$ of 9.9 and 8.6 ng/ml, respectively (p-value for model fit=0.06). Removing the next three highest exposure groups did not improve model fit. However, if the highest five dose groups are removed, model fit improves substantially but the BMD_{01} and $BMDL_{01}$ change only slightly (9.8 and 8.5 ng/ml, respectively; p-value for model fit=0.97).

ORs for elevated TC

NOAEC/LOAEC method: The ORs from Steenland et al. (2009) for having an elevated TC level are shown in Table 6.1.7. Samples sizes in each quartile were not provided so they were estimated using the total sample size divided by four. The mean PFOA concentrations in each quartile were also not provided, so OEHHA used the category midpoints. An exception to this was quartile 4. Here a value of 507 ng/ml was used since another publication involving a subsample of the C8 cohort (Vieira et al., 2013) showed that this was likely to be the most

common exposure level, or close to the most common exposure level, in participants with modeled exposures above 67 ng/ml, the lower cut-off point of the 4th quartile in Steenland et al. (2009). This estimate had no effect on the selection of the NOAEC or LOAEC or on BMD calculations (see below). As seen in Table 6.1.7, the OR in the second quartile is statistically significant, and the LOAEC is 19.9 ng/ml (rounded from 19.85 ng/ml).

BMD method: BMD calculations were not performed using these data because both the glst and US EPA BMDS require information on the prevalence of the outcome in each exposure group, data that were not provided. In some instances, ORs can be used to estimate disease prevalence. In this study however, the prevalence of the outcome was fairly high (15%) and it is unclear whether, and by how much, ORs might lead to an overestimation of risk or prevalence.

Table 6.1.7. Odds ratios for elevated serum TC by quartiles of serum PFOA (ng/ml) from Steenland et al. (2009). The LOAEC is highlighted in blue.

Quartile	PFOA[1]	N	OR	95% CI
1	6.55	11,574	1.00	Ref
2	19.85	11,574	1.21	1.12-1.31
3	46.75	11,574	1.33	1.23-1.43
4	507	11,574	1.38	1.28-1.50

Abbreviations: CI, 95% confidence interval; OR, odds ratio; Ref, reference
[1] Category midpoints for PFOA serum concentrations (ng/ml) except highest quartile based on data presented in Vieira et al. (2013)

Summary: PFOA PODs

A summary of the PODs for PFOA derived from the candidate studies identified by OEHHA are shown in Table 6.1.8. These PODs ranged from 2.8 to 19.9 ng/ml (mean = 10.9 ng/ml). The lowest value of 2.8 ng/ml was the $BMDL_{10}$ for a decrease in antibody levels in response to influenza vaccine from the study by Abraham et al. (2020). Although this value was within the range of the PFOA levels observed in this study, it was associated with a large BMD:BMDL ratio (e.g., >5). The highest value was the LOAEC for ORs for elevated serum TC from the C8 cohort (Steenland et al., 2009). Aside from the Steenland et al. (2009) LOAEC for elevated TC, the highest PODs were for some of the NOAECs for vaccine response from Abraham et al. (2020). This is not surprising given this study's small sample size and low statistical power. After excluding the two LOAECs in Table 6.1.8, the PODs ranged from 2.8 to 19.4 ng/ml, with a mean of 10.4 ng/ml.

Table 6.1.8. Summary of potential PODs for PFOA

Candidate studies	Effect	Method	POD/notes
Immunotoxicity			
Abraham et al. (2020)	Diphtheria antibody levels	NOAEC/LOAEC	NOAEC = 18.9 ng/ml
	Hib antibody levels	NOAEC/LOAEC	NOAEC = 19.4 ng/ml
	Tetanus antibody levels	NOAEC/LOAEC	NOAEC = 18.9 ng/ml

Candidate studies	Effect	Method	POD/notes
	Diphtheria antibody levels	Knee function	NOAEC = 16.2 ng/ml
	Hib antibody levels	Knee function	NOAEC = 12.2 ng/ml
	Tetanus antibody levels	Knee function	NOAEC = 16.9 ng/ml
	Diphtheria antibody levels	BMDS: Hill model	BMD_{10} = 17.0 ng/ml $BMDL_{10}$ = 11.4 ng/ml
	Hib antibody levels	BMDS: Hill model	BMD_{10} = 16.8 ng/ml $BMDL_{10}$ = 2.8 ng/ml
	Tetanus antibody levels	BMDS: Hill model	BMD_{10} = 20.0 ng/ml $BMDL_{10}$ = 6.5 ng/ml
Faroe Islands (Grandjean et al., 2017a; Budtz-Jorgensen and Grandjean, 2018)	Diphtheria antibody levels	NOAEC/LOAEC	NOAEC = 4.75 ng/ml
	Tetanus antibody levels	NOAEC/LOAEC	Data not available
	Diphtheria antibody levels	BMDS: Hill and Piecewise models	$BMDL_{05}$ outside observed range; large BMD:BMDL ratio
	Tetanus antibody levels	BMDS: Hill and Piecewise models	$BMDL_{05}$ outside observed range; large BMD:BMDL ratio
Liver Toxicity			
Darrow et al. (2016)	Mean ALT levels	NOAEC/LOAEC	NOAEC = 8.6 ng/ml
	Mean ALT levels	BMDS: multiple models, all exposure levels	Large BMD:BMDL ratios; non-convergence
	Mean ALT levels	BMDS: Hill model, quintile 5 excluded	BMD_{01} = 14.1 ng/ml $BMDL_{01}$ = 8.0 ng/ml
	Elevated ALT ORs	NOAEC/LOAEC	Inconsistent dose-response curve – used Gallo et al. (2012)
	Elevated ALT ORs	glst	Inconsistent dose-response curve – used Gallo et al. (2012)
	Elevated ALT ORs	BMDS	Inconsistent dose-response curve – used Gallo et al. (2012)
Gallo et al. (2012)	Mean ALT levels	NOAEC/LOAEC	Data in figure form – used Darrow et al. (2016)
	Mean ALT levels	BMDS	Data in figure form – used Darrow et al. (2016)

Candidate studies	Effect	Method	POD/notes
	Elevated ALT ORs	NOAEC/LOAEC	**NOAEC = 9.8 ng/ml**
	Elevated ALT ORs	glst	BMD_{01} = 12.4 ng/ml $BMDL_{01}$ = 10.6 ng/ml
	Elevated ALT ORs	BMDS: Hill model	BMD_{01} = 11.8 ng/ml $BMDL_{01}$ = 10.6 ng/ml
Lipid Concentrations			
Lin et al. (2019)	Mean TC levels	NOAEC/LOAEC	NOAEC = 4.2 ng/ml
	Mean TC levels	BMDS: Hill model	BMD_{05} = 5.5 ng/ml $BMDL_{05}$ = 4.6 ng/ml
Dong et al. (2019)	Mean TC levels	NOAEC/LOAEC	Information not provided
	Mean TC levels	BMDS	Information not provided
	Mean TC levels	Hybrid model	BMD_{10} = 10.5 ng/ml $BMDL_{10}$ = 5.6 ng/ml
Steenland et al. (2009)	Mean TC levels	NOAEC/LOAEC	No NOAEC LOAEC = 9.8 ng/ml
	Mean TC levels	BMDS: Hill model, deciles 1-5	BMD_{01} = 9.8 ng/ml $BMDL_{01}$ = 8.5 ng/ml
	Elevated TC ORs	NOAEC/LOAEC	No NOAEC LOAEC = 19.9 ng/ml
	Elevated TC ORs	BMDS/glst	Prevalence data not provided

OEHHA selected the NOAEC of 9.8 ng/ml for elevated ALT from the Gallo et al. (2012) study as the POD for its PFOA ADD calculations. While this study does not provide the lowest POD, it does offer the following advantages for dose-response and risk assessment calculations.

Very large sample size (N=46,452). This is by far the largest of the candidate studies OEHHA reviewed for its POD calculations. The very large sample size helps reduce the probability that findings are due to chance, allows for the detection of relatively subtle effect sizes, and helps to increase the likelihood that study findings are broadly generalizable. In addition, because of the large sample size, effect estimates could be examined in a relatively large number of exposure categories (i.e., deciles in this case) with good statistical power. This allowed for a more precise determination of the NOAEC compared to some of the smaller studies OEHHA considered for its POD calculations.

Valid method for assessing exposure. Exposure was assessed using a single measured serum concentration of PFOA in each participant. Serum measurements are a commonly used and widely accepted method for assessing PFOA exposure. The long half-life of PFOA in serum suggests that a single serum measurement is likely to provide an accurate and precise

indication of a person's true long-term exposure. Based on the methodologic details provided in the study publication there is no indication that the serum samples were collected, or PFOA concentrations were measured, in a way that would have differed between those with higher or lower ALT levels. Because of this, the major effect of misclassification of PFOA exposure would most likely be non-differential and most likely bias results towards the null (i.e., in the direction of finding no effect) (Jurek et al., 2005). Non-differential misclassification of exposure could have biased some of the ORs in the middle exposure categories (i.e., in deciles 2-9) away from 1.0. However, OEHHA could find no evidence that this bias was substantial, and a number of other, lower-exposure studies (Gleason et al., 2015; Jain and Ducatman, 2018b; Salihovic et al., 2018; Attanasio, 2019; Nian et al., 2019) identified statistically significant PFOA-ALT associations at PFOA concentrations similar to the LOAEC in Gallo et al. (2012).

Clinically relevant outcome. The cutoff points used to define elevated ALT in Gallo et al. (2012) were based on clinical reference levels published by the International Federation of Clinical Chemistry and Laboratory Medicine (Schumann et al., 2002). ALT concentrations above these reference levels have been associated with increases in both liver-related mortality and all-cause mortality (Kwo et al., 2017). For example, based on participants from US NHANES 1999-2008, Ruhl and Everhart (2012) found that ALT levels >30 IU/L in men or >19 IU/L in women were associated with a >8-fold increase in mortality from liver disease (hazard ratio = 8.2; 95% CI, 2.1-31.9). In addition, a study by Lee et al. (2008) involving 47,182 residents of Olmsted County, MN, found ALT concentrations >45 IU/L for men and >29 IU/L for women were associated with a 34% increase in mortality from all causes (SMR = 1.34; 95% CI, 1.05-1.71). These findings do not necessarily mean that people with PFOA exposures above a certain level will have an 8.2-fold higher risk of death from liver disease or a 34% higher risk of death overall. However, elevated ALT is an indicator of liver toxicity. As such, these findings highlight the potential clinical importance of having high ALT and the importance of the pathological processes that cause these elevated ALT levels. The clinical relevance of these ALT levels is also highlighted by the fact that the American College of Gastroenterology recommends that people with ALT levels >33 IU/L in men and >25 IU/L in women undergo a clinical evaluation, including a physical exam, screening for alcohol and hepatotoxic medication use, testing for viral hepatitis, assessment for nonalcoholic fatty liver disease and alcoholic liver disease, and screening for hereditary liver diseases (Kwo et al., 2017).

Consistency of findings. The Gallo et al. (2012) findings linking PFOA to elevated levels of ALT are not only consistent with research done in other study areas and other study populations (see Appendix 7, Table A7.5), these findings are also internally consistent. In this and the Darrow et al. (2016) study (which used mostly the same study participants), PFOA-ALT associations were examined using a variety of different approaches and analyses, and all provided evidence that PFOA was associated with increased ALT. These approaches and analyses included using both modeled and measured PFOA exposures; evaluating PFOA and ALT as both continuous and categorical variables; assessing PFOA exposure as both cumulative lifetime and yearly annual average exposure; examining other biomarkers of liver toxicity such as GGT and bilirubin; and performing subgroup analyses based on age, sex, and occupational exposure. Overall, the internal and external consistency of the Gallo et al. (2012) findings provides very strong evidence that the associations identified in this study are real (Bradford Hill, 1965).

No major confounding identified. The findings in Gallo et al. (2012) were adjusted or otherwise controlled for a number of factors that could potentially affect ALT levels, including age, sex, alcohol intake, socioeconomic status, fasting, race, smoking, BMI, exercise, and

insulin resistance. These include some of the most prevalent and strongest risk factors for liver disease. A number of other factors can affect ALT levels including use of certain medications, viruses, other chemical exposures, or genetic disorders. However, OEHHA could not find convincing evidence that these are prevalent enough, or related strongly enough to both ALT and PFOA, to cause the elevated ORs seen in Gallo et al. (2012) (Axelson, 1978; Schlesselman, 1978).

Effects are unlikely to be solely due to PFOS. A particular concern for studies of PFOA is the potential for confounding by other PFAS. This is especially true for confounding by PFOS since in many populations, PFOA and PFOS exposures are highly correlated and PFOS is the predominant PFAS exposure. OEHHA evaluated the possibility that PFOS, and not PFOA, was responsible for the associations identified in Gallo et al. (2012) and found this to be unlikely for a number of reasons.

First and perhaps most importantly, animal data have shown that PFOA can cause liver toxicity independent of exposure to other PFAS, including PFOS. Second, the C8 studies involved an area that had high levels of PFOA contamination, and mean serum concentrations of PFOA were higher than those of PFOS (mean serum levels of 83.0 and 23.3 ng/ml, respectively). By itself, the fact that PFOA was the predominant PFAS exposure here does not ensure that it caused the effects reported in Gallo et al. (2012). However, the fact that PFOA is known to cause liver toxicity in laboratory animals, combined with the fact that PFOA exposures in this area were very high, is highly suggestive that PFOA was the causative agent.

The third piece of evidence that the PFOA findings of Gallo et al. (2012) were due to PFOA and not PFOS was that the correlation between these two agents in the C8 study area was only moderate, well below the correlations seen in other studies. For example, correlation coefficients for PFOA and PFOS as high as 0.64 have been reported in US NHANES (Calafat et al., 2007b). In the C8 cohort, the serum PFOA-PFOS correlation coefficient was 0.32 (Steenland et al., 2009). Another important finding is that the magnitude of the association between PFOS and ALT was not markedly higher than that seen with PFOA. In fact, it was a little lower (i.e., regression coefficients of 0.020 for lnPFOS (ng/ml) and lnALT (IU/L), and 0.022 for lnPFOA (ng/ml) and lnALT (IU/L)). These findings are important because the likelihood that a factor will cause major confounding is not simply related to whether or not that factor is associated with both the exposure and the outcome of interest, but more importantly, its related to the relative magnitude of these associations (Axelson, 1978). Overall, the fact that the association between PFOS and ALT was not markedly stronger than that between PFOA and ALT, combined with the fact that PFOA and PFOS were only moderately correlated, make it highly unlikely that PFOS was an important cause of the effects attributed to PFOA in this study.

One method to evaluate whether the associations between the potential confounding factor and the exposure and outcome of interest are strong enough to cause major confounding is to compare the main study results before and after statistically adjusting for that factor. Although the authors of the Gallo et al. (2012) paper did not present PFOA-ALT results adjusted for PFOS, an illustration of the likely impact that this adjustment would have had can be seen in another C8 study. In the C8 study examining PFAS and serum TC (Steenland et al., 2009), the effect size for PFOS was much larger than that seen for PFOA (e.g., regression coefficients of 0.01112 for lnPFOA (ng/ml) and 0.02660 for PFOS (ng/ml), respectively, with serum lnTC (mg/dl)). Here, because the magnitude of the association with TC is much stronger for PFOS than for PFOA, one might not be surprised if confounding by PFOS could have caused the association reported for PFOA. However, when the authors of this study adjusted the PFOA-TC

results for PFOS, the PFOA-TC effect size was only reduced by 20-30%, and the overall trend of increasing TC with increasing PFOA remained (more detailed results were not provided in the publication). The most likely reason the PFOA-TC effect did not decrease even further (e.g., to zero) after this adjustment is that the PFOA-PFOS correlation was only moderate, and not strong enough to cause major confounding. For ALT, because the effect of PFOS was not larger than that for PFOA, the impact of confounding by PFOS is likely to be even less than the 20-30% seen in the analysis of TC.

The results of Lin et al. (2010) provide another example of the likely impact of confounding by PFOS or other PFAS on the relationship between PFOA and ALT. This study involved a cross-sectional evaluation of several PFAS and serum liver enzymes in 2,216 adults (18 years of age or older) in the 1999-2000 and 2003-2004 US NHANES. An NOAEC, LOAEC, or BMD was not developed from this study because the main categorical results were not adjusted for potentially important confounders. However, the authors did report regression coefficients for associations between ALT and PFOA and PFOS in analyses that were adjusted for age, gender, race/ethnicity, smoking, alcohol drinking, education, BMI, insulin resistance, metabolic syndrome, and iron saturation status. Results were presented both in analyses where PFOA and PFOS were entered into the linear regression models on their own, and in analyses where these agents were entered together (as well as with PFHxS and PFNA). The results of these analyses are shown in Table 6.1.9. As seen, while the regression coefficient for PFOA increased after adjustment for PFOS, the regression coefficient for PFOS decreased after adjustment for PFOA. As a whole, these results provide further evidence that PFOA can affect ALT levels, and that this effect is not strongly confounded by PFOS.

Table 6.1.9. Linear regression coefficients (β) for association between serum log-PFOA or log-PFOS (ng/ml) and serum ALT (IU/L) in Lin et al. (2010)

Analysis	Log-PFOA	Log-PFOS
Each PFAS entered into the model on its own	β=1.86 p=0.005	β=1.01 p=0.066
Each PFAS entered into the model with other PFAS	β=2.19 p=0.009	β=-0.19 p=0.769

No obvious selection bias. The C8 study has been estimated to include >80% of all residents of the affected areas. This high participation rate suggests that major errors due to selection bias are highly unlikely. In other studies, participation rates were much lower or were unclear or not reported (e.g., Lin et al. (2019)).

NOAEC is available. In some of the studies and outcome metrics that OEHHA considered for POD calculations, LOAECs were available but NOAECs were not. While this is not a fatal flaw, using a LOEAC rather than an NOAEC would add additional uncertainty to the ADD calculations.

Appropriate study design. The Gallo et al. (2012) analyses were cross-sectional, and a common criticism of cross-sectional studies is the possibility of reverse causation. However, OEHHA could not find any convincing evidence or plausible reason to believe that the alterations in serum ALT levels, or the liver diseases that typically cause them, would cause widespread and major increases in serum PFOA concentrations. This is particularly clear when looking at the Gallo et al. (2012) analyses of mean ALT concentrations by increasing categories

of PFOA exposure. Here, while the ALT levels increased by approximately 11% from the first to the tenth decile of PFOA, the mean PFOA levels across these deciles increased by over 5,000%. OEHHA was not able to identify any evidence or any plausible mechanism by which such a relatively small increase in serum ALT (or the underlying factors that might cause these relatively small increases) could cause such a huge increase in serum PFOA.

Another criticism of cross-sectional studies is that because they measure exposure and outcome at the same time, the most relevant exposure period might be missed. This is especially important in scenarios where the latency period between the exposure and outcome is long. However, as discussed above, the half-life of PFOA in human serum is several years. As such, a measurement of PFOA at a single point in time likely represents PFOA exposure over a fairly extended period of time. In addition, the half-life of ALT in plasma is short: about 47 hours (Kim et al., 2008). ALT levels typically rise rapidly (within a few hours or days) after a toxic exposure begins (Kim et al., 2008), and fall rapidly after exposure cessation (Curtis and Sivilotti, 2015; McGovern et al., 2015). Overall, the long half-life of serum PFOA, combined with the acute nature of ALT as a marker of hepatic injury, suggest that the cross-sectional approach used in Gallo et al. (2012) is a valid method for assessing PFOA-ALT associations.

Acceptable Daily Dose Calculation

To calculate the acceptable daily dose (ADD), which is an estimated maximum daily dose of a chemical that can be consumed by humans for an entire lifetime without toxic effects, the POD is divided by the composite uncertainty factor (UF). The ADD for PFOA was calculated using the NOAEC of 9.8 ng/ml for elevated ALT from the Gallo et al. (2012) study and the clearance factor of 0.28 ml/kg-day discussed in Chapter 4, with a UF of √10 for intraspecies variation. The clearance factor was used to convert the POD, which was expressed as a serum concentration of PFOA (in units of ng/ml), into a daily intake rate in units of nanograms per kilogram of body weight per day (ng/kg-day). OEHHA typically applies an intraspecies UF of 30 when deriving an ADD from an animal study, and this can be reduced to 1 when using human data. Here, the intraspecies UF is reduced to √10 rather than 1 for the following reasons. The Gallo et al. (2012) study involved a very large population of adults and the participation rate was about 80%. This large sample size and high participation rate suggests the study participants were a good representation of the population of the study area as a whole, and likely included a diverse group of people in terms of ages, health status, smoking and other chemical exposures, nutrition, socioeconomic status, and other factors. The study did not include children. While a few studies have identified associations between PFOA and ALT concentrations in children, the most consistent evidence linking PFOA to ALT has been in adults, and OEHHA could not find other convincing evidence that the hepatotoxic effects of PFOA are likely to be substantially greater in children than adults. In addition, since PFOA is not metabolized readily, there are not likely large kinetic differences between children and adults. A UF of √10 for intraspecies variation rather than 1 was applied because the C8 study population was not diverse in terms of race or ethnicity. In addition, it did not examine other potential susceptibility factors such as obesity or genetics. Some data suggest that obesity or certain genetic polymorphisms might increase susceptibility to PFAS (Ghisari et al., 2017; Jain and Ducatman, 2018b; Jain and Ducatman, 2019; Wen et al., 2019a). However, these data were limited (for a variety of reasons) and OEHHA was unable to identify consistent or convincing evidence that this susceptibility might be greater than a factor of √10. Finally, there is strong evidence for immunotoxicity of PFOA. The NOAECs from the immunotoxicity studies ranged from 4.75 to 19.4 ng/ml for response to vaccination in children. In the Faroe Islands study, depressed response to childhood vaccinations was seen at mean serum levels of 6.1 ng/ml, lower than the

mean serum concentration identified as the NOAEC for elevated ALT in Gallo et al. (2012). OEHHA chose not to use PODs from these studies because of the limited sample sizes and associated uncertainty in the data. However, these studies point to the potential for immunotoxicity to occur below the NOAEC for elevated ALT in adults. This is another reason a UF for intraspecies variation larger than 1 is warranted.

ADD = (POD × CF) ÷ UF = (9.8 ng/ml × 0.28 ml/kg-day) ÷ √10 = **0.87 ng/kg-day**.

Animal Studies

Liver, immune system, thyroid, and developmental/reproductive toxicities are the major noncancer effects for PFOA in experimental animals. Of these effects, liver toxicity appears to be the most sensitive endpoint. OEHHA identified PODs from the animal studies for comparison with those derived using human studies. Among the animal studies that exhibited a dose-response, Table 6.1.10 lists candidate critical studies for PFOA that report serum/plasma levels of PFOA and that OEHHA considers are of sufficient quality. However, because there are sufficient human data available to calculate an ADD and further develop a health-protective concentration without the uncertainty of extrapolating from animals to humans, OEHHA did not derive ADDs from these animal studies.

Table 6.1.10. Candidate critical studies for noncancer effects of PFOA in animals

Reference	Sex/Species	Dose/Route of Exposure/Duration	Endpoint	NOAEL/LOAEL (mg/kg-day)	NOAEL/LOAEL/ BMDL (mg/L)
Liver Toxicity					
Yu et al. (2016)	Male BALB/c mice (5/dose)	0, 0.5, or 2.5 mg/kg-day via oral infusion for 28 days	↑ relative liver weight	NOAEL: 0.5	NOAEL: 29.3 $BMDL_{1SD}$: 22.6
Li et al. (2017b)	Male and female BALB/c mice (30/sex/ dose)	0, 0.05, 0.5, or 2.5 mg/kg-day via gavage for 28 days	hepatic mitochondrial membrane potential changes, apoptosis, oxidative DNA damage	LOAEL: 0.05	LOAEL: 0.97 $BMDL_{1SD}$: 0.11 (↑ p-53 levels)
Guo et al. (2019)	Male BALB/c mice (12/dose)	0, 0.4, 2, or 10 mg/kg-day via gavage for 28 days	↑ relative liver weight, hepatocellular hypertrophy, and karyolysis	LOAEL: 0.4	LOAEL: 13 $BMDL_{1SD}$: 1.71
Blake et al. (2020)	Pregnant CD-1 mice (11-13 dams/dose)	0, 1, or 5 mg/kg-day via gavage from ED1.5 to ED11.5 or ED17.5	liver effects (↑ cell death) in dams, ED11.5	LOAEL: 1	LOAEL: 25.4 $BMDL_{05}$: 3.0

BMD modeling (BMDS, version 2.7) was performed on the candidate critical study datasets in Table 6.1.10, using plasma/serum concentration as the dose metric, and the results are summarized in Table 6.1.11.

Table 6.1.11. BMD modeling of noncancer endpoints from candidate PFOA animal studies

Study	Endpoint	Model	Goodness of fit p-value	BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)
Yu et al. (2016)	↑ relative liver weight	Linear[a]	0.901	32.1	22.6
Li et al. (2017b)	↑ p-53 levels	Hill	0.8453	0.15	0.11
Li et al. (2017b)	↑ mitochondrial membrane potential changes	Poor model fit			
Li et al. (2017b)	↑ caspase-9 levels	Poor model fit			
Li et al. (2017b)	↑ 8-OHdG	Poor model fit			
Guo et al. (2019)	↑ relative liver weight	Exponential4	0.413	2.55	1.71
Blake et al. (2020)	↑ relative liver weight (ED11.5)	Linear[a]	0.204	16.4	12.9
Blake et al. (2020)	↑ relative liver weight (ED17.5)	Linear	0.708	7.0	5.1
Blake et al. (2020)	↑ liver cell death (ED11.5)	Gamma[a]	0.995	6.4[b]	3.0[b]

[a] Additional models produced the exact same results
[b] This dataset was modeled with a benchmark response of 5% above background, therefore the values represent BMD_{05} and $BMDL_{05}$
8-OHdG, 8-hydroxy-2-deoxyguanosine; ED, embryonic day

The LOAEL of 0.05 mg/kg-day from Li et al. (2017b) for hepatic mitochondrial membrane potential changes and increased apoptosis and oxidative DNA damage corresponds to a serum concentration of 0.97 mg/L. These endpoints were also frequently observed in in vitro studies (Wu et al., 2017; Orbach et al., 2018; Sun et al., 2019b; Xu et al., 2019b). While these endpoints are not frank effects, they may be considered upstream effects in the continuum of changes resulting in liver toxicity, which includes hepatocyte hypertrophy and increased liver weight as reported in the Li et al. (2017b) study. BMD modeling of increased p-53 levels, a biomarker of apoptosis, from the Li et al. (2017b) resulted in a $BMDL_{1SD}$ of 0.11 mg/L.

A NOAEL of 0.003 mg/kg-day was identified from the van Esterik et al. (2016) study (Table 5.5.1), based on reduced female pup body weight on PND 4 in animals exposed to PFOA during gestation and lactation. However, serum concentrations were not reported in this study, and due to the complexity of the dosing scheme (PFOA was administered to dams during pregnancy and lactation, making it difficult to determine whether the effect was due to in utero exposure, lactational exposure, or both), and lack of a reliable kinetic model, serum concentrations could not be determined. Therefore, this study is not considered as a candidate critical study.

6.1.2. Perfluorooctane Sulfonic Acid

Human Studies

Based on its review of the available human studies, OEHHA has determined that the sensitive noncancer endpoints for PFOS are immunotoxicity and alterations in lipid metabolism or production. Several studies have also linked PFOS to adverse effects related to the thyroid gland; however, these findings are too inconsistent from study to study to make firm conclusions regarding these outcomes. Research in laboratory animals has shown that PFOS can cause hepatoxicity. However, the human epidemiologic evidence linking PFOS to increases in liver enzyme levels or other hepatotoxic effects is not as robust or consistent as that seen for PFOA. Associations with several reproductive and developmental outcomes have also been reported. While the positive associations reported in some of these studies are cause for concern, overall, the findings from study to study were also somewhat inconsistent, and OEHHA was unable to identify studies that could be used to accurately evaluate the dose-response patterns for these outcomes.

In the following sections, OEHHA presents the human studies that were considered as candidate studies for POD determination.

Immunotoxicity

A number of animal and human studies have provided evidence that PFOS can increase the risks of immune-related diseases or otherwise adversely affect the immune system. With regards to human studies, increased serum levels of PFOS have been linked to diminished antibody levels in response to vaccinations for diphtheria, tetanus, rubella, and mumps (Granum et al., 2013; Stein et al., 2016b; Grandjean et al., 2017a; Grandjean et al., 2017b; Pilkerton et al., 2018). These findings have been seen in both adults and children, although the greatest effect sizes and most consistent results have been reported in children. For example, decreases in tetanus and diphtheria antibody concentrations of >30% have been reported for each doubling of PFOS serum concentration in children from the Faroe Islands (Grandjean et al., 2017a) (Table 5.1.1). A few studies have identified associations between PFOS and immune-related diseases such as asthma, eczema, and lower respiratory tract infections (Dalsager et al., 2016; Goudarzi et al., 2017; Qin et al., 2017; Averina et al., 2019; Manzano-Salgado et al., 2019; Kvalem et al., 2020) although the evidence for these outcomes is less consistent than that seen for diminished vaccine response.

For immunotoxicity, OEHHA selected the studies of PFOS and diminished response to diphtheria and tetanus vaccinations performed in the Faroe Islands cohorts as candidate critical studies. Clear associations between PFOS and diminished antibody response to diphtheria, influenza, and tetanus vaccines were not seen in the recently published study in one-year old children by Abraham et al. (2020). The exact reasons for the inconsistency between the Faroe Islands studies and the Abraham et al. (2020) study for PFOS are unknown although the latter involved a much more limited age range (Abraham et al. (2020) only included one-year-old children, while the Faroe Islands studies followed children from birth up to age 17 years old). In addition, Abraham et al. (2020) involved only cross-sectional analyses, while the Faroe Islands studies included both cross-sectional and prospective analyses. Finally, Abraham et al. (2020) had far fewer children than the Faroe Islands cohorts. The Faroe Islands studies, and the PODs that can be derived from them, are discussed below.

Faroe Islands studies:

NOAEC/LOAEC method: Associations between serum PFOS concentrations and antibody levels in response to diphtheria and tetanus vaccinations in children were assessed in two Faroe Islands cohorts (Grandjean et al., 2017a). Details of these studies are provided in Appendix 7, Table A7.4. Categorical data, which can be used to develop NOAECs, were not available from the peer-reviewed publications but results for diphtheria have been obtained from a draft report by the European Food Safety Authority (EFSA, 2020). These are shown in Table 6.1.12. As seen in this table, the LOAEC and NOAEC are 26.0 and 20.6 ng/ml, respectively. The LOAEC and NOAEC developed based on PFOS deciles were similar. Although the decrease in antibody response in quintile 3 was statistically significant, the analyses by exposure deciles showed a large degree of variability in responses around the quintile 3 exposure levels (e.g., for PFOS levels between 14 and 20 ng/ml). As such, OEHHA selected quintile 5 rather than quintile 3 as the LOAEC, and quintile 4 rather than quintile 2 as the NOAEC. Categorical data for tetanus were not available.

Table 6.1.12. Mean serum diphtheria antibody concentrations at age 7 by quintiles of serum PFOS (ng/ml) at age 5 from the Faroe Islands cohorts (EFSA, 2020). The NOAEC is highlighted in green.

Quintile	PFOS[1]	N	Mean[2]	SD	p-value
1	11.5	86	0.25	1.81	Ref
2	14.9	86	-0.07	1.91	0.32
3	17.4	86	-0.48	1.89	0.01
4	20.6	86	-0.16	1.90	0.15
5	26.0	86	-0.39	1.94	0.03

Abbreviations: Ref, reference category; SD, standard deviation
[1] Mean PFOS serum concentrations (ng/ml) in each quintile
[2] Log2 values of mean serum antibody concentrations

BMD method: As reviewed in Chapter 5 and shown in Table 5.1.4, BMD calculations have been performed by the Faroe Islands study investigators (Budtz-Jorgensen and Grandjean, 2018). Using a piecewise model with a 5% decrease in antibody levels as the BMR, the authors reported a BMD_{05} and $BMDL_{05}$ of 3.57 and 0.72 ng/ml, respectively, for tetanus antibody, and a BMD_{05} and $BMDL_{05}$ of 1.21 and 0.54 ng/ml, respectively, for diphtheria antibody. Both of these BMDLs are well below the lowest PFOS serum concentration observed in this study. OEHHA performed its own BMD modeling and obtained results with large BMD:BMDL ratios (e.g., >5) and BMDLs that were also well below the range of observed values. The most likely reason the BMD:BMDL ratios were so large was the high degree of variability (as indicated by the large SDs) in antibody levels seen in each PFOS exposure category.

Serum Lipid Concentrations

A number of human studies have identified associations between increasing serum concentrations of PFOS and increasing serum concentrations of total cholesterol (TC). OEHHA considered the following studies for dose-response analyses of PFOS and TC.

- Dong et al. (2019)
- Steenland et al. (2009)
- Frisbee et al. (2009)

- Starling et al. (2014)

These studies were selected because each provided some evidence of a statistically significant association between PFOS and TC; each involved a relatively large sample size (i.e., had sufficient statistical power); each adjusted or otherwise accounted for the strongest and most prevalent risk factors for elevated TC levels (e.g., age, gender, BMI, and socioeconomic status); each used validated and well accepted methods for assessing both exposure and outcome (i.e., serum concentrations of PFOS and TC); none showed evidence of selection bias; and each presented results in a format that could potentially be used for dose-response assessment (e.g., ORs or mean TC levels by categories of serum PFOS).

Dong et al. (2019):

Using data from US NHANES for the years 2003-2014 on 8,948 adults, Dong et al. (2019) calculated a BMD and BMDL for PFOS and TC using a hybrid approach (Crump, 1995). Here, the cut-off point for elevated TC was set at the upper 5th percentile of TC values in the lowest PFOS exposure group (the actual TC value at this cutoff point was not provided), and the BMR was defined as a 10% increase in the number of people with TC values above this level. Further details on this analysis are provided in Section 5.3.4. Using this method, Dong et al. (2019) reported a BMD_{10} and $BMDL_{10}$ of 44.2 and 24.1 ng/ml, respectively (Table 5.3.5). Key variables or other key results such as the cut-off point used to define elevated TC or the model fit parameters were not provided.

Steenland et al. (2009) and Frisbee et al. (2009):

Steenland et al. (2009) performed a cross-sectional investigation on the relationship between serum PFOS and serum lipid concentrations in 46,294 adults from the C8 cohort. Details of this study are provided in the PFOA dose-response assessment above. Results were presented for analyses using both PFOS and TC as categorical and continuous variables. Increases in mean serum TC levels were seen in increasing deciles of serum PFOS. The regression coefficients representing the difference in lognormal TC values between deciles 2-10 and decile 1 were 0.01, 0.01, 0.03, 0.03, 0.04, 0.04, 0.05, 0.06, and 0.06, respectively (these results are reported in the footnotes of Table 3 in Steenland et al. (2009)). Overall, these coefficients indicate a pattern of increasing TC levels with increasing PFOS exposure although standard errors or p-values were not provided. Adjusted mean TC concentrations by deciles of PFOS with 95% confidence intervals were presented in figure form, and this also shows a clear pattern of increasing TC concentrations with increasing PFOS exposure.

The authors also calculated ORs for having an elevated TC concentration for PFOS quartiles 2-4 compared to quartile 1. Here, a TC value of ≥240 mg/dl was used to define elevated TC. This value has historically been used for defining high TC (US DHHS, 2005), and 15% of the study population were above this level. The results of these analyses are shown in Table 6.1.13. Samples sizes in each quartile were not provided, so they were estimated using the total sample size divided by four. Here, the OR in quartile 2 is statistically significant, and the LOAEC is 16.4 ng/ml. BMD calculations were not performed using these data because both the glst and US EPA BMDS require an estimate of the disease prevalence in each exposure group, data that were not provided. In some instances, ORs can be used to estimate disease prevalence. In this study however, the prevalence of the outcome was fairly high (e.g., >10%) and it is unclear whether, and by how much, the ORs here might lead to an overestimation of risk or prevalence.

Table 6.1.13. Odds ratios for an elevated serum TC level by quartiles of serum PFOS concentrations (ng/ml) from Steenland et al. (2009). The LOAEC is highlighted in blue.

Quartile	PFOS[1]	N	OR	95% CI
1	6.6	11,574	1.00	Ref
2	16.4	11,574	1.14	1.05-1.23
3	23.8	11,574	1.28	1.19-1.39
4	34.0	11,574	1.51	1.40-1.64

Abbreviations: CI, 95% confidence interval; OR, odds ratio; Ref, reference

[1] Category midpoints for PFOS serum concentrations (ng/ml) except for the highest quartile which is the midpoint based on digitized data from Figure 3 of Steenland et al. (2009)

While the Steenland et al. (2009) study only included adults, another study in the C8 area performed the same analyses in children. Frisbee et al. (2009) was a cross-sectional study in the C8 area that measured serum PFOS and serum lipid concentrations in 12,476 children and adolescents ages 1-18 years old. Both studies enrolled participants and collected serum samples in the same years (2005-2006). Mean (±SD) serum PFOA and PFOS concentrations were 69.2 (±111.9) ng/ml and 22.7 (±12.6) ng/ml, respectively, which were similar to those reported in the C8 study of adults discussed above (Steenland et al., 2009). In linear regression models with adjustments for age, sex, BMI, fasting, and exercise, increasing PFOS serum concentrations were associated with increases in TC and LDL. The actual regression coefficients were not provided but the authors reported that the mean differences in TC and LDL levels in the 5th compared to the 1st quintile of PFOS were 8.5 mg/dl and 5.8 mg/dl, respectively. For PFOA, the corresponding values were lower: 4.6 mg/dl and 3.8 mg/dl, respectively. Figures showing clear increases in mean TC and LDL levels with increasing quintiles of PFOS were provided. Somewhat similar patterns were seen for PFOA, although the magnitude of these associations appear less strong than for PFOS. Mean HDL concentrations also seemed to generally increase with increasing quintiles of PFOS, although the dose-response pattern was more variable (i.e., the increases were not monotonic across all exposure levels).

ORs for having elevated TC by quintiles of PFOS were presented, and are shown in Table 6.1.14. Here, the cut-off for elevated TC was based on the American Heart Association guideline for children of >170 mg/dl (Benjamin et al., 2017). ORs were presented for PFOS quintiles 2-5, with quintile 1 as the reference group. Quintile cut-off points or medians were not provided, but the medians could be estimated based on digitization of the article's Figure 3. As seen, the LOAEC was 16 ng/ml, which is very close to the LOAEC of 16.4 ng/ml seen in the C8 adults.

Table 6.1.14. Odds ratios for elevated serum TC by quintiles of serum PFOS (ng/ml) from Frisbee et al. (2009). The LOAEC is highlighted in blue.

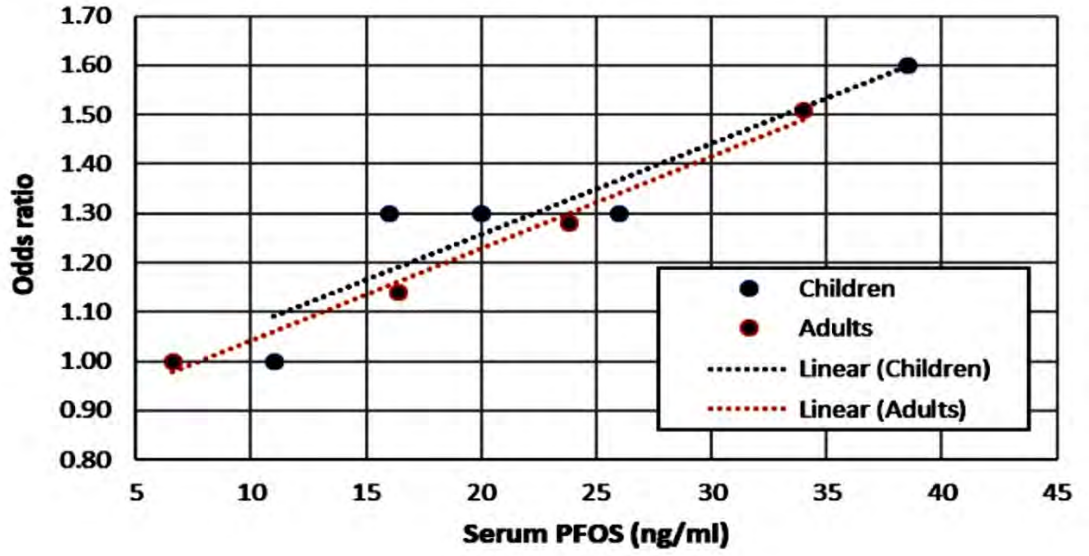
Quintile	PFOS[1]	N	OR	95% CI
1	11	2,495	1.0	Ref
2	16	2,495	1.3	1.1-1.4
3	20	2,495	1.3	1.2-1.5
4	26	2,495	1.3	1.2-1.6
5	38	2,495	1.6	1.4-1.9

Abbreviations: CI, 95% confidence interval; OR, odds ratio; Ref, reference

[1] Category medians for PFOS serum concentrations (ng/ml) based on digitized data from the paper's Figure 3

In order to help explore whether children might be more susceptible to the lipid-altering effects of PFOS than adults, OEHHA compared the PFOS-TC ORs reported in Steenland et al. (2009) to those in Frisbee et al. (2009). The ORs from both studies are shown in Figure 6.1.1. Unweighted linear regression slopes are also shown and fit both sets of data well (R^2s >0.80). As seen, the dose-response slopes for adults and children are very similar, suggesting that based on PFOS serum levels, children are not more susceptible to the effects of PFOS on TC than adults.

Figure 6.1.1. Odds ratios for elevated serum TC by quantiles of serum PFOS (ng/ml) in adults Steenland et al. (2009) and children Frisbee et al. (2009) from the C8 study area



Starling et al. (2014):

This was a cross-sectional analysis of 891 pregnant women enrolled in the Norwegian Mother and Child (MoBa) Cohort Study in 2003–2004. Non-fasting plasma samples were obtained at mid-pregnancy and analyzed for 19 different PFAS including PFOA and PFOS. Plasma concentrations of TC, LDL, HDL, and TGs were also measured. Median (IQR) PFOA and PFOS concentrations were 2.25 (1.66-3.03) ng/ml and 13.03 (10.31-16.60) ng/ml, respectively. The authors reported that each lognormal increase in PFOS was associated with an increase in TC of 8.96 mg/dl (95% CI, 1.70-16.22). These results were adjusted for age, pre-pregnancy BMI, nulliparous or inter-pregnancy interval, duration of breastfeeding, parity, education, smoking at mid-pregnancy, gestational weeks at blood draw, and oily fish consumption. The association between PFOA and TC was not statistically significant (each lognormal increase was associated with a 2.58 mg/dl (95% CI, -4.32-9.47) increase in TC.

The mean differences in TC for PFOS quartiles 2-5 compared to quartile 1 are shown in Table 6.1.15. As seen, none of these mean differences was statistically significant. As such, no NOAEC or LOAEC could be identified.

OEHHA entered the data in Table 6.1.15 into the US EPA BMDS, using various dose-response models. The SDs of the mean differences were estimated using the confidence intervals provided in the publication and the equations above. The absolute mean TC concentration (in mg/dl) in PFOS quartile 1 was provided in the publication. Using this, the absolute mean TC concentrations in the other PFOS quartiles could be estimated by adding the mean differences in TC reported for these other quartiles to the absolute mean TC concentration in quartile 1.

The mean difference in TC in PFOS quartile 4 compared to PFOS quartile 1 of 7.59 mg/dl represents about a 3.7% increase in mean TC across these quartiles. Because of this, OEHHA selected a 1% increase in TC, rather than a 5% or 10% increase, as the BMR. Using this BMR, the Hill model gave a BMD_{01} and $BMDL_{01}$ of 14.5 and 12.3 ng/ml, respectively (p-value for model fit=0.37). Fit was not improved with the other models.

A potential weakness of the Starling et al. (2014) study for dose-response assessment is that a statistically significant association was seen between PFOS and **increasing** concentrations of HDL (the “good cholesterol”). For example, the increases in TC, LDL, and HDL for each lognormal increase in PFOS were 8.96 mg/dl (95% CI, 1.70-16.22), 6.48 (95% CI, −0.07-13.03) and 4.39 (95% CI, 2.37-6.42), respectively. Since HDL is a component of TC, these results suggest that a fairly substantial portion of the effect seen for TC is due to HDL and therefore may not necessarily be adverse. Another potential weakness is that PFOS was highly correlated with several other PFAS (several Spearman correlation coefficients >0.60). In addition, the authors noted that the PFOS-TC association was attenuated after adjustment for these other PFAS. Further details of this particular analysis were not provided, making it difficult to evaluate the overall importance of this effect.

Table 6.1.15. Mean difference and absolute mean serum TC concentrations by quartiles of serum PFOS concentrations (ng/ml) from Starling et al. (2014)

			Mean differences (mg/dl)				Absolute means (mg/dl)		
Quartile	PFOS[1]	N	Mean difference	CI_L	CI_U	SD	Mean	CI_L	CI_U
1	5.2	223	0	Ref		41.26	207.59	Ref	
2	11.7	223	-3.35	-10.34	3.64	37.64	204.24	197.25	211.23
3	14.8	223	3.06	-4.93	11.05	43.02	210.65	202.66	218.64
4	20.5	223	7.59	-0.42	15.60	43.13	215.18	207.17	223.19

Abbreviations: CI_L, lower 95% confidence interval; CI_U, upper 95% confidence interval; Ref, reference; SD, standard deviation

[1] The midpoint of each PFOS category for quartiles 1-3. For quartile 4, the upper border was not provided so the midpoint between the 75th and 95th percentile was used.

Summary: PFOS PODs

A summary of the PODs derived from the candidate studies identified by OEHHA are shown in Table 6.1.16. These PODs ranged from 12.3 to 24.1 ng/ml in serum (mean = 17.9 ng/ml). After removing the two LOAECs (both from C8 studies), the range remained the same but the average changed slightly (19.0 ng/ml). The lowest POD was the $BMDL_{01}$ of 12.3 ng/ml calculated using the data in Norwegian pregnant women from Starling et al. (2014). The highest was the $BMDL_{10}$ of 24.1 ng/ml generated by Dong et al. (2019) from the data in adults in US NHANES using the hybrid approach.

Table 6.1.16. Summary of potential PODs for PFOS

Candidate studies	Effect	Method	POD/notes
Immunotoxicity			
Faroe Islands (Grandjean et al., 2017a; Budtz-Jorgensen and Grandjean, 2018)	Diphtheria antibody levels	NOAEC	NOAEC = 20.6 ng/ml
	Tetanus antibody levels	NOAEC	Data not available
	Diphtheria antibody levels	BMDS: Hill and Piecewise models	$BMDL_{05}$ outside observed range; large BMD:BMDL ratios
	Tetanus antibody levels	BMDS: Hill and Piecewise models	$BMDL_{05}$ outside observed range; large BMD:BMDL ratios
Lipid Concentrations			
Dong et al. (2019)	Mean TC levels	NOAEC/LOAEC	Information not provided
	Mean TC levels	BMDS	Information not provided

Candidate studies	Effect	Method	POD/notes
	Mean TC levels	Hybrid model	BMD_{10} = 44.2 ng/ml $BMDL_{10}$ = 24.1 ng/ml
Steenland et al. (2009)	Elevated TC ORs	NOAEC/LOAEC	No NOAEC **LOAEC = 16.4 ng/ml**
	Elevated TC ORs	BMDS/glst	Prevalence data not provided
Frisbee et al. (2009)	Elevated TC ORs	NOAEC/LOAEC	No NOAEC LOAEC = 16 ng/ml
	Elevated TC ORs	BMDS/glst	Prevalence data not provided
Starling et al. (2014)	Mean TC levels	NOAEC/LOAEC	Categorical results not statistically significant
	Mean TC levels	BMDS: Hill model	BMD_{01} = 14.5 ng/ml $BMDL_{01}$ = 12.3 ng/ml

OEHHA selected the LOAEC of 16.4 ng/ml from the Steenland et al. (2009) study of PFOS and elevated TC in adults from the C8 study area as the basis of its ADD calculations. This study offers the following advantages for dose-response and risk assessment calculations.

Very large sample size (N=46,294). This is by far the largest of the candidate studies OEHHA reviewed for its PFOS POD calculations. The very large sample size helps reduce the probability that findings are due to chance, allows for the detection of relatively subtle effect sizes, and helps to increase the likelihood that study findings are mostly generalizable. In this study, associations were evaluated in PFOS quartiles that included over 10,000 people each. This large size allowed for the calculations of ORs that were much more precise than in any other study.

Valid method for assessing exposure. This study assessed PFOS exposure using a well-accepted and validated method: serum PFOS concentrations. The long half-life of PFOS in serum suggests that a single serum measurement is likely to provide an accurate and precise indication of a person's true long-term exposure. In addition, there was no indication in this study that the serum samples were collected, or that PFOS concentrations were measured, in a way that would likely lead to differential bias and false positive effects. As mentioned for PFOA, non-differential misclassification of exposure can potentially bias ORs in the middle exposure categories (i.e., quartiles 2-3) away from 1.0. However, given the very large sample size of this study and the highly precise ORs that were reported, it is unlikely that this particular bias was large enough to have had a major impact on the statistical significance of the OR at the LOAEC.

Clinically relevant outcome. The cutoff point used to define elevated TC in Steenland et al. (2009) was based on a clinical reference level published by the American Heart Association (Benjamin et al., 2017). A TC level ≥240 mg/dl is a well-known risk factor for heart disease and stroke (Benjamin et al., 2017). The OR at the LOEAC was 1.14 (95% CI, 1.05-1.23). Although ORs can overestimate prevalence ratios when outcomes are common, this OR still likely represents a greater than 10% increase in the number of people with high TC at this exposure level. This is important because exposure levels near the LOAEC (16.4 ng/ml) are common in

the US general population (Dong et al., 2019). As such, this >10% increase likely represents large numbers of people. Overall, while the relatively small changes in mean TC levels seen with increasing PFOS exposure levels may not affect many people on an individual basis, the population effects of these small changes, given that TC is a major risk factor for cardiovascular disease, are likely to be widespread and large.

The two major components of TC are LDL (the "bad" cholesterol) and HDL (the "good" cholesterol). OEHHA selected TC rather than LDL for its critical effect because more studies evaluated TC than LDL, and a somewhat more robust, detailed, and consistent body of evidence was seen for this outcome. Given this, it should be noted that a number of studies have identified associations between PFOS and LDL (Table A7.9; US EPA (2016d)), and these support OEHHA's conclusion that the effects of PFOS on TC are adverse. In Steenland et al. (2009), the most informative results for PFOS and LDL and HDL were presented in the publication's Figures 4 and 5. It can be clearly seen in these figures that PFOS was strongly associated with LDL but not with HDL. This is strong evidence that the effects seen for TC are primarily due to LDL (i.e., its "bad" component) and should therefore be considered adverse.

Consistency of findings. Associations between PFOS and TC were seen both in the C8 study of adults (Steenland et al., 2009) and in the C8 study of children (Frisbee et al., 2009). The results of these two studies, and the PODs derived from them, were almost identical. In addition, the authors of these two studies used multiple different methods to evaluate associations (e.g., linear regression analyses, ORs), and the results of all of these methods were consistent with a statistically significant association between PFOS and TC. The results of these two studies are also consistent with a large number of other studies that have identified associations between PFOS and TC, or related outcomes like LDL, which are reviewed in Section 5.3. A number of studies in non-pregnant adults published after OEHHA's initial literature search (up to January 2, 2020) have also identified evidence of associations between PFOS and increasing TC and LDL (see Appendix 7, Table A7.29). These include three large high quality studies (Canova et al., 2020; Fan et al., 2020; Li et al., 2020). Full references provided in Table A7.29). These studies involved mostly general population-based samples, used serum levels to assess both PFOS and lipid levels, included adjustments for multiple relevant confounders, and involved excellent statistical power. Overall, these studies provide strong additional support that PFOS alters serum lipid levels.

No major confounding identified. The findings in Steenland et al. (2009) were adjusted for a number of factors that could potentially affect TC levels, including age, gender, BMI, socioeconomic status, exercise, smoking, and alcohol consumption. People who were taking cholesterol-lowering medications were excluded from the study. The authors also reported that findings were similar in people who did and did not fast before serum collection. A number of other factors can affect TC levels (e.g., certain genetic disorders, diabetes), but OEHHA found no indication that these were prevalent enough, or related strongly enough to both TC and PFOS, to cause important confounding. Consumption of a high fat diet or high total caloric intake could potentially be related to both elevated TC and higher PFOS exposure, but these could also be in the causal pathway. In addition, they are strongly related to the factors that were controlled for (BMI, smoking, and exercise) and therefore unlikely to have been fully responsible for the PFOS-TC associations reported in this study.

LOAEC unlikely to be affected by PFOA. Potential confounding by PFOA is a concern in this particular study because of the substantial environmental contamination of PFOA that occurred in the study area. While average PFOS serum levels in the study participants were similar to

those seen in NHANES (a mostly general population sample), serum levels of PFOA were markedly higher than general population levels. For example, while the mean (±SD) serum concentrations of PFOA and PFOS in the Steenland et al. (2009) study were 80.3 (±236.1) ng/ml and 22.4 (±14.8) ng/ml, respectively, the corresponding values in US NHANES during approximately the same years were 4.6 and 25.3 ng/ml, respectively (Nelson et al., 2010). Importantly, the correlation between PFOS and PFOA was only modest (R=0.32) in the C8 study area. As noted above, this is markedly lower than the correlations seen in other studies. In addition, the magnitude of the association between PFOS and TC appeared to be generally greater than that for PFOA and TC. For example, the regression coefficients for each lognormal increase in exposure was 0.02660 (SD=0.00140; $p < 0.05$) for PFOS, but >2-times lower for PFOA (β=0.01112; SD=0.00076; $p < 0.05$). Similarly, the OR for elevated TC in 4th versus the 1st quartile of exposure was lower for PFOA than for PFOS (ORs of 1.38 (95% CI, 1.28-1.50) and 1.51 (95% CI, 1.40-1.64), respectively). Overall, the fact that the associations between PFOA and both the exposure of interest here (PFOS) and outcome of interest here (TC) were only modest suggests that PFOA is unlikely to be the sole cause of the elevated ORs seen between PFOS and TC. In fact, the authors did note that, "When both PFOA and PFOS were considered together in the same model for total cholesterol, the effect of each was attenuated (20%–30%), but both continued to show the same monotonic, or nearly monotonic, trend of increasing cholesterol with increasing fluorocarbon." Further details of this particular analysis were not provided. However, given the very large sample size of this study (N=46,294), it is unlikely that a 20-30% reduction in the effect sizes would have had an important change in the statistical significance of the OR reported at the LOAEC.

No obvious selection bias. The C8 study has been estimated to include >80% of all residents of the affected areas. This high participation rate suggests that major errors due to selection bias are highly unlikely. In other studies, participation rates were much lower or participation rates were unclear or not reported, making it more difficult to evaluate the potential impact of selection bias in those studies.

Reverse causality unlikely. The Steenland et al. (2009) analyses were cross-sectional, and a common criticism of cross-sectional studies is the possibility of reverse causation. However, most of the convincing evidence regarding PFAS and reverse causation has involved other health outcomes (e.g., kidney function, time to pregnancy, birth weight, and early menopause) (Fei et al., 2012; Dhingra et al., 2017). With regards to serum lipids, one hypothesis is that higher serum cholesterol levels could lead to higher serum PFAS concentrations through the displacement of protein-bound PFAS from β-lipoprotein or albumin (Kerger et al., 2011). However, while PFAS can bind to human albumin (Han et al., 2003; Chen and Guo, 2009; Kerger et al., 2011), the major transport proteins for cholesterol in blood are lipoproteins, not albumin (Feingold and Grunfeld, 2018). And, while cholesterol binding to β-lipoprotein is significant (e.g., to form LDL), PFAS binding to lipoproteins is fairly minimal (Butenhoff et al., 2012c).

Another hypothesis is that serum lipid and serum PFOS concentrations are related because both bile acids (an important elimination route for cholesterol) and PFOS undergo significant enterohepatic circulation and share the same transport proteins involved in this process (Zhao et al., 2015a). In fact, studies in humans have shown that blocking the enterohepatic circulation of bile acids using cholestyramine, which can increase the fecal elimination of cholesterol, can also increase the fecal elimination of PFOS (Genuis et al., 2010; Genuis et al., 2013). Importantly though, whether this effect can lead to large changes in PFOS serum concentrations or large changes in the body burden of PFOS is unknown. This is important

because the associations seen in Steenland et al. (2009) involved large differences in the serum concentrations of PFOS but only small differences in the serum concentrations of TC. For example, as seen in the publication's Figure 3, an approximately 1,000% increase in serum PFOS was associated with only about a 5% increase in serum TC. This suggests that if enterohepatic circulation was responsible for the Steenland et al. (2009) findings, this mechanism would have to have an extremely large impact on serum PFOS, something that OEHHA was unable to find quantitative evidence to support.

OEHHA identified additional evidence that the associations between PFAS and serum cholesterol levels identified by Steenland et al. (2009) and others are not due to reverse causality. Some of this evidence involves PFOA rather than PFOS. However, the associations between TC and PFOS reported by Steenland et al. (2009) were very similar to those they reported for TC and PFOA. Because of this, and because OEHHA could not find any rational explanation why a true causal association would exist for one of these chemicals but reverse causality would be important for the other, evidence relating to either PFOS or PFOA are presented here.

The evidence that PFAS-TC associations are not due to reverse causality include the following:

- In Everds and Kennedy (2015), PFOA was administered orally to two groups of hamsters, one fed a normal diet and one fed a high fat diet, and serum PFOA concentrations were then compared between these two groups. PFOA was administered in the form of ammonium perfluorooctanoate, at doses of 0.1, 1.0, and 10 mg/kg, for 30 days. The researchers reported that while serum cholesterol levels were about 40-100% higher in the hamsters fed a high fat diet, the differences in serum PFOA concentrations between the hamsters on high fat and normal diets at each PFOA intake level was small (generally <10%). Overall, this finding argues against the hypothesis that increases in serum lipids can cause major increases in serum PFOA.

- Associations between PFOA and TC in the C8 study area have not only been seen in analyses based on actual measured serum PFOA levels, but also in analyses based on modeled PFOA exposures. In Winquist and Steenland (2014), estimates of PFOA concentrations in local air, surface water, and groundwater were generated using an environmental fate and transport model. These were combined with the study participant's residential history, drinking water sources, water consumption rates, and occupational exposures, and entered into a toxicokinetic model to generate yearly PFOA serum concentration estimates for each participant (N=32,254). Participants were defined as having high cholesterol if they reported taking cholesterol-lowering medications, and the year this condition started was defined as the year the participant reported that they were first told by a medical care provider they had high cholesterol. Analyses were adjusted for age, sex, education, race, smoking, and BMI. The researchers found that increasing quintiles of modeled cumulative PFOA exposure were associated with hazard ratios for high cholesterol of 1.00 (reference), 1.24 (95% CI, 1.15-1.33), 1.17 (95% CI, 1.09-1.26), 1.19 (95% CI, 1.11-1.27), 1.19 (95% CI, 1.11-1.28) (p-test for trend=0.005). Overall, because these analyses were based on external metrics of PFOA exposure and not on serum PFOA concentrations, the associations reported here would not be due to any direct effect that serum lipids would have on serum PFOA and therefore would not be due to reverse causality.

- With regards to PFOS, the Steenland et al. (2009) researchers evaluated reverse causality by comparing PFOS serum concentrations in people taking cholesterol-lowering medications to PFOS serum concentrations in people not taking these medications. These analyses were adjusted for gender, age, BMI, race, and other factors. The rationale behind this analysis was that most people who were taking cholesterol-lowering medications would be doing so for reasons unrelated to their PFOS exposure (e.g., elevated cholesterol levels due to diet, lack of exercise, or genetics). Because of this, if higher serum lipid levels did indeed cause higher serum PFOS levels, the use of these medications (and the decreased cholesterol levels that would be associated with this use) would be expected to lead to a major decrease in serum PFOS. However, the authors reported that serum PFOS concentrations were not lower in those taking cholesterol-lowering medications, providing evidence that higher serum lipid levels were not causing higher serum PFOS.

- Finally, perhaps the strongest evidence that reverse causation was not responsible for the effects seen in Steenland et al. (2009), is that an association between PFOS and higher TC levels has also been reported in a prospective study done in the C8 study area. In Fitz-Simon et al. (2013), 560 of the adult participants in the C8 study who provided serum for PFOS and TC measurements in 2005-2006, also provided serum for PFOS and lipid measurements in 2010. Over this time, the geometric mean serum concentration of PFOS in these participants fell from 18.5 ng/ml in 2005-2006 to 8.2 ng/ml in 2010. The authors found that a 50% decrease in serum PFOS over the study period was associated with a 3.2% (95% CI, 1.6-4.8) decrease in serum TC and a 5.0% (95% CI, 2.5-7.4) decrease in serum LDL. A statistically significant change in HDL was not seen. These findings provide strong evidence that reverse causality is not responsible for the PFOS-TC associations seen in the C8 area because there is a well-known reason why the PFOS exposures decreased in this study, and this reason is unrelated to any changes in serum lipids. In fact, serum TC and LDL levels in subjects in the lowest PFOS exposure group actually increased. Rather, the cause of the decreased PFOS levels was the major reductions in the production, use, and exposure to PFOS that took place throughout the US just before and during the years of the study (Dong et al., 2019; ATSDR, 2021). Given these well-known reasons for the declining PFOS exposure, the idea that the decreases in serum PFOS seen in this study were caused by declining TC or LDL levels is implausible.

Overall, based on the findings of Fitz-Simon et al. (2013) and the other data presented above, OEHHA has concluded that it is highly unlikely that reverse causality was responsible for the PFOS-TC associations reported by Steenland et al. (2009) and others.

Appropriate study design. Another criticism of cross-sectional studies is that because they measure exposure and outcome at the same time, the most relevant exposure period might be missed. However, as discussed above for PFOA, the half-life of PFOS is several years. As such, a single serum measurement of PFOS is likely to represent exposure over the long-term. In addition, the half-life of LDL cholesterol in plasma is fairly short, about three days (Daniels et al., 2009), and TC and LDL levels typically fall within days or weeks after beginning cholesterol-lowering medications. Overall, the long half-life of serum PFOS, combined with the short half-life of serum cholesterol suggest that the cross-sectional approach used in Steenland et al. (2009) is a valid method for assessing the dose-response relationship between PFOS and TC.

Acceptable Daily Dose Calculation

The ADD was calculated using the LOAEC of 16.4 ng/ml from the Steenland et al. (2009) study of PFOS and elevated TC in adults from the C8 study area, and applying the clearance factor of 3.9×10^{-4} L/kg-day (or 0.39 ml/kg-day) discussed in Chapter 4. An uncertainty factor (UF) of √10 for intraspecies variation was also applied in these calculations. The Steenland et al. (2009) study involved a very large population of adults and the participation rate was >80%. This large sample size and high participation rate suggests the study participants were a good representation of the study area as a whole, and likely included a diverse group of people in terms of ages, health status, smoking and other chemical exposures, nutrition, socioeconomic status, and other factors. As discussed above, a separate study in this area examined the relationship between PFOS and TC in children (Frisbee et al., 2010), and OEHHA's evaluation found that based on serum levels, the PFOS-TC relationship was essentially the same in children as in adults. A UF of √10 rather than 1 for intraspecies variation was applied because the C8 study population was not diverse in terms of race or ethnicity. In addition, it did not examine other potential susceptibility factors such as obesity or genetics. Some data suggest that obesity or certain genetic polymorphisms might increase susceptibility to PFAS (Ghisari et al., 2017; Jain and Ducatman, 2018b; Jain and Ducatman, 2019; Wen et al., 2019a). However, these data were limited (for a variety of reasons) and OEHHA was unable to identify consistent or convincing evidence that this susceptibility might be greater than a factor of √10.

OEHHA also applied the LOAEC to NOAEC UF of √10 because the Steenland et al. (2009) ORs involved an LOAEC rather than an NOAEC. A full LOAEC to NOAEC UF of 10 was not used for this ADD calculation because the very large sample size and the very precise nature of the ORs allowed the study to detect relatively small effect sizes with excellent statistical power. As such, an LOAEC could be detected at a fairly low PFOS exposure level. In fact, the LOAEC occurred in the second highest PFOS exposure quartile, which had a range of 13.3 to 19.5 ng/ml. These levels are very close to those commonly seen in the general US population (Dong et al., 2019). Overall, given the very high precision of the Steenland et al. (2009) results, it seems highly unlikely that a future study (at least one done in the near future) will identify an NOAEC with good precision that is 10-times lower than the Steenland et al. (2009) LOAEC.

The ADD for PFOS was calculated as:

ADD = (POD × CF) ÷ UF = (16.4 ng/ml × 0.39 ml/kg-day) ÷ 10 = **0.64 ng/kg-day**.

Animal Studies

Similar to PFOA, the major noncancer effects of PFOS in experimental animals are liver, immune system, thyroid, and developmental/reproductive toxicities. Liver, thyroid, and immune system toxicity appear to be the most sensitive endpoints. OEHHA derived ADDs from animal studies for comparison with those derived from human studies. Among the animal studies that exhibited a dose-response, Table 6.1.17 lists candidate critical studies for PFOS that report serum/plasma levels of PFOS and that OEHHA considers are of sufficient quality. BMD modeling was performed on the candidate critical study datasets in Table 6.1.17, using plasma/serum concentration as the dose metric, and the results are presented in Table 6.1.18. As with PFOA, ADDs are not calculated for PFOS due to the availability of human data for derivation of a health-protective concentration.

Table 6.1.17. Candidate critical studies for noncancer effects of PFOS in animals

Reference	Sex/Species	Dose/Route of Exposure/Duration	Endpoint	NOAEL/LOAEL (mg/kg-day)	NOAEL/LOAEL/ BMDL (mg/L)
Liver Toxicity					
Xing et al. (2016)	Male C57BL/6 mice (10/dose)	0, 2.5, 5, or 10 mg/kg-day via gavage for 30 days	↑ relative liver weight	LOAEL: 2.5	LOAEL: 70.2 $BMDL_{1SD}$: 8.9
Lai et al. (2018)	Female CD-1 mice (≥4/dose)	0, 0.3, or 3 mg/kg-day via gavage for 7 weeks	↑ liver triglycerides	LOAEL: 0.3	LOAEL: 33.8 $BMDL_{1SD}$: 11.2
NTP (2019b)	Male and female Sprague Dawley rats (10/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	↑ relative liver weight	LOAEL: 0.312	Males LOAEL: 23.7 $BMDL_{1SD}$: 4.7 Females LOAEL: 30.5 $BMDL_{1SD}$: 44.1
Immunotoxicity					
Zhong et al. (2016)	Pregnant C57BL/6 mice (12/dose)	0, 0.1, 1, or 5 mg/kg-day via gavage from GD 1-17	↓ splenic natural killer cell activity in male pups at 8 weeks of age	NOAEL: 0.1	NOAEL: 3.79
Dong et al. (2009)	Male c57/BL6 mice (10/dose)	0, 0.008, 0.083, 0.417, 0.833, or 2.08 mg/kg-day via gavage for 60 days	↓ plaque forming cell response	NOAEL: 0.008	NOAEL: 0.674 $BMDL_{1SD}$: 5.1 $BMDL_{10}$: 0.75
Thyroid Toxicity					
NTP (2019b)	Male and female Sprague Dawley rats (10/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	↓ free and total T4	LOAEL: 0.312 (both sexes)	Males LOAEL: 23.7 $BMDL_{1SD}$: 4.8 (free T4) 5.2 (total T4)[a] Females LOAEL: 30.5 $BMDL_{1SD}$: 12.1 (free T4)[a] 8.2 (total T4)

GD, gestation day; T4, thyroxine
[a] Highest dose group excluded in BMD modeling

While the NOAEL for immunotoxicty from Zhong et al. (2016) was lower than the BMDLs from the modeled datasets in Table 6.1.17, the reported serum values in pups (measured at 4 weeks and 8 weeks of age) would not be representative of the serum concentrations during the dosing period (GD1-17). Pups at 4 and 8 weeks of age would be expected to have lower serum concentrations than during the period of active dosing.

Table 6.1.18. BMD modeling of noncancer endpoints from candidate PFOS animal studies

Study	Endpoint	Model	Goodness of fit p-value	BMD_{1SD} (mg/L)	$BMDL_{1SD}$ (mg/L)
Xing et al. (2016)	↑ relative liver weight	Linear	0.483	12.8	8.9
Lai et al. (2018)	↑ liver triglycerides	Linear	0.053	15.5	11.2
NTP (2019b)	↑ relative liver weight (females)	Exponential2	0.374	55.1	44.1
NTP (2019b)	↑ relative liver weight (males)	Exponential4	0.679	6.9	4.7
NTP (2019b)	↓ free T4 (males)	Exponential4	0.224	7.5	4.8
NTP (2019b)	↓ total T4 (males)[a]	Exponential4	0.829	7.9	5.2
NTP (2019b)	↓ free T4 (females)[a]	Exponential4	0.330	20.8	12.1
NTP (2019b)	↓ total T4 (females)	Exponential4	0.763	12.5	8.2
Dong et al. (2009)	↓ plaque forming cell response[a]	Exponential5	0.742	10.0	5.1
Dong et al. (2009)	↓ plaque forming cell response[a]	Hill	0.776	1.56[b]	0.750[b]

[a] Highest dose group excluded
[b] Data modeled with a BMR of 10%, therefore these values correspond to BMD_{10} and $BMDL_{10}$

OEHHA identified Dong et al. (2009) as the critical toxicity study for deriving a notification level (NL) recommendation for the noncancer effects of PFOS (OEHHA, 2019). At the time, BMD modeling of the data for plaque forming cell response from (Dong et al., 2009) did not provide any adequate models. Therefore, the NOAEL of 0.008 mg/kg-day, corresponding to a serum concentration of 0.674 mg/L, was selected as the POD. The data are summarized in Table 6.1.19. Here, an additional analysis of this dataset indicates an acceptable model fit can be obtained with BMD modeling when the highest dose is excluded. Excluding the highest dose is appropriate for this dataset since there is a 260-fold difference between the lowest and the highest dose, and inclusion of the highest dose data appeared to compromise the data fit in the low dose range.

Table 6.1.19. Plaque forming cell response in male C57BL/6 mice exposed to PFOS via oral gavage for 60 days (Dong et al., 2009)

Dose (mg/kg-day)	Serum Concentration (mg/L)	Plaque Forming Response[a] (PFC/10^6 spleen cells)
0	0.048 ± 0.014[b]	597 ± 64[b] (202)
0.008	0.674 ± 0.166	538 ± 52 (164)
0.083	7.132 ± 1.039	416 ± 43* (136)
0.417	21.638 ± 4.410	309 ± 27* (85)
0.833	65.426 ± 11.726	253 ± 21* (66)
2.08	120.670 ± 21.759	137 ± 16* (51)

[a] Data taken from New Jersey DWQI (2018). Authors state they received numerical data via personal communication with GH Dong.
[b] Mean ± SEM (N=10/dose); SD in parentheses
* p <0.05, reported by study authors

OEHHA typically uses a BMR of one standard deviation (1 SD) for continuous data when it is uncertain what level of response is biologically significant (US EPA, 2012). This results in a $BMDL_{1SD}$ of 5.1 mg/L (Table 6.1.18). However, when the SDs are relatively large, as for the Dong et al. (2009) dataset (Table 6.1.19), a BMR of 1 SD may represent a much larger change than 10%, which was historically a level to which a 1 SD change was comparable (Davis et al., 2011). Thus, OEHHA also modeled the data using a BMR of 10%, which resulted in a $BMDL_{10}$ of 0.75 mg/L.

6.2. Cancer Dose-Response Analyses and Cancer Potency Derivation

6.2.1. Perfluorooctanoic Acid

Because several high quality human studies were available, dose-response analyses were performed using human rather than animal data. As discussed above, the strongest and most consistent human evidence linking PFOA to cancer involves studies of kidney cancer. Based on evaluations of statistical power, generalizability, potential bias and confounding, and other factors, OEHHA selected the human studies by Shearer et al. (2021) and Vieira et al. (2013) for cancer dose-response analyses. Descriptions of these two studies, and evaluations of their potential strengths and weaknesses are provided in the following sections. Two other epidemiologic studies identified associations between PFOA and kidney cancer (Steenland and Woskie, 2012; Barry et al., 2013). The high exposure occupational study by Steenland and Woskie (2012) was not used for dose-response analysis because information on a range of exposures more relevant to the general population were available from the Shearer et al. (2021) and Vieira et al. (2013). The study by Barry et al. (2013) was not used for dose-response analysis because it was performed in the same study area as the Vieira et al. (2013) study and these two studies likely involved a number of the same participants. Vieira et al. (2013) was selected over the Barry et al. (2013) because it presented dose-response data using a more appropriate exposure metric (discussed in further detail below).

Shearer et al. (2021):

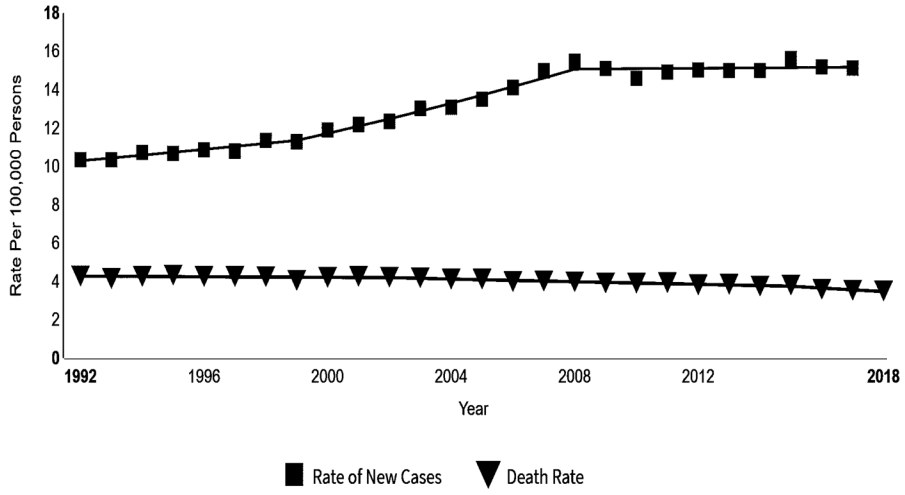
Study design: The study by Shearer et al. (2021) is a case-control study nested within the National Cancer Institute's (NCI) Prostate, Lung, Colorectal, and Ovarian Screening Trial (PLCO). The PLCO is a randomized clinical trial of the use of serum biomarkers for cancer screening. Participants were recruited via convenience sampling from 10 medical centers at various locations throughout the US (Table 6.1.17). The PLCO trial included approximately 150,000 people, with half of all participants randomized to the screening arm, and half randomized to the control arm. Participants in the screening arm had serum samples collected once at baseline during the years 1993-2002. These samples were processed and frozen within two hours of collection and stored at −70°C. A questionnaire was administered at baseline and was used to collect information on demographic variables and other cancer risk factors including smoking, BMI, medical history, and occupations. Participants were followed annually for an average of 8.9 years (range, 2-18 years). Incident cases of RCC (International Classification of Diseases-Oncology (ICD-O)-2 C64.9) were ascertained during the years 1996-2014 by medical record review of suspected cancers reported in the annual questionnaires, or from physicians or relatives, the National Death Index (NDI), or local cancer registries (Liao et al., 2017). The cases in the Shearer et al. (2021) study included all of the participants of the screening arm of the PLCO trial who were newly diagnosed with RCC during the follow-up period (N=326). All cases were histopathologically confirmed. Controls were selected from among participants of the PLCO trial screening arm who had never had RCC. These controls were individually matched to the RCC cases by age at enrollment, sex, race/ethnicity, study center, and year of blood draw. Concentrations of PFOA, PFOS, and other PFAS were measured in the baseline serum samples by the Centers for Disease Control and Prevention in the year 2018 using on-line solid phase extraction liquid chromatography isotope dilution tandem mass spectrometry. Laboratory personnel were blinded to the case-control status of the participants. Two case-control pairs were excluded due to missing PFAS concentrations. Results were presented for total PFOA and total PFOS, which were the sum of the concentrations of their respective isomers (i.e., n-PFOA and sum of the branched PFOA isomers for total PFOA, and n-PFOS and sum of perfluoromethylheptane sulfonic acid isomers for total PFOS). The study was funded by the National Cancer Institute (NCI), the US Department of Defense, and the National Institutes of Health (NIH).

Renal cell carcinoma (RCC): Kidney cancer is among the top ten cancers diagnosed in the US each year (ACS, 2020a). The American Cancer Society (ACS) has estimated that 73,820 new cases of kidney cancer were diagnosed in 2019, and that 14,770 deaths from kidney cancer occurred that year. The 5-year survival rate for kidney cancer is about 75%. RCC is the most common type of kidney cancer, and approximately 90% of all kidney cancers are RCCs. The major risk factors for kidney cancer are excess body weight and smoking, and the ACS has estimated that about half of all kidney cancers could be prevented by eliminating these two risk factors. Other risk factors include high blood pressure, chronic renal failure, and occupational exposure to certain chemicals such as trichloroethylene (TCE).

The incidence rate of kidney cancer in the US increased approximately 1-2% per year from 1992 to 2008. Since 2008, this rate has leveled off (Figure 6.2.1). The same general pattern has been seen for RCC, but with an average annual increase of more than 3% in the 12 years before 2008 (Saad et al., 2019). The exact reasons for the increases prior to 2008 are unknown but may be related to earlier detection through the increased use of abdominal CT scans, increasing obesity, or increases in certain occupational exposures, hypertension, or other risk factors (Saad et al., 2019). The reason for the leveling off after 2008 is also unknown. It is

unlikely that this leveling off is entirely due to changes in smoking or obesity since declines in smoking (and subsequent declines in lung cancer) began decades before 2008, and rates of obesity in the US have continued to increase well beyond 2008. Interestingly, US production and serum levels of PFOA declined sharply 5-10 years prior to the leveling off of RCC rates. Although it is unknown if and how much this leveling off may be due to decreases in PFOA exposure in the US, similar latency patterns following exposure cessation have been seen for other carcinogens, including smoking (Tindle et al., 2018).

Figure 6.2.1. Yearly incidence and death rates from kidney cancer in the US (SEER, 2020)



Demographics of the participants in Shearer et al. (2021): The RCC cases in the Shearer et al. study were unevenly distributed among the various recruitment centers, although no single site contributed more than 26% of all cases (Table 6.2.1). Compared to controls, cases were more likely to be obese (35.5% vs. 23.5%) and more likely to have hypertension (43.5% vs. 33.3%). Similar proportions of cases and controls were never, former or current smokers, and roughly 90% of both cases and controls were either former or never smokers.

Table 6.2.1. Number and percentage of cases and controls in Shearer et al. (2021) and in the PLCO trial by study center

	Shearer et al. (2021)		PLCO trial[1]
Center	**Controls N (%)**	**Cases N (%)**	**All participants N (%)**
Georgetown (Washington, DC)	15 (4.6)	15 (4.6)	8,108 (5.2)
Colorado	20 (6.2)	20 (6.2)	13,165 (8.5)
Hawaii	9 (2.8)	9 (2.8)	10,847 (7.0)
Henry Ford (Michigan)	37 (11.4)	37 (11.4)	24,665 (15.9)
Minnesota	84 (25.9)	84 (25.9)	28,862 (18.6)
Washington University (Missouri)	33 (10.2)	33 (10.2)	15,042 (9.7)
University of Pittsburgh (Pennsylvania)	40 (12.4)	40 (12.4)	16,930 (10.9)
University of Utah	27 (8.3)	27 (8.3)	14,387 (9.3)
Marshfield (Wisconsin)	46 (14.2)	46 (14.2)	16,740 (10.8)
University of Alabama	13 (4.0)	13 (4.0)	6,188 (4.0)

1. Data from Gren et al. (2009)

Exposure levels: The Shearer et al. (2021) serum samples were collected from 1993 to 2002. Geometric mean PFOA levels were somewhat higher in the later years of sample collection (geometric mean PFOA concentrations in the controls were 4.0, 4.3, and 4.6 ng/ml for the years 1993-95, 1996-97, and 1998-2002, respectively). The geometric mean in the 1998-2002 period is very close to the median PFOA level reported for participants age 60 and over in the 1999-2000 NHANES (4.8 ng/ml; 95% CI, 4.3-5.1 ng/ml), the first time PFOA was measured in a US nationwide survey. The similarity of these levels suggest that the Shearer et al. (2021) controls are a good representation of the general US population in terms of PFOA exposure.

Statistical analyses: Associations between PFOA and RCC were analyzed using baseline serum PFOA concentration as either a continuous or a categorical variable. In the categorical analyses, categories of PFOA were based on the quartiles of serum PFOA in the controls, and ORs for RCC were calculated using the lowest PFOA category as the reference group. ORs were adjusted for age, sex, race/ethnicity, study center, year of blood draw, estimated glomerular filtration rate (eGFR), BMI, smoking status, hypertension, prior freeze-thaw cycles of samples, and calendar year of blood draw. Wald tests were used to test for linear trends across the adjusted ORs, and were performed by modeling the within-category median of each PFOA quartile as a continuous variable.

In the analysis of serum PFOA as a continuous variable, PFOA was log2 transformed, and a linear regression analysis was used to calculate the RCC OR for each doubling of serum PFOA. Nonlinear trends were assessed by modeling logOR and the log2-transformed PFOA concentrations using a natural spline with three degrees of freedom.

Results: The ORs for the association between serum PFOA and RCC in the categorical analyses are shown in Table 6.2.2. A statistically significant increase in the odds of RCC was seen when comparing the highest to the lowest exposure category (OR = 2.63; 95% CI, 1.33-5.20). Although the ORs in the 2nd and 3rd highest exposure categories were not statistically significant, they were >1.0 and the p-test for trend across the ORs was statistically significant (p = 0.007).

Table 6.2.2. ORs for the association between PFOA serum concentrations and RCC in Shearer et al. (2021)

Exposure category (ng/ml)	Exposure category midpoint (ng/ml)	OR_a[1]	CI_L	CI_U	Case	Control	OR_u	SE
<4.0	2.0	1.00	Ref		47	81	1.00	
≥4-5.5	4.7	1.47	0.77	2.80	83	79	1.81	0.33
>5.5-7.3	6.4	1.24	0.64	2.41	69	83	1.43	0.34
>7.3-27.2	17.3	2.63	1.33	5.20	125	81	2.66	0.35

Abbreviations: CI_L, lower 95% confidence interval of OR_a; CI_U, upper 95% confidence interval of OR_a; OR_a, adjusted odds ratio; OR_u, unadjusted odds ratio; Ref, reference category; SE, standard error of the logOR
[1] p-trend = 0.007

In the analysis of PFOA as a continuous variable, a statistically significant association was seen between log2 PFOA and the odds of RCC, such that a doubling of PFOA was associated with an RCC OR of 1.71 (95% CI, 1.23-2.37). Analyses of log2-transformed PFOA using natural splines found no evidence of a significant non-linear relationship between PFOA and RCC risk.

The following sections discuss OEHHA's evaluations of the likelihood the Shearer et al. (2021) findings could have been due to bias (exposure misclassification, outcome misclassification, or selection bias) or confounding.

Exposure misclassification: Exposure was based on a single serum PFOA measurement in each participant. Significant bias could occur if people's serum PFOA concentrations change dramatically in a few days or months. As mentioned previously, serum measurements are a commonly used and widely accepted method for assessing PFOA exposure (NTP, 2016), and the half-life for PFOA in serum is in the order of years (Olsen et al., 2007; Mogensen et al., 2015b). This long half-life suggests that a single serum measurement is likely to provide an accurate and precise indication of a person's long-term PFOA exposure. Another important aspect of the Shearer et al. (2021) study is that the serum samples were collected years before cancer diagnoses. This prospective collection of exposure data helps alleviate concerns about reverse causation. In addition, the laboratory personnel measuring the PFOA serum concentrations were blinded to the case or control status of the study participants, which helps reduce concerns about researcher bias. Overall, there is no indication that the serum samples were collected, or that PFOA concentrations were measured, in a way that would have differed between the RCC cases and the controls. Because of this, potential misclassification of PFOA exposure, particularly in the highest exposure category, would most likely be non-differential, and therefore most likely bias results towards the null (i.e., in the direction of finding no association) (Jurek et al., 2005). Non-differential misclassification of exposure could have biased the ORs in the second and third PFOA quartiles away from 1.0. However, there is no indication that this bias would have a major impact on the dose-response slopes.

Outcome misclassification: Under or over-diagnosis of RCC is also possible, but major bias from this is unlikely. Although the Shearer et al. (2021) publication provided little information about case ascertainment, many of the details of this process can be found in other publications on the PLCO trial (Hayes et al., 2000; Prorok et al., 2000; Liao et al., 2017). Over-diagnosis of RCC is unlikely since all of the cases were histologically confirmed. Under-diagnosis (i.e., missed cases) is possible. However, because the serum samples used to measure PFOA were collected before cancer diagnosis, bias from missed cases is most likely non-differential and thus most likely towards the null. In addition, as discussed below in more detail, a comparison of the number of RCC cases in the Shearer et al. (2021) study to the number of cases expected based on US national rates suggests that under-diagnosis of RCC was not a major problem in this study.

Selection bias: The Shearer et al. (2021) study involved a convenience sample of mostly white non-Hispanic participants, and information on household income, education, or other socioeconomic indicators were not presented. While it is possible that the risks of RCC caused by PFOA vary by race, ethnicity, or socioeconomic status, OEHHA was unable to find any evidence showing that this is the case.

Follow-up rates were also not provided in the Shearer et al. (2021) publication. However, one way to evaluate the adequacy of follow-up is to compare the number of RCC cases ascertained in this study to the number of cases expected based on US national rates. Because Shearer et al. (2021) is essentially a population-based study, the PFOA levels in this study should be close to those in the general US population, and the number of cases should be similar to what would be expected based on US national rates.

The RCC rate in the US for the age groups included in the Shearer et al. (2021) study is about 50 cases per 100,000 person-years (Saad et al., 2019; SEER, 2020). Given an underlying cohort of approximately 74,000 people used for the Shearer et al. (2021) study, and an average follow-up period of 8.9 years, the number of cases expected based on US rates is:

74,000 people × 8.9 years × 50 cases per 100,000 person-years = 329 cases.

Although this number is only an estimate, its similarity to the actual number of cases in the Shearer et al. (2021) study (N=326) suggests that under-reporting, under-ascertainment, or poor follow-up were not major problems in this study.

Confounding: The ORs developed by Shearer et al. (2021) were adjusted for all of the important known risk factors for RCC including age, sex, BMI, smoking, and hypertension (IARC, 2019; ACS, 2020b)(. They were also adjusted for eGFR (high vs. low), which is a marker of non-malignant renal disease. The authors also presented a number of subgroup analyses that can be used to evaluate potential confounding or other forms of bias. Here, PFOA-RCC ORs were similar across subgroups of age, gender, BMI, hypertension (yes vs. no), eGFR (high vs. low), time from blood draw to cancer diagnosis (2-8 years vs. ≥8 years), and previous freeze-thaw cycle (none vs. ≥1). The similarity of the ORs within each of these subgroups is evidence that none of these factors caused major confounding. The PFOA-RCC OR was higher in current and former smokers than in never smokers. However, the confidence intervals for these ORs were wide and the difference between them was not statistically significant ($p = 0.24$).

Other known or possible risk factors for kidney cancer include certain chemical or occupational exposures such as arsenic (Ferreccio et al., 2013), TCE (IARC, 2014), asbestos (Smith et al., 1989), and cadmium (IARC, 2012b); medications such as acetaminophen or non-steroidal anti-inflammatory drugs (Karami et al., 2016); or genetic disorders such as von Hippel-Lindau disease or Birt-Hogg-Dube syndrome. Most of these known genetic disorders are too rare to cause important confounding. Arsenic is unlikely to cause confounding since, although it is strongly related to renal pelvis cancer, it is not strongly related to RCC (Ferreccio et al., 2013).

With regards to cadmium and asbestos, several studies have reported associations between these agents and kidney cancer. However, these findings are not consistent across all studies, and IARC has not established cadmium and asbestos as a sufficient cause of kidney cancer (IARC, 2012a; IARC, 2012b). TCE is an established cause of kidney cancer (IARC, 2014). Several studies, have reported an association between acetaminophen use and kidney cancer (Karami et al., 2016). Because there is little to no evidence that PFOA exposure is strongly related to asbestos, cadmium, or TCE exposure or to acetaminophen use, it is very unlikely that these agents could have caused the elevated ORs reported by Shearer et al. (2021). Even if there was some relationship between PFOA and these agents, the magnitude of these relationships would have to be very high, to the point of being implausible, to cause the PFOA-RCC OR of 2.63 reported for the highest PFOA exposure category in Shearer et al. (2021). The reason for this is that most studies linking asbestos, cadmium, TCE, and acetaminophen to kidney cancer report relative risks near 2.0, only in participants with very high exposure levels, levels that do not occur or are very rare outside of certain occupations or unusual medical settings (IARC, 2012a; IARC, 2012b; IARC, 2014). Relative risks for lower exposures to these agents are generally below 2.0. Because the relative risks between these agents and kidney cancer are not very high, and because only a small percentage of the general population are highly exposed to them, it is very unlikely they had important confounding effects (Axelson,

1978). Even if every person in the upper PFOA category was highly exposed to TCE, cadmium, asbestos, or acetaminophen and every person in the lower PFOA reference category was not (an extreme and implausible scenario), it would still be incredibly unlikely that confounding by these agents would cause the OR of 2.63 reported for the higher PFOA category. Smaller differences in TCE, cadmium, asbestos, and acetaminophen use or exposure between people in the higher and lower PFOA categories would only have small impacts on ORs. For example, given a relative risk between asbestos and RCC of 2.0 (Smith et al., 1989) and a prevalence of high asbestos exposure of about 4% in the general population (ATSDR, 2018b), even a 2-fold higher level of asbestos exposure in the highest PFOA exposure quartile would only cause an OR of about 1.04 (Axelson, 1978). This is well below the OR of 2.63 reported by Shearer et al. (2021).

One potential concern is the somewhat high percentage of RCC cases from the Minnesota study site: 25.9% of all RCC cases were from this site. In contrast, only 18.6% of all PLCO study participants were from the Minnesota site. The reason for this difference is unknown. The Minnesota study site is somewhat close to a former PFOA production plant, where high occupational exposures and contamination of local drinking water sources have been well documented (Raleigh et al., 2014; MPCA, 2020). The researchers did not specifically define the catchment area for the Minnesota portion of the study. However, although these exposed areas were nearby, there were many highly populated uncontaminated areas also nearby, and there was no indication that recruitment at this study site was restricted to people living or working in the contaminated area. The Shearer et al. (2021) authors did not report serum PFOA levels for each of the 10 study sites individually, but did report that median levels in participants from the Upper Midwest region (65% of whom were from the Minnesota study site) were somewhat lower than those from the other study regions. That is, the median serum PFOA levels for participants from the Upper Midwest, Western/Southern, and Eastern regions were 3.9, 4.6 and 4.3 ng/ml, respectively. The lower levels in the region containing Minnesota suggests that the large majority of the participants from the Minnesota site were not from the contaminated area and that PFOA exposures directly related to the former PFOA production facility were not a major driver of the Shearer et al. (2021) results.

The high percentage of cases from Minnesota could be due to confounding within the Minnesota site recruitment area, but this is unlikely. Kidney cancer rates for the years 2003-2017 (the years available) in the counties that include or are near the Minnesota study site (Hennepin, Ramsey, Dakota, and Washington Counties) are similar to those in Minnesota and in the US as a whole (Minnesota Department of Health, 2020; SEER, 2020). The fact that kidney cancer rates in these counties were not elevated argues against the presence of an unknown potent and widespread confounder in the Minnesota study area. Because the Shearer et al. (2021) researchers both matched and adjusted results by study site, any RCC risk factor that was greater in the Minnesota area than in the other recruitment areas is unlikely to have caused major confounding (Pearce, 2016). Confounding might still occur if the levels of an RCC risk factor varied within the Minnesota study area. However, this would only cause major confounding if the risk factor was potent (that is, a potent cause of RCC), widespread, strongly correlated with PFOA exposure, and not already adjusted for in the statistical analyses. This is unlikely since the most widespread and potent risk factors for RCC or kidney cancer were already included in the statistical adjustments, including age, sex, obesity, smoking, and hypertension. Major confounding by TCE, cadmium, asbestos, and acetaminophen is also highly unlikely for the same reasons given above.

Potential confounding of the Shearer et al. (2021) results by other PFAS was also evaluated. Serum concentrations of PFOA were correlated with serum concentrations of PFOS and PFHxS (Spearman correlation coefficients of 0.62 and 0.42, respectively). These correlations raise concerns that some of the effect reported for PFOA might actually be due to these other PFAS. To evaluate this possibility, the authors presented PFOA-RCC ORs both adjusted and unadjusted for PFOS and PFHxS. They also presented PFOS-RCC ORs adjusted and unadjusted for PFOA and PFHxS, and PFHxS-RCC ORs adjusted and unadjusted for PFOA and PFOS. The results of these analyses are shown in Table 6.2.3. As seen, the PFOA-RCC ORs changed only slightly with adjustment for PFOS and PFHxS. Although the CIs were wider after adjustment, this was expected given the correlations between these PFAS. The finding that adjusting for PFOS or PFHxS had little effect on the PFOA ORs provides good evidence that the elevated ORs reported for PFOA were not due to these other PFAS.

Table 6.2.3. ORs for serum PFOA, PFOS, PFHxS concentrations and RCC adjusted and unadjusted for other PFAS (Shearer et al., 2021)

				Without PFAS adjustment[a]		With PFAS adjustment[b]	
PFAS	**Exposure**	**Cases**	**Controls**	**OR**	**95% CI**	**OR**	**95% CI**
PFOA	<4.0	47	81	1.00	Ref	1.00	Ref
	≥4.0-5.5	83	79	1.47	0.77-2.80	1.41	0.69-2.90
	>5.5-7.3	69	83	1.24	0.64-2.41	1.12	0.52-2.42
	>7.3-27.2	125	81	2.63	1.33-5.20	2.19	0.86-5.61
	Continuous[c]	324	324	1.71	1.23-2.37	1.68	1.07-2.63
PFOS	≤26.3	60	81	1.00	Ref	1.00	Ref
	>26.3-38.4	82	81	1.67	0.84-3.30	1.24	0.59-2.57
	>38.4-49.9	61	81	0.92	0.45-1.88	0.53	0.22-1.24
	>49.9-154.2	121	81	2.51	1.28-4.92	1.14	0.45-2.88
	Continuous[c]	324	324	1.39	1.04-1.86	0.92	0.60-1.42
PFHxS	≤2.2	75	88	1.00	Ref	1.00	Ref
	>2.2-3.4	74	83	1.41	0.75-2.64	1.28	0.66-2.51
	>3.4-5.5	88	76	1.14	0.59-2.20	0.89	0.43-1.85
	>5.5-37.4	87	77	2.07	1.06-4.04	1.46	0.67-3.18
	Continuous[c]	324	324	1.27	1.03-1.56	1.12	0.88-1.43

Abbreviations: CI, confidence interval; Exposure, serum concentration of each PFAS in ng/ml; OR, odds ratio; Ref, reference category

[a] Adjusted for age, sex, race/ethnicity, study center, year of blood draw, eGFR, BMI, smoking status, history of hypertension, prior freeze-thaw cycles, and calendar year of blood draw

[b] Adjusted for all of the factors listed above plus adjustment for other PFAS (entered as log2-transformed concentrations of PFOA, PFOS, and PFHxS)

[c] Analysis of each PFAS as a continuous variable (entered in the model as log2 PFAS)

Similar findings were seen in analyses stratified by high, medium, and low levels of PFOS and PFHxS. That is, PFOA-RCC ORs comparing the upper tertile (>6 ng/ml) to the lower tertile (≤4 ng/ml) of PFOA were similar in participants with higher and lower levels of these other agents.

CIs widened in these analyses but this was expected given the smaller sample sizes. Overall, these analyses provide additional evidence that the elevated ORs for PFOA were unlikely due to these other agents. The elevated RCC ORs for PFOS and PFHxS mostly disappeared after adjustment for PFOA, suggesting that these other agents are not strongly related to RCC. Several other PFAS were also measured but these were either not associated with RCC or were not strongly correlated with PFOA, and therefore unlikely to have caused major confounding.

Vieira et al. (2013):

Study design: The Vieira et al. (2013) study was a cancer registry-based case-control study that took place in the C8 study area. The cancers of interest included kidney, pancreatic, testicular, and liver cancers. These were selected by the researchers because they had been linked to PFOA in previous animal and human studies. The controls were all other cancer types. The study area encompassed the six contaminated public water districts and the 13 counties in Ohio and West Virginia that surround the DuPont Washington Works PFOA facility. Initially, all incident cancer cases diagnosed from 1996 through 2005 in the Ohio counties of Athens, Meigs, Gallia, Washington, and Morgan and the West Virginia counties of Wood, Mason, Wirt, Putnam, Jackson, Pleasants, Ritchie, and Cabell were obtained from the Ohio Cancer Incidence Surveillance System (OCISS) and the West Virginia Cancer Registry (WVCR), respectively. However, only the OCISS provided the participants addresses, which could be used to develop individual estimates of PFOA exposure.

Initially, the OCISS provided the names of 9,402 cancer cases. According to the authors, 745 cases of oral cavity, pharynx, esophagus, larynx, and stomach cancer, and Hodgkin lymphoma were excluded because there were too few cases for meaningful analysis (<100 cases each), or they had not been previously investigated in relation to PFOA in animal toxicologic studies or occupational mortality studies. Seven hundred and fifty two participants could not be geocoded at the address level and were also excluded. Fifteen cases under the age of 15 years old were also excluded, leaving 7,869 Ohio participants in the study. Information on residences prior to cancer diagnoses were not available.

The exposure assessment in this study was based on estimates of serum PFOA concentrations at the time of diagnosis and 10 years before diagnosis. Because the residences prior to cancer diagnosis were unknown, the latter was done under the assumption that the participant lived at the address at diagnosis for at least ten years prior to diagnosis. Exposure was based on modeled estimates of each participant's annual average PFOA serum concentration. The model used to develop these estimates incorporated information on facility emissions, fate and transport characteristics of PFOA, and hydrogeological properties of the study area. These were then used to estimate yearly PFOA air and water concentrations throughout the exposed water districts. This information was then linked to each participant's residence and to standard assumptions about water intake, body weights, and PFOA half-life, to estimate the yearly average annual PFOA serum concentrations. Information on individual water intake (e.g., the amount of tap water consumed, bottled water use, etc.) or on occupational exposures were not included. These models were only applied to those living in the exposed water districts. Those living in one of the included counties, but outside of an exposed water district, were assigned to the "unexposed" reference category. Cumulative exposure was assessed by summing the yearly serum PFOA exposure estimates for the ten years prior to cancer diagnosis.

Statistical analysis: For participants residing in one of the exposed water districts, PFOA exposure was categorized into groups of "low," "medium," and "high" based on the tertile cutoff points in these participants. Because there was a large break in the distribution at 110 µg/L, participants in the upper 10% of modeled serum PFOA levels were removed from the "high" category and placed into a "very high" category. Logistic regression was used to calculate ORs for PFOA and kidney cancer, using all participants except those with kidney, pancreas, testicular, and liver cancer as controls. Odds ratios were adjusted for age, sex, diagnosis year, smoking status and type of insurance.

Results: The ORs for the association between modeled annual average PFOA serum concentrations 10 years prior to cancer diagnosis and kidney cancer in Vieira et al. (2013) are shown in Table 6.2.4. A statistically significant increase in the odds of kidney cancer was seen 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. The corresponding ORs were similar in the high and very high categories of cumulative exposure (2.0 and 2.1, respectively) but were slightly lower (1.8 and 1.7, respectively) in analyses without the 10-year lag. P-values for trends or analyses using continuous estimates of PFOA exposure were not provided.

Table 6.2.4. Adjusted ORs for modeled serum PFOA concentrations and kidney cancer in Vieira et al. (2013)

Category	Serum PFOA	Case	Control[1]	OR	95% CI
"Unexposed"	Unknown	187	5,957	1.0	Ref
Low	3.7-12.8	11	446	0.8	0.4-1.5
Medium	12.9-30.7	17	455	1.2	0.7-2.0
High	30.8-109	22	339	2.0	1.3-3.2
Very High	110-655	9	142	2.0	1.0-3.9

Abbreviations: CI, confidence interval; Serum PFOA, modeled serum PFOA concentration in ng/ml; OR, odds ratio; Ref, reference category
1. Controls were people with cancers other than kidney, pancreatic, testicular, and liver cancer

The following sections discuss OEHHA's evaluations of the likelihood the Vieira et al. (2013) findings could have been due to bias (exposure misclassification, outcome misclassification, or selection bias) or confounding.

Exposure misclassification: A previous validation study found that the measured serum PFOA levels and estimates of serum PFOA based on models similar to those used in Vieira et al. (2013) were reasonably well correlated (Spearman's R = 0.67). This correlation improved only slightly when information on tap water consumption was incorporated (R = 0.69). Overall, the model tended to under-predict the measured serum PFOA concentrations by about 10-20%. One exception involved people from Little Hocking, the water district with the highest PFOA exposures. Here the model appeared to over-estimate measured levels by about 20-30% (Shin et al., 2011).

The likely degree to which exposure misclassification would affect the ORs reported by Vieira et al. (2013) was evaluated using the methods presented by Greenland (1998), with separate analyses run for model sensitivity and model specificity. Since the researchers assessed exposure using the same methods in cases and controls, most errors from exposure

misclassification are likely to be non-differential and therefor have biased the ORs to the null. Analyses using the methods presented by Greenland (1998) show that in most instances, errors in model sensitivity (i.e., those that would be associated with model under-prediction) are likely to have had only small effects on the ORs. For example, correcting for a model sensitivity of 70% would only change the ORs in the very high PFOA category from 2.0 to about 2.1. Errors in model sensitivity would include issues such as occupational exposures that were missed, people who lived in unexposed areas but worked in exposed areas, or people with high PFOA exposures from their diet. Model under-prediction involving participants in the Belpre water district might have biased the OR in the "high" category upwards. However, simulations performed by OEHHA aimed at correcting this show that this bias is also likely to be small.

Errors related to model specificity would occur if participants with lower PFOA exposures were mistakenly assigned to the higher exposure categories (i.e., model over-prediction). This might occur if participants in exposed areas consumed bottled water with low PFOA levels, or if participants moved from an unexposed area to an exposed area just prior to cancer diagnosis. Since there are greater numbers of subjects in the unexposed category than in the higher exposure categories in Vieira et al. (2013), errors related to model specificity can have a greater impact on ORs than errors in model sensitivity. Importantly though, as noted above, the exposure model used in Vieira et al. (2013) tended to under-predict rather than over-predict PFOA exposures, suggesting that errors in specificity are less common than errors in sensitivity. As mentioned, decreases in model specificity could be caused by missing information on tap water source or bottled water use. However, as noted, this information had little effect on model validation results. Model over-prediction did seem to occur in participants from the Little Hocking water district (Shin et al., 2011). However, major bias from this is unlikely since the modeled serum levels in these participants were among the highest in this study, such that the large majority were probably already placed in the highest exposure category.

Outcome misclassification: Reporting of newly diagnosed cancer cases to the OCISS is mandatory in Ohio, and the percentage of all cancers that were kidney cancers in the Vieira et al. (2013) study was similar to the percentage of these cancers in the US as a whole (2.63% vs. 2.58%, respectively, for the years 1996-2005). This suggests that under-reporting or under-ascertainment of kidney cancers was not a major problem in this study.

The Shearer et al. (2021) study provides strong evidence that PFOA is associated with RCC. However, it is unknown whether PFOA is also associated with other, non-RCC, kidney cancer types. Because Vieira et al. (2013) included all kidney cancer types, bias might have occurred if non-RCC kidney cancers are unrelated to PFOA. In other words, ORs could be biased towards the null if kidney cancer types unrelated to PFOA were included in the "case" group. Since it is unknown whether or not PFOA is related to non-RCC kidney cancers, it is unknown whether or not this bias actually occurred. However, this bias, if it did occur, is likely to be small since the large majority (approximately 90%) of all kidney cancers are RCCs. In order to evaluate the likely magnitude of this bias (if it did occur), OEHHA performed simulations in which 10% of all kidney cancers were removed from the Vieira et al. (2013) data, with the percentage removed from each exposure category inversely proportional to the OR for each exposure category. Based on this simulation, correcting for this bias would likely only change the ORs in the high and very high categories from 2.0 to about 2.15.

Selection bias: The use of cancer controls in cancer case-control studies offers several important advantages over the use of noncancer population-based controls (Smith et al., 1988). Regardless, bias of ORs towards the null might occur if some of the control cancers are caused

by PFOA. However, Vieira et al. (2013) excluded participants from the control group who had cancers which have been previously associated with PFOA exposure in animal and human studies, including pancreas, testes, and liver cancers. Sensitivity analyses in which these cancers were included in the control group gave similar results.

As reviewed above, there is some evidence that PFOA may be associated with testicular cancer or certain subtypes of breast cancer. However, there is no evidence that these associations are strong enough or prevalent enough to cause major bias in the Vieira et al. (2013) results.

Confounding: The findings in Vieira et al. (2013) were adjusted for age, sex, diagnosis year, smoking status and type of insurance, the latter being an indicator of socioeconomic status. Data on obesity were not available. However, obesity rates in the C8 study area were similar to those in the region as a whole (Frisbee et al., 2009), and no evidence could be found that modeled estimates of serum PFOA were strongly related to obesity in this area. Similarly, no evidence exists that other possible kidney cancer risk factors like cadmium, arsenic, TCE, asbestos, or acetaminophen are prevalent enough or strongly enough related to PFOA exposure in this area to cause major confounding. With regards to confounding by other PFAS, there is no established association between PFOS or any other PFAS and kidney cancer, based on this review of the literature; correlations between PFOA and other PFAS in the C8 study area were only moderate (Frisbee et al., 2009). As such, important confounding by other PFAS is unlikely. The information presented above for the (Shearer et al., 2021) study also shows that confounding of PFOA-kidney cancer relationships by PFOS and other PFAS is unlikely.

Criteria for causal inference

Overall, the Shearer et al. (2021) and Vieira et al. (2013) studies meet most, if not all, of the criteria commonly used to evaluate causal inference (Hill, 1965).

Chance: Several of the key results in Shearer et al. (2021) are statistically significant, including the OR for the highest PFOA exposure category, the test for trend in the categorical analysis, and the OR in the analysis of PFOA as a continuous variable. The ORs in the two highest exposure categories in the Vieira et al. (2013) study are also statistically significant. The low p-values or confidence intervals excluding 1.0 associated with each of these findings show that the increases in RCC or kidney cancer related to PFOA reported in these studies are unlikely due to chance.

Temporality: The serum samples used to assess PFOA exposure in Shearer et al. (2021) were collected years before the kidney cancers in the study were diagnosed. In Vieira et al. (2013), modeled exposures were estimated for the year ten years prior to cancer diagnosis. Overall, because the exposure assessments in both of these studies were based on PFOA exposures prior to cancer diagnosis, the likelihood of reverse causality (i.e., the likelihood that having kidney cancer might lead to higher serum or modeled PFOA exposures) is small.

Dose-response: Both the linear test for trend in the categorical analysis and the results of the analysis with PFOA as a continuous variable in the Shearer et al. (2021) study are consistent with the presence of a dose-response relationship. While a demonstrated dose-response relationship is not a *sine qua non* for causality, a large number of other established carcinogens exhibit similar dose-response relationships (i.e., increases in cancer risk associated with increasing levels of exposure). Formal analyses of dose-response were not presented in Vieira et al. (2013), although the ORs for the two highest exposure categories were increased and

statistically significant, which is also consistent with a dose-response relationship between PFOA and kidney cancer.

Bias and confounding: The evaluations of information bias (exposure and outcome misclassification) and selection bias discussed above suggest that these potential issues are either minor, or are very unlikely to have caused the positive associations seen in these two studies. Similarly, evaluations of confounding, including potential confounding by all of the known major risk factors for kidney cancer, as well as confounding by other PFAS, show that these factors are also highly unlikely to have caused the Shearer et al. (2021) and Vieira et al. (2013) results.

Biologic plausibility: Findings from a number of other studies support the biologic plausibility of the Shearer et al. (2021) and Vieira et al. (2013) results. This includes the results of studies linking PFOA to non-malignant kidney disease (US EPA, 2016a). These results show that PFOA can not only reach the kidney but can also cause kidney toxicity. The biologic plausibility of the Shearer et al. (2021) and Vieira et al. (2013) findings is also supported by research in laboratory animals, which has shown that, while not specific for kidney cancer, PFOA can cause cancer in mammalian species (Chapter 5). In addition, studies have linked PFOA to mechanisms leading to cancer or key characteristics of carcinogens (Chapter 5 and Appendix 8), which also support the plausibility of the Shearer et al. (2021) and Vieira et al. (2013) findings.

Consistency: Another criterion for causal inference is consistency, both internal and external. The similarity of the Shearer et al. (2021) findings when PFOA was analyzed as either a categorical or a continuous variable, as well as the similarity of the findings across several different subgroups (e.g., subgroups based on age, gender, eGFR, and smoking), all highlight the internal consistency of these results. The similarity of findings in the Vieira et al. (2013) study across different exposure metrics (cumulative vs. annual average, with and without 10-year lags) and with different control groups (i.e., control groups with and without kidney, liver, pancreas, and testicular cancer cases) highlights the internal consistency of the results of this study.

The Shearer et al. (2021) and Vieira et al. (2013) findings are also consistent with two of the other human studies of PFOA and kidney cancer. As described in Chapter 5, OEHHA identified seven human studies of PFOA and kidney cancer. Two of these studies are not informative, either because of the ecologic nature of the exposure data (Mastrontonio et al., 2017), or because of the very small number of cases (Girardi and Merler, 2019). Four of the remaining five studies reported statistically significant associations between PFOA and kidney cancer or RCC incidence (Barry et al., 2013; Vieira et al., 2013; Shearer et al., 2021) or mortality (Steenland and Woskie, 2012). These four studies were very different in terms of study populations, sample sizes, exposure assessment, outcome metrics, statistical analyses, and other study design features. These differences are detailed in Table 6.2.5. Despite all of these differences, each of these four studies identified a strong association between PFOA and kidney cancer.

Table 6.2.5. Differences between the four epidemiologic studies identifying an association between PFOA and kidney cancer

Study criteria	Shearer et al. (2021)	Vieira et al. (2013)	Barry et al. (2013)	Steenland and Woskie (2012)
Study design	Case-control with population controls	Case-control with cancer controls	Retrospective cohort	Retrospective cohort
Population	US population: 10 sites	Exposed population: C8 study area	Exposed population: C8 study area	Occupational: DuPont facility
Sample size	Cases: 324 Controls: 324	Cases: 246 Controls: 7,339	Cases: 105 Cohort: 32,254	Cases: 12 Cohort: 5,791
Exposure assessment	Serum PFOA	Residential exposure model - serum	Residential exposure model - serum	Inhalation exposure model - serum
Exposure categorization	Quartiles and continuous	Contaminated vs. uncontaminated districts; annual average – five categories	Cumulative exposure, continuous and quartiles; 0 and 10-year lags	Job exposure matrix, exposure model cumulative serum
Statistical adjustments	Age, sex, race/ethnicity, study center, year of blood draw, eGFR, smoking, hypertension, freeze-thaw cycles	Age, sex, diagnosis year, smoking, and insurance	Smoking, alcohol, sex, education, and age	Standardized by age and sex
Outcome	Renal cell cancer incidence (ICD-0–2 C64.9)	Kidney cancer incidence (ICD codes not provided)	Kidney cancer incidence (ICD codes not provided)	Kidney cancer mortality (ICD9 189.0–189.2)

eGFR, estimated glomerular filtration rate; ICD, International Classification of Disease

Elevated mortality from kidney cancer was found in the DuPont occupational cohort studied by Steenland and Woskie, 2012 (standardized mortality ratio (SMR) = 2.68; 95% CI, 1.5-5.24). These results were not adjusted for smoking, but major confounding by smoking is unlikely since the SMR for lung cancer was not elevated (SMR = 0.75; 95% CI, 0.48-1.11). TFE was also used at this facility. TFE is classified by IARC as probably carcinogenic to humans (Group 2A), primarily based on increases in kidney cancer, liver cancer, testicular cancer, and leukemia in rodents (IARC, 2017b). It has been hypothesized that because TFE is highly volatile and explosive, it is well controlled and appreciable exposures during normal operations would have been unlikely (Steenland and Woskie, 2012). However, data for any air, biologic, or other monitoring for TFE at the DuPont facility were not located. To date, only one study has investigated the association between TFE and cancer in humans (Consonni et al., 2013). This retrospective cohort study included several facilities in North America and Europe where TFE was used, including the DuPont facility investigated in Steenland and Woskie (2012). In fact, 40% of the Consonni et al. (2013) cohort were DuPont workers. The kidney cancer SMR in the "medium" group of cumulative TFE exposure was elevated (SMR = 2.58; 95% CI, 0.95-5.62, N=6 cases). Risks in the "high" exposure group were below 1.0 (SMR = 0.81; 95% CI, 0.10-2.93) although the numbers of cases was small (N=2). The authors attempted to separate out the individual risks of TFE and PFOA, but the exposures to these agents were too highly correlated to provide meaningful results. Eighty-eight percent of all workers exposed to TFE were also exposed to PFOA, and every worker exposed to PFOA was also exposed to TFE. In the only other occupational study of PFOA and kidney cancer, in a facility where TFE was used infrequently, the relative risk estimates for kidney cancer mortality and incidence were all near or below 1.0 (Raleigh et al., 2014). However, in the two studies of the population surrounding

the DuPont facility, where significant drinking water contamination occurred, some evidence of increased kidney cancer risks were found in both studies. For example, in the cancer registry study by Vieira et al. (2013), kidney cancer ORs of 2.0 (95% CI, 1.3-3.2) and 2.0 (95% CI, 1.0-3.9) were reported in the highest two categories of PFOA exposure. Since TFE is highly volatile, this population is unlikely to have had high and prolonged TFE exposure. In addition, elevated TFE exposure is also unlikely in the population based study by Shearer et al. (2021), which also identified a strong association between PFOA and RCC. Overall, the findings of clear associations between PFOA and kidney cancer in studies where TFE exposures are most likely very low or non-existent suggests that important confounding by TFE is unlikely.

The occupational study by Raleigh et al. (2014) did not find an association between PFOA and kidney cancer. This study examined cancer mortality and incidence in 4,668 workers at a 3M facility in Cottage Grove, Minnesota. This facility produced ammonium perfluorooctanoate (APFO), the ammonium salt of PFOA. SMRs for the period 1960 to 2008 were calculated using Minnesota state rates as the reference. The authors also calculated hazard ratios (HR) for kidney cancer mortality and incidence using non-PFOA exposed workers (N=4,359) from a nearby 3M facility in St. Paul, Minnesota as the reference group. The St. Paul facility manufactured tapes and abrasives. The specific chemicals used to produce these items were not described. Only 200 employees reported for work duties at both locations.

The exact reason why the findings of the Raleigh et al. (2014) study differed from those of most other studies of PFOA and kidney cancer is unknown, but several possibilities exist. One relates to chance and the relatively small numbers of cases in this study. Raleigh et al. (2014) included only six kidney cancer deaths (with only one in the highest exposure category), and only 16 incident kidney cancer cases (with only four in the highest exposure category). For kidney cancer incidence, the HR in the highest exposure category was 0.73 (95% CI, 0.21-2.48). Although this HR was below 1.0, the wide CI shows that this result is imprecise. In fact, the difference between this HR and the relative risk (RR) estimate reported for the most highly exposed workers in Steenland and Woskie (2012), the other occupational study, (SMR = 2.66; 95% CI, 1.15-5.24) was not statistically significant ($p = 0.08$). Overall, the small numbers of kidney cancer cases, and the imprecise results highlight the possibility that the Raleigh et al. (2014) study could have missed a true association because of chance.

Another possibility is confounding. No data were available from either the Cottage Grove or St. Paul facility on smoking, BMI, or any other known risk factor for kidney cancer except age and sex. Using Minnesota state rates as the reference population, the SMRs in the Cottage Grove facility for all causes (0.85; 95% CI, 0.80-0.90) and for all cancers (0.87; 95% CI, 0.78-0.97) were below 1.0, which likely indicates a significant healthy worker effect. In addition, differences between the SMRs for the Cottage Grove and St. Paul facilities suggest that the latter may not have been an appropriate comparison group. For example, the all cause (0.98; 95% CI, 0.94-1.03 and all cancer (1.04; 95% CI, 0.95-1.13) SMRs for the St. Paul facility were higher than those for Cottage Grove. In fact, the SMRs for almost all individual cancers other than kidney cancer, as well as the SMRs for diabetes, ischemic heart disease, and cerebrovascular disease were all higher for St. Paul than for Cottage Grove. Overall, the higher SMRs seen in the St. Paul workers for outcomes not known to be associated with PFOA show that these workers were generally less healthy than the Cottage Grove workers, and provide evidence that the St. Paul workers were not an appropriate comparison group.

Another reason why the Raleigh et al. (2014) study may have missed a true association could relate to the methods used to assess exposure. Exposure assessment was based on modeled

estimates of PFOA air concentrations in the workplace. Although ground water contamination has been well documented near the Cottage Grove facility (MPCA, 2020), no information was available on non-work related residential exposures. For workers in the APFO production area, the exposure models incorporated industrial hygiene data (205 personal and 659 area samples from 1977-2000), job titles, and the proportion of time spent in the exposed areas. PFOA air levels were expressed as a daily time weighted average (TWA) in mg/m^3 for each job title. Cumulative exposure was then estimated by summing the daily TWAs for all years and all jobs each employee worked at the facility. Exposures prior to 1977 were extrapolated from estimated exposure levels in 1977-2000 using differences in annual APFO production levels. Exposures in non-production areas were based only on expert judgments and physical proximity to the APFO production area. St. Paul workers were assigned an air level one order of magnitude below the non-chemical division workers in Cottage Grove in order to account for non-work related exposure (i.e., residential exposures).

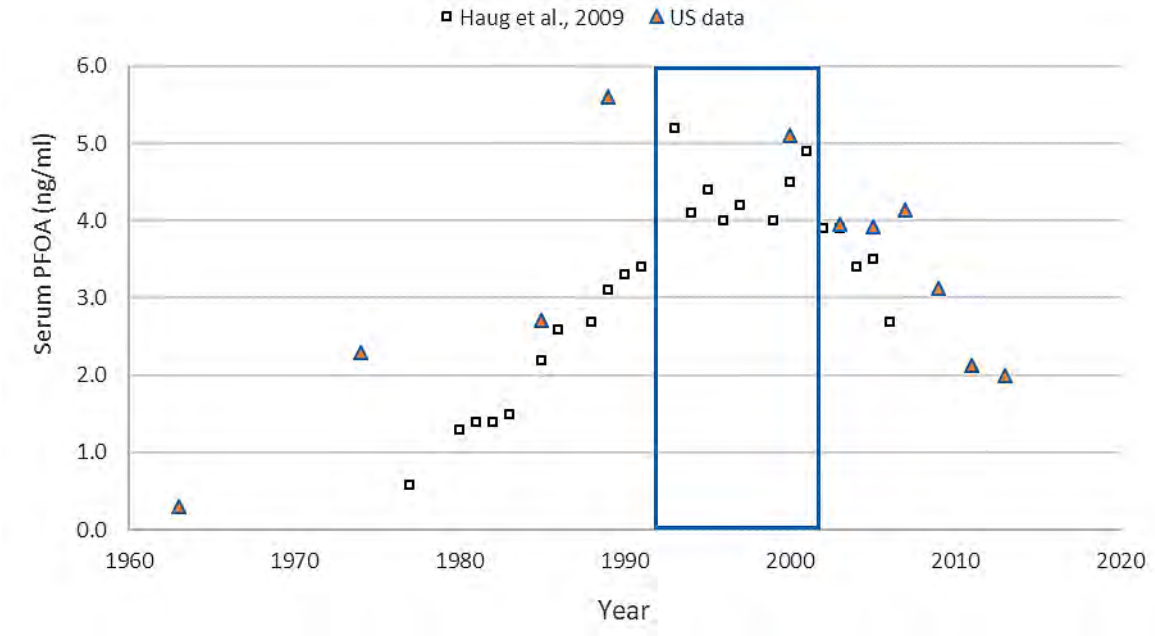
One potential problem with this method of exposure assessment is that little to no information is available on the degree to which inhaled PFOA is absorbed in humans or the inter-individual factors that might affect this absorption. Another potential problem is that the PFOA exposure estimates in both the non-production workers at the Cottage Grove facility and in all of the St. Paul facility workers were not based on actual PFOA measurements. In addition, no validation data were presented showing how well the estimates of airborne PFOA concentrations correlated with actual serum PFOA measurements. Data are presented showing that a sub-sample of Cottage Grove APFO production workers had high levels of serum PFOA (e.g., 282 to 2,538 ng/ml) but these involved a relatively small number of samples (N=148), and they were not used as part of the exposure model or for formal model validation. In contrast, the exposure assessment in the occupational study by Steenland and Woskie (2012), which did find an association between PFOA and kidney cancer, was based on modeled serum (not air) PFOA concentrations, and 2,125 serum measurements in over 1,308 workers were used to develop this model. Overall, the accuracy with which the exposure estimates in the Raleigh et al. (2014) study represent true internal exposure is unknown. This is especially important given the relatively small numbers of kidney cancer cases in this study and the possibility that misclassification of the exposure in only a few workers could have a large impact on study results.

Overview of exposure assessment: peak exposure

As described in this section, the exposure data used in both the Vieira et al. (2013) and Shearer et al. (2021) studies appear to be good indicators of the peak lifetime PFOA exposure for most of the participants in these two studies. As mentioned above, the PFOA serum levels measured in Shearer et al. (2021) were very similar to those in NHANES, suggesting that the exposure patterns in the participants of this study were about the same as those in the US as a whole. US nationwide survey data of PFOA serum levels are not available prior to 1999-2000. However, based on PFOA production levels, data from smaller studies, and subsequent NHANES data, peak serum PFOA levels in the US appeared to have occurred at the about same time that the serum samples were collected in Shearer et al. (2021). That is, PFOA serum levels in the US appeared to have gradually increased from the 1950's until peaking in the early 1990's (Olsen et al., 2005; Calafat et al., 2007a; D'Eon and Mabury, 2011a; Kato et al., 2011; Olsen et al., 2012; Dong et al., 2019). They then remained at these peak levels until about the year 2002, after which they began to steadily decline. Figure 6.2.2 shows serum PFOA concentrations over a 28-year period in 57 pooled serum samples from the Norwegian Institute of Public Health (Haug et al., 2011a) as well as median serum PFOA concentrations

from several studies in the US (Olsen et al., 2005; Calafat et al., 2007a; Kato et al., 2011; Wang et al., 2011; Jain, 2018). These data show that peak PFOA exposure in the US probably occurred from about 1990 to 2002. This period includes the years (1993-2002) in which the serum samples were collected in Shearer et al. (2021). This suggests that the PFOA measurements in the Shearer et al. (2021) study are likely a good representation of the lifetime peak exposure in most of the study's participants. While a few participants may have had higher exposures before or after this period, the large magnitude of this peak, the long half-life of PFOA in serum, and the fact that there are relatively few widespread high exposure sources in the US (e.g., like the C8 area) all suggest that this number is likely to be small.

Figure 6.2.2. PFOA serum levels by year in pooled samples from the Norwegian Institute of Public Health (Haug et al., 2009) and from studies in the US (Olsen et al., 2005; Calafat et al., 2007a; Kato et al., 2011; Wang et al., 2011; Jain, 2018). The blue rectangle area represents the years of serum sample collection in (Shearer et al., 2021)



The exposure data used in Vieira et al. (2013) are also likely a good indicator of the lifetime peak exposure in most of this study's participants. In this study, PFOA exposure was based on modeled estimates occurring 10 years before cancer diagnosis. Since cancers were diagnosed during the years 1996-2005, this exposure period represents the years 1986-1995. Shin et al. (2011) used a retrospective fate and transport model to predict yearly PFOA water concentrations for the six municipal water systems in the C8 area for the years 1951-2010. These models incorporated data on historic emission rates from the local PFOA production facility, physicochemical properties of PFOA, and local geologic and meteorological data. Although actual water measurements were not available for all years, predicted levels correlated well with measured levels for the years these data were available. Overall, these models show that the peak exposure period in the C8 area occurred during the years 1985-2002, a period that includes 1986-1995 exposure period used in Vieira et al. (2013). As such, as with the Shearer et al. (2021) study, the exposure data used Vieira et al. (2013) are probably also a good indicator of the lifetime peak PFOA exposure in the large majority of this study's participants.

Summary of study selection

In conclusion, four human studies (Steenland and Woskie, 2012; Barry et al., 2013; Vieira et al., 2013; Shearer et al., 2021) with adequate data to evaluate an association between PFOA and kidney cancer all reported strong evidence supporting a true causal association between PFOA and this cancer type. Evaluations of chance, bias, confounding, dose-response, consistency, and biologic plausibility all support these findings. There are a number of potential reasons, as detailed above, why a fifth study, the Raleigh et al. (2014) study, could have missed a true effect. Overall, based on these analyses, OEHHA concludes that the positive associations identified in most of the studies of PFOA and kidney cancer are real, and that PFOA is a cause of kidney cancer in humans.

Of the four human studies identifying associations between PFOA and kidney cancer, the Vieira et al. (2013) and Shearer et al. (2021) studies were selected for dose-response assessment and cancer slope factor (CSF) calculations. The study by Steenland and Woskie (2012) was not selected because of its relatively small sample size, and because it involved kidney cancer mortality and very high occupational exposures, exposures that are much higher than those seen in the general population. In contrast, information on kidney cancer incidence and on exposures closer to those seen in the general population were available in the two selected studies. The study by Barry et al. (2013) was not selected because it only presented kidney cancer ORs for categories of cumulative exposure. As discussed above, there was no evidence that cumulative exposure provides a better metric for assessing PFOA-related cancer risks than the metrics of peak exposure used in the Shearer et al. (2021) and Vieira et al. (2013) studies.

Generalizability

Several of the studies identifying links between PFOA and kidney cancer discussed were done in populations that were mostly, although not exclusively, Caucasian. As such, the findings of these studies may not be generalizable to every person in the US. Importantly though, this does not invalidate the elevated risks that were identified in these studies. Currently it is unknown whether PFOA or PFOS cancer risks might vary by race, ethnicity, or any other related factor. There is currently not sufficient data to make conclusive statements about whether the risks in certain groups of people are higher than those currently reported. And if they are higher, there is currently no data available to accurately quantify this increase.

Cancer Slope Factor (CSF) Calculations

Methods

This section describes the method used by OEHHA to calculate CSFs based on data from the Shearer et al. (2021) and Vieira et al. (2013) studies. This method is similar to that used by OEHHA for its PHG for arsenic and to that used by the US EPA for its CSF calculations for TCE (OEHHA, 2004; US EPA, 2011b). Both of these involved data from human case-control studies. The underlying model involves a linear regression between PFOA exposure and cancer relative risk. This model is described in detail below. Other methods considered for calculating CSFs and the cancer health protective concentration are reviewed in Appendix 12.

Calculating the dose-response slope: The dose-response slope between PFOA and cancer for each selected study was calculated using a linear regression analysis, which took the following basic form:

$$RR = bx + 1 \quad (\textit{Equation 1}).$$

Here, RR is the relative risk between PFOA and kidney cancer (or RCC) in each non-reference category. The variable b is the slope between the excess RR (that is, RR − 1) and PFOA dose (x). This slope, b, can be estimated using the following equation (Rothman, 1986; US EPA, 2011b):

$$b = \frac{\Sigma\, w_j x_j RR_j - \Sigma\, w_j x_j}{\Sigma\, w_j x_j^2} \quad (\textit{Equation 2})$$

where, x_j is the PFOA exposure level in each non-reference category and w_j is the weight applied to each RR. These weights are the inverse of the variance of each RR_j. Since the incidence of kidney cancer is relatively low and because the cases and controls were matched on age in both studies, the ORs presented in both studies can be used as a good approximation of the underlying RR_js.

The advantages of Equation 2 over a simple linear regression are that each OR is weighted by its precision (i.e., the inverse of its variance), and the precisions of these ORs can be used to calculate a 95% CI around b.

The variances of the ORs were estimated from their CIs using the following equation (US EPA, 2011b):

$$Var(OR_j) = OR_j^2 \times \left(\frac{\ln(CI_{Uj}) - \ln(CI_{Lj})}{2 \times 1.96} \right)^2 \quad (\textit{Equation 3})$$

where CI_{Uj} and CI_{Lj} are the upper and lower 95% CIs, respectively, of each non-reference category OR.

In Vieira et al. (2013), individual estimates of exposure were only available for the Ohio participants. Because of this, only the dose-response data from the Ohio participants were used in the cancer slope factor calculations for this study. Because both the Shearer et al. (2021) and Vieira et al. (2013) studies presented serum PFOA levels in each exposure category as a range, OEHHA assigned the midpoint of each range to its respective exposure category. However, in Equation 2, the dose in the lowest exposure category is assumed to be zero and is therefore ignored. That is, the intercept is set at a dose of zero and an RR of 1.0. To do this, the midpoint of the lowest exposure category was subtracted from the midpoint of each exposure category for each study. These adjusted dose levels were then used as x_j in the equations above. Because the model is linear and the same value is being subtracted from each dose level, this adjustment will have no effect on b.

The standard error (SE_b) and lower and upper 95% CIs (CI_{Lb}, and CI_{Ub}, respectively) of b were estimated using the following equations:

$$SE_b = \sqrt{}\,(1 \div \Sigma w_j x_j^2) \quad (\textit{Equation 4})$$

$$95\%\ CI_b = b \pm (1.96 \times SE_b) \quad (\textit{Equation 5}).$$

Calculating the CSF: For each study, the CSF was first calculated as the excess cancer risk associated with each ng/ml increase in serum PFOA (CSF_{serum}). This was then combined with

the clearance rate (CL) discussed in Chapter 4 to calculate a CSF_{intake}, which is the excess cancer risk associated with each ng/kg-day intake in PFOA.

The CSF_{serum} was calculated by first converting the linear regression model discussed above from the RR scale to the absolute risk scale. This was done by starting with the following two equations:

$RR = bx + 1$ (*Equation 1)*

$RR = (R_E + R_O) \div R_O$ (*Equation 5)*

where R_E is the excess risk associated with a PFOA serum concentration of x, and R_O is the baseline risk, that is, the risk of RCC or kidney cancer in an unexposed or lower exposure reference group.

Combining Equations 1 and 5 gives:

$(R_E + R_O) \div R_O = bx + 1$ (*Equation 6).*

Solving Equation 6 for excess risk per PFOA serum concentration (i.e., solving for $R_E \div x$) gives the equation for CSF_{serum}:

$CSF_{serum} = R_E \div x = bR_O$ (*Equation 7).*

Incorporating information on PFOA clearance (CL) gives the equation for CSF_{intake}:

$CSF_{intake} = CSF_{serum} \div CL$ (*Equation 8)*

where CL is in units of ml/kg-day.

As seen in Equation 7, the decision of what value to use as R_O will have an impact on the CSFs. Commonly, the risk in a study's unexposed or lower exposure reference group is used as the R_O. However, because the studies selected by OEHHA are both case-control studies, direct estimates of absolute risk are not available. Because of this, cancer risks in the general US population were used as the R_Os. For Vieira et al. (2013), the lifetime risk of kidney cancer in US males of 0.0202 was used as the R_O (ACS, 2020a). The lifetime risk in males was used since this is about 2-times higher than that in females (0.0202 vs. 0.0101, respectively) (ACS, 2020a). For Shearer et al. (2021), R_O was the lifetime risk of RCC in US males, which was estimated by multiplying the lifetime risk of kidney cancer in US males by the percentage of all kidney cancers that are the RCC subtype (90%). This gives an R_O of $0.0202 \times 90\% = 0.0182$. These values are likely to overestimate the risk in a truly unexposed population since everyone in the US has had some PFOA exposure (Calafat et al., 2007a) and therefore may have some excess risk from these exposures. However, US lifetime cancer risks have been used in risk assessments of other chemicals that, like PFOA, also have widespread exposure (e.g., arsenic, benzene). In addition, sensitivity analyses show that using lower values for R_O will have only a small effect on the CSFs and therefore only a small effect on the health-protective concentration calculated from these CSFs (Appendix 12).

Integrating the two study CSFs: Because both the Vieira et al. (2013) and Shearer et al. (2021) studies were determined to be of good quality, without evidence for major bias or confounding, an overall CSF_{intake} was calculated by taking the geometric mean of the CSF_{intake}s from each

study. Although the dose-response slopes calculated from these two studies differed, this difference was not statistically significant (discussed in further detail below).

In some cancer risk assessments, the upper 95% CI of the dose-response slopes are used to help account for possible inter-study variance. Here however, the central estimates of the slopes (i.e., the slopes themselves) were used. This is because the results of two separate studies, including one involving ten separate study sites, were combined to develop the final overall CSF. This combination of different studies and different study sites should help account for much of the variance likely to occur across different PFOA-kidney cancer studies.

Results

A simple regression analysis shows that the linear model represented by Equation 1 provides a good fit to the Shearer et al. (2021) data (R^2 = 0.95) (Figure 6.2.3). For Vieira et al. (2013), model fit improved dramatically when the highest dose level was excluded (Figure 6.2.4). Based on this improved fit, and because the PFOA levels in the highest dose category in Vieira et al. (2013) are well above those seen in the very large majority of the US population, the highest dose group in Vieira et al. (2013) was excluded in the subsequent dose-response and CSF calculations.

Figure 6.2.3. Simple linear regression analysis of serum PFOA and RCC ORs from Shearer et al. (2021)

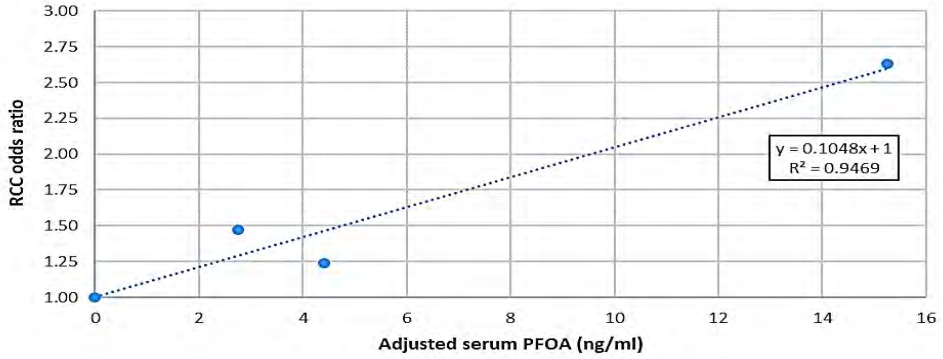
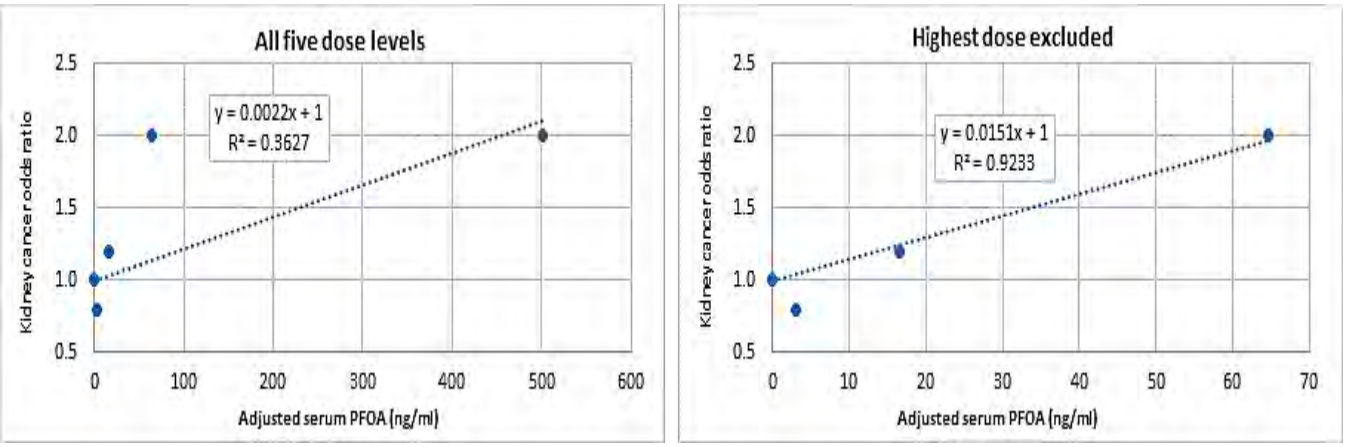


Figure 6.2.4. Simple linear regression analysis of serum PFOA and kidney cancer ORs from Vieira et al. (2013) with and without the highest exposure (dose) category



The key data used in the CSF calculations for both the Vieira et al. (2013) and Shearer et al. (2021) studies are shown in Table 6.2.6, and the results for equations 2, 4, 5, 7, and 8 are shown in Table 6.2.7. The geometric mean of the CSF_{intake} values from the Vieira et al. (2013) and Shearer et al. (2021) studies is **0.0026 (ng/kg-day)$^{-1}$** of PFOA intake.

Table 6.2.6. Data used in the CSF calculations for the Shearer et al. (2021) and Vieira et al. (2013) studies

Study	x_j	OR_j	CI_{Lj}	CI_{Uj}	var(OR_j)	w_j
Shearer et al. (2021)	0.00	1.00	Ref			
	2.75	1.47	0.77	2.80	0.234	4.267
	4.41	1.24	0.64	2.41	0.176	5.685
	15.26	2.63	1.33	5.20	0.837	1.195
Vieira et al. (2013)	0.00	1.0	Ref			
	3.05	0.8	0.4	1.5	0.073	13.743
	16.60	1.2	0.7	2.0	0.103	9.682
	64.70	2.0	1.3	3.2	0.211	4.734

Abbreviations: CI_{Lj}, lower 95% confidence interval of OR_j; CI_{Uj}, upper 95% confidence interval of OR_j; OR_j, odds ratio; Ref, reference group; var(OR_j), variance of OR_j; w_j, the weight applied to each OR_j; x_j, adjusted PFOA serum level in ng/ml

Table 6.2.7. Data and results for Equations 2, 4, 5, 7, and 8

	Shearer et al. (2021)	Vieira et al. (2013)
$\Sigma(w_j x_j OR_j)$	96.32	839.04
$\Sigma(w_j x_j)$	55.06	508.96
$\Sigma(w_j x_j^2)$	421.21	22614.68
b	0.0980	0.0146
SE_b	0.0487	0.0066
CI_{Lb}	0.0025	0.0016
CI_{Ub}	0.1935	0.0276
R_O	0.0182	0.0202
CSF_{serum}	0.00178	0.00029
CL	0.28	0.28
CSF_{intake}	0.00637	0.00105
CSF_{intake} geometric mean = 0.0026		

Abbreviations: b, regression slope from Equation 2; CL, PFOA clearance in mg/kg-day; CL_{Lb}, lower 95% confidence interval of b; CL_{Ub}, upper 95% confidence interval of b; CSF_{intake}, excess cancer risk per ng/kg-day of PFOA intake; CSF_{serum}, excess cancer risk per ng/ml of serum PFOA; OR_j, odds ratio; w_j, the weight applied to each OR_j; x_j, adjusted PFOA serum level in ng/ml

6.2.2. Perfluorooctane Sulfonic Acid

There is sufficient evidence to consider and critically evaluate the liver and pancreatic tumors in male and female rats exposed to PFOS for CSF derivation. First, the two chronic bioassays reported in Butenhoff et al. (2012b) are of sufficient quality (appropriate length, suitable number of animals per dose, adequate reporting, etc.) to warrant consideration as critical studies.

Second, recent studies of PFOA by NTP (2020) provide additional support for considering carcinogenicity as a critical endpoint for PFOS. In its cancer bioassays, NTP (2020) showed that chronic exposure to PFOA led to a significant increase in hepatocellular adenomas and/or carcinomas in male rats (data presented in Table 5.7.3), which is similar to the carcinogenic effects of PFOS reported by Butenhoff et al. (2012b). The similarity in molecular structure between PFOS and PFOA suggests that these chemicals may have comparable biological activities and, in fact, their noncancer toxicity profiles are similar, with the liver, thyroid, and immune and developmental/reproductive systems being major targets of both chemicals. Moreover, International Agency for Research on Cancer (IARC, 2017a) designated PFOA possibly carcinogenic to humans (Group 2B). PFOS also produced a positive trend for pancreatic carcinomas in male rats (Butenhoff et al., 2012b), which is a critical tumor type in PFOA-exposed male rats in the NTP (2020) bioassay (Table 5.7.3. It should be noted that the highest administered dose in the (Butenhoff et al., 2012b) PFOS bioassay (0.984 mg/kg-day) was essentially the same as the lowest administered dose in the NTP (2020) PFOA bioassay (1.0 mg/kg-day). This suggests that the Butenhoff et al. (2012b) studies are less sensitive than the NTP (2020) studies, and that the modest, but significant, tumor incidences observed (when compared against the NTP (2020) PFOA data) are the result of overall lower administered doses.

Third, there is evidence to indicate that PFOS is genotoxic and has multiple key characteristics of carcinogens (see Appendix 8 for greater detail). PFOS likely acts through multiple MOAs, and presently, the evidence does not support excluding genotoxicity as a possible MOA for carcinogenicity. As such, because the carcinogenicity MOA is uncertain, PFOS is evaluated using linear extrapolation.

Hepatocellular tumors in male and female Sprague Dawley rats and pancreatic islet cell tumors in male rats from Butenhoff et al. (2012b) were evaluated for CSF derivation. BMD modeling was conducted using serum concentrations as the dose metric, and results are presented in Appendix 10 and summarized in Table 6.2.8.

Table 6.2.8. BMD modeling results for tumors in male and female Sprague Dawley rats following exposure to PFOS in the diet for two years (Butenhoff et al., 2012b)

	$BMDL_{05(animal)}$ (mg/L)	$BMDL_{05(human)}$ (mg/kg-day)[a]	CSF_{human} $(mg/kg\text{-}day)^{-1}$	Model p-value	Polynomial Degree
Males – liver	24.8	0.0054	9.3	0.1999	1
Males - pancreatic	24.4	0.0053	9.4	0.6540	1
Males – liver and pancreatic combined	14.7	0.0032	**15.6**	-	-
Females - liver	33.5	0.0069	7.2	0.5862	1

[a] Rounded numbers are presented in the table, but the cancer slope factor is derived from unrounded values.

The combined incidence of liver and pancreatic tumors in male rats produced the lowest $BMDL_{05}$ value of 14.7 mg/kg-day. To convert the $BMDL_{05}$ of 14.7 mg/L to an HED, the plasma concentration is first multiplied by a human PFOA clearance factor of 3.9×10^{-4} L/kg-day. The resulting value is 0.0057 mg/kg-day. To estimate an HED from animal data that would result in an equal lifetime risk of cancer, OEHHA uses body weight (BW) scaling to the ¾ power (OEHHA, 2009). This adjustment accounts for interspecies differences in toxicokinetics and toxicodynamics. Because TK differences have already been accounted for by using plasma concentration as the dose metric instead of administered dose, the $BMDL_{05}$ only needs modification for toxicodynamic differences ($BW^{1/8}$ adjustment). The equation is provided below.

$$BMDL_{05(human)} = BMDL_{05(animal)} \times (BW_{animal}/BW_{human})^{1/8}$$

Applying the $BW^{1/8}$ adjustment, where the time-weighted average male rat body weights is 0.687 kg (female rat body weight is 0.412 kg) (from Thomford (2002)), and the adult human body weight is the default of 70 kg, the human $BMDL_{05}$ is 0.0032 mg/kg-day for the combined tumor incidence in males. This BMDL results in a human CSF of **15.6 (mg/kg-day)$^{-1}$**.

7. HEALTH-PROTECTIVE DRINKING WATER CONCENTRATIONS

7.1. Noncancer Health-Protective Drinking Water Concentrations

7.1.1. Perfluorooctanoic Acid

To calculate a health-protective concentration for a chemical, the ADD is converted to a concentration in drinking water that accounts for the total exposure to the chemical that people receive from using tap water. The relative source contribution (RSC) is the proportion of exposures to a chemical attributed to tap water (including inhalation and dermal exposures, e.g., during showering), as part of total exposure from all sources (including food and air pollution). The RSC values typically range from 20 to 80 percent (expressed as 0.20 to 0.80), and are determined based on available exposure data. The default RSC of 0.2 is selected because there is not enough data to determine specific exposure patterns for PFOA (see Appendix 4 for more details). In addition to drinking water, there are several other sources of PFOA that may contribute to exposure in the general population, including air, soil, food, and consumer and industrial products. PFOA released to air may adsorb to airborne particles and travel long distances (US EPA, 2016b). Additionally, the use of PFOA in many consumer products and its environmental persistence has led to the presence of PFOA in indoor air and dust. In fact, (US EPA, 2016b) reports that the most common exposure routes of PFOA are diet and indoor dust. Thus, an RSC of 0.2 is appropriate, and consistent with RSCs used by other agencies, including US EPA and the State of New Jersey.

Oral ingestion is the primary route of exposure for PFOA in drinking water. PFOA is not very volatile in its ionized form (its predominant form in water) (Johansson et al., 2017), so inhalation of PFOA directly from drinking water is not anticipated to be a major route of exposure. Dermal absorption is also not anticipated to be a significant route of exposure from typical household uses of tap water. Ionized PFOA penetrates skin poorly compared to the neutral form, and PFOA should remain ionized in the stratum corneum due to its buffering capacity (Franko et al., 2012).

PFOA can permeate mouse and human skin in vitro, and its absorption following dermal application in mice in vivo was demonstrated by Franko et al. (2012). However, a time-course of >5 hours is needed for PFOA to penetrate full-thickness human skin, and this exposure scenario is unlikely to occur from typical household uses of tap water.

Because oral ingestion is considered to be the only significant route of drinking water exposure, a lifetime average drinking water intake rate of 0.053 L/kg-day (OEHHA, 2012) is used to determine the noncancer health-protective concentration, which is calculated using the following formula:

$$C = ADD \times RSC \div DWI,$$

where:

ADD = acceptable daily dose, in ng/kg-day,

RSC = relative source contribution of 0.2, and

DWI = daily water intake rate of 0.053 L/kg-day.

Thus, based on the increased risk of elevated ALT in humans reported by Gallo et al. (2012), a health-protective concentration is calculated as follows:

C = (0.87 ng/kg-day × 0.2) ÷ 0.053 L/kg-day = 3.28 ng/L or **3 ppt** (rounded).

7.1.2. Perfluorooctane Sulfonic Acid

Calculating the health-protective concentration for PFOS uses the same procedure as for PFOA. The default RSC of 0.2 is selected because there is not enough data to determine specific exposure patterns to PFOS. In addition to drinking water, there are several other sources of PFOS that may contribute to exposure in the general population, including air, soil, food, and consumer and industrial products. PFOS released to air may adsorb to airborne particles and travel long distances (US EPA, 2016d). Additionally, the use of PFOS in many consumer products and its environmental persistence has led to the presence of PFOS in indoor air and dust. As with PFOA, US EPA (2016d) reports that the most common exposure routes of PFOS are diet and indoor dust. Thus, an RSC of 0.2 is appropriate (see Appendix 4 for details), and consistent with RSCs used by other agencies, including US EPA and the State of New Jersey.

Oral ingestion is the primary route of exposure for PFOS in drinking water. Volatilization of the predominant anionic form in water (pKa <1.0) is not expected to occur (HSDB, 2020b).

Dermal absorption is also not anticipated to be a significant route of exposure from typical household uses of tap water, based on its physicochemical similarities to PFOA. However, no specific studies could be identified that addressed absorption of PFOS following dermal exposure. ATSDR (2021) reports the results of an unpublished single-dose dermal absorption study in rabbits, where potassium PFOS or its diethanolamine salt (at doses up to 20 µg/kg) was applied to clipped, intact skin (Johnson et al., 1995a,b, as reported by ATSDR (2021)). Compared to controls, no increase in organic fluoride in the liver was detected, suggesting that PFOS was not absorbed.

Because oral ingestion is considered to be the only significant route of drinking water exposure, a lifetime average drinking rate of 0.053 L/kg-day (Appendix 3 (OEHHA, 2012)) is used to determine the noncancer health-protective concentration. Thus, based on the increased total cholesterol in humans reported by Steenland et al. (2009), a health-protective concentration is calculated as follows:

C = (0.64 ng/kg-day × 0.2) ÷ 0.053 L/kg-day = 2.42 ng/L or **2 ppt** (rounded).

7.2. Cancer Health-Protective Drinking Water Concentrations

7.2.1. Perfluorooctanoic Acid

Because exposure to PFOA is mainly through oral ingestion, the calculation of a cancer-based health-protective concentration is based only on exposure through ingestion of drinking water. Age sensitivity factors were not applied because the NTP (2020) animal bioassay showed no increased risks in combined adenomas and carcinomas from perinatal exposure compared to exposures later in life. The cancer-based health-protective concentration for PFOA can be calculated as:

$C = R \div (CSF_{intake} \times DWI)$,

where:

R = default excess cancer risk level of one in one million, or 10^{-6}

CSF_{intake} = cancer potency in units of excess risk per ng/kg-day intake of PFOA

DWI = time weighted drinking water intake, lifetime average.

For PFOA, the cancer-based health-protective concentration is:

$C = 10^{-6} \div (0.0026\ (ng/kg\text{-}day)^{-1} \times 0.053\ L/kg\text{-}day) = 0.00729$ ng/L, equivalent to **0.007 ppt** (rounded).

The cancer health-protective concentration of 0.007 ppt is selected as the PHG and should protect against the noncancer effects of PFOA since it is lower than the 3 ppt level for noncancer effects.

7.2.2. Perfluorooctane Sulfonic Acid

As described in the noncancer health-protective concentration derivation, oral ingestion is the primary route of exposure to PFOS in drinking water, and inhalation and dermal exposures are considered negligible.

When determining cancer risk, OEHHA typically applies ASFs to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). However, ASFs were not included when deriving the cancer health-protective concentration for PFOA because the NTP (2020) study provided evidence that early-life exposure did not increase tumor incidences later in life. Because it is anticipated that PFOS behaves in a similar manner as PFOA, OEHHA is excluding ASFs in the health-protective concentration derivation for cancer.

Since oral ingestion is considered to be the only significant route of drinking water exposure to PFOS, a lifetime average drinking rate of 0.053 L/kg-day (OEHHA, 2012) is used, along with the CSF of 15.6 $(mg/kg\text{-}day)^{-1}$ determined in Chapter 6, to calculate the health-protective concentration, C, for carcinogenic effects of PFOS as follows:

$C = 10^{-6} \div (15.6\ (mg/kg\text{-}day)^{-1} \times 0.053\ L/kg\text{-}day) = 1.2 \times 10^{-6}$ mg/L

C = 1 ng/L or **1 ppt** (rounded).

The cancer health-protective concentration of 1 ppt is selected as the PHG and should protect against the noncancer effects of PFOS since it is lower than the 2 ppt level for noncancer effects.

8. RISK CHARACTERIZATION

PFOA and PFOS are ubiquitous, and biomonitoring data from California have shown that these compounds are found in the serum samples of nearly all participants examined. Because exposure to these chemicals is so prevalent and elimination times are so long, it is critical to understand the toxicity associated with these compounds, and their impacts on human health.

In the development of the currently proposed PHGs and noncancer health-protective concentrations, OEHHA employed a thorough and methodical approach. OEHHA conducted a comprehensive analysis of the human epidemiology literature, and updated the animal toxicity evaluation to include recent studies and endpoints not captured in the 2019 NL recommendations for these chemicals.

In humans, exposure to environmental levels of PFOA is associated with numerous adverse health effects, including decreased vaccine response, increased liver enzyme levels (indicative of hepatotoxicity), increased cholesterol levels, and kidney cancer. Similarly, PFOS is associated with decreased vaccine response and increased cholesterol levels. The animal toxicity data for PFOA and PFOS support the human epidemiologic studies, with multiple studies reporting immunotoxicity, liver toxicity, and cancer in rodents. Furthermore, animal studies reported several additional toxicity endpoints, including thyroid toxicity, reproductive/developmental toxicity, and, for PFOS, neurotoxicity.

In most cases, humans were more sensitive than animals to PFOA and PFOS, which eliminated the uncertainty in extrapolating animal data to humans. Thus, human data were used to derive the proposed PHG for PFOA and the noncancer health-protective concentrations for PFOA and PFOS. The proposed PHG of 0.007 ppt for PFOA is based on increased risk of kidney cancer in humans (Vieira et al., 2013; Shearer et al., 2021). This level should protect against noncancer toxicity as well, as it is lower than the health-protective concentration of 3 ppt, based on elevated ALT levels in humans. Similarly, the PHG of 1 ppt for PFOS is based on increased liver and pancreatic tumor incidence in a two-year rat study (Butenhoff et al., 2012b). The PHG should be protective for all noncancer toxicity endpoints, as it is lower than the health-protective concentration of 2 ppt, based on elevated total cholesterol in humans.

In this assessment, OEHHA critically evaluated the parameters used to derive the PHGs. These include toxicokinetic parameters, and exposure parameters, such as drinking water intake rates and the relative source contribution. These issues are summarized below:

- **Toxicokinetics.** Due to the long half-lives of PFOA and PFOS in humans, serum concentration is the preferred dose metric for dose-response analysis. TK approaches were developed to convert serum levels of PFOA or PFOS in humans to an external oral dose, and to convert serum concentrations in animals to human equivalent doses, on which the PHGs and health-protective concentrations can be based. OEHHA evaluated several different approaches to address this issue, including multiple PK models, the use of reported serum/plasma concentration as the primary dose metric, and the development of chemical-specific clearance factors. OEHHA's evaluations of PK models indicate that models may produce inaccurate results outside of the optimized range, which may increase uncertainty rather than reduce it. OEHHA determined that using reported serum/plasma concentrations is the least uncertain method and is generally precise when compared against modeled concentrations. To estimate external dose from serum concentration, OEHHA derived human clearance factors for PFOA and

PFOS, based on human exposure studies. OEHHA's clearance factors are larger than the clearance factors derived by US EPA (US EPA, 2016b; US EPA, 2016d). Finally, selecting epidemiologic studies as critical studies for derivation of the noncancer health-protective concentrations bypasses the need for interspecies conversions and additional TK adjustments

- **Relative source contribution.** OEHHA thoroughly evaluated the relative source contribution, following US EPA's Exposure Decision Tree Approach, and determined that there is not enough information to estimate relevant sources of exposure quantitatively, particularly for California residents. Therefore, OEHHA utilized the default RSC of 20% for PFOA and PFOS.
- **Drinking water intake rate.** To derive the PHGs and noncancer health-protective concentrations for PFOA and PFOS, OEHHA used a time-weighted lifetime average drinking water intake rate of 0.053 L/kg-day (OEHHA, 2012). OEHHA evaluated US EPA's recently updated water consumption rates published in the Exposure Factors Handbook (US EPA, 2019). While US EPA's updated water intake rates are based on newer data (NHANES, 2005-2010) than those used by OEHHA (CSFII, 1994-1996, 1998), they do not capture the continued trend of increased water consumption both nationwide and in California. OEHHA's preliminary analysis of newer NHANES data (2015-2016) supports the use of the drinking water intake rates developed previously (OEHHA, 2012). Furthermore, California water consumption rates may differ from national rates due to climate and lifestyle factors. Additionally, OEHHA's drinking water rates are more protective for infants, who may be at greater risk of adverse health effects than the general population due to their greater exposure to drinking water contaminants on a body weight basis.

Mechanistic evidence, some key uncertainties, and other issues also considered in the development of the proposed PHGs and health-protective concentrations for PFOA and PFOS are summarized below:

- **Genotoxicity.** There is some positive evidence of genotoxicity for PFOA and PFOS. For PFOA, the evidence of mutagenicity is limited, but chromosomal effects and DNA damage have been observed both in vivo and in vitro. For PFOS, there is some evidence of mutagenicity, and positive evidence of chromosomal effects and DNA damage. Therefore, genotoxicity cannot be dismissed as a possible mode of action for PFOA and PFOS.
- **Human relevance of PPARα.** PFOA is a known activator of PPARα and there has been considerable discussion in the scientific literature that carcinogenesis in rodents mediated by activation of PPARα is not relevant to humans, as PPARα activation in humans is not known to induce tumors. However, there is evidence in PPARα knockout animals that PFOA induces PPARα-independent toxicity, including carcinogenesis. Additionally, toxicity is observed at doses that do not activate PPARα. This indicates that PFOA acts via multiple mechanisms.
- **Interspecies extrapolation.** To estimate the cancer risk for PFOS based on rodent data, OEHHA would generally use the interspecies scaling factor of body weight to the ¾ power to account for TK and toxicodynamic differences between rodents and humans that might cause differences in tumorigenic response. However, because serum levels of PFOS were used in the cancer dose-response analysis and the chemical is not metabolically active in humans or rodents, the differences in interspecies TK are inherently included. Therefore, only body weight scaling adjustment for toxicodynamics

was applied. The difference between scaled and unscaled CSFs was approximately two-fold for rat-based values.

The California State Water Resources Control Board reported levels of PFOA and PFOS in drinking water from April 2019 to June 2020, with mean values (including non-detects) ranging from 4.79-5.71 ppt for PFOA and 9.7-11.5 ppt for PFOS. These levels are higher than the draft PHGs and HPCs calculated in this draft document from human studies.

Other Regulatory Standards and Advisory Levels

Below is a list of drinking water regulatory standards and advisory levels from US EPA and other states.

Table 8.1. Summary of state and federal drinking water regulations and advisory levels for PFOA and PFOS

State/Organization	Chemical	Regulatory/Advisory Value
US EPA	PFOA (individual and combined sum with PFOS)	HA – 70 ppt
	PFOS (Individual and combined sum with PFOA)	HA – 70 ppt
Connecticut	Sum of PFOA and PFOS (and PFHxS, PFNA, and PFHpA)	AL – 70 ppt
Massachusetts	Sum of PFOA and PFOS (and and PFHxS, PFNA, PFDA and PFHpA)	MCL – 20 ppt
Michigan	PFOA	MCL – 8 ppt
	PFOS	MCL – 16 ppt
Minnesota	PFOA	HRL – 35 ppt
	PFOS	HBV – 15 ppt
New Hampshire	PFOA	MCL – 12 ppt
	PFOS	MCL – 15 ppt
New Jersey	PFOA	MCL – 14 ppt
	PFOS	MCL – 13 ppt
New York	PFOA	MCL – 10 ppt
	PFOS	MCL – 10 ppt
Vermont	Sum of PFOA and PFOS (and PFHxS, PFNA, and PFHpA)	MCL – 20 ppt
Washington	PFOA	Draft AL – 10 ppt
	PFOS	Draft AL – 15 ppt

AL, action level; HA, health advisory; HBV, health based value; HRL, health risk limit; MCL, maximum contaminant level

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APPENDIX 1. LITERATURE SEARCH STRATEGY

Literature search and screening methods

OEHHA's librarian conducted a search of the literature on the toxicity of PFOA and PFOS. The goal was to identify peer-reviewed open-source and proprietary journal articles, print and digital books, reports, and gray literature that potentially reported relevant information on the toxicity of these chemicals. Searches were limited to literature published from January 2016 to the time the searches were executed in September 2019. One animal toxicity study, Blake et al. (2020), was identified after OEHHA's initial literature review (published or identified between January and September 2020). Detailed descriptions of the literature search strategy for epidemiologic studies, based on outcome of interest, can be found in Appendix 7.

Search process

PubMed MeSH browser was used to identify subject headings, other index terms and synonyms for PFOA and PFOS and the concept of toxicity. Preliminary searches were run and results evaluated to identify additional relevant search terms. The resulting search strategy was executed in PubMed.

Toxicity studies

Search as executed in PubMed (PubMed) on September 4, 2019.

Set #	Strategy	Results	Notes
1	("perfluorooctane sulfonic acid"[nm] OR PFOS [Tiab] OR "perfluoroalkyl sulphonate"[tiab] OR "perfluoro-n-octanesulfonic" [Tiab] OR "perfluoroctane sulfonic" [Tiab] OR "perfluorooctane sulfonic" [Tiab] OR perfluoroctanesulfonic [Tiab] OR perfluorooctanesulfonic [Tiab] OR "perfluorooctane sulphonic" [Tiab] OR "perfluoroctane sulphonic" [Tiab] OR perfluoroctanesulphonic [Tiab] OR perfluorooctanesulphonic [Tiab] OR "perfluorooctane sulfonate" [Tiab] OR "perfluoroctane sulfonate" [Tiab] OR perfluorooctanesulfonate [Tiab] OR perfluoroctanesulfonate [Tiab] OR "perfluorooctane sulphonate" [Tiab] OR "perfluoroctane sulphonate" [Tiab] OR perfluoroctanesulphonate [Tiab] OR perfluorooctanesulphonate [Tiab] OR "perfluorooctanyl sulfonate" [Tiab] OR "perfluorooctanyl sulphonate" [Tiab] OR perfluoroctylsulfonic [Tiab] OR "heptadecafluoro-1-octanesulfonic" [Tiab] OR "heptadecafluoro-1-octane sulfonic" [Tiab] OR "heptadecafluorooctane sulfonic" [Tiab] OR "heptadecafluoroctane sulfonic" [Tiab] OR "heptadecafluorooctane sulfonic" [Tiab] OR heptadecafluorooctanesulfonic [Tiab] OR "heptadecafluorooctane-1-sulphonic" [Tiab] OR "heptadecafluorooctane sulphonic" [Tiab] OR "1-octanesulfonic acid" [Tiab] OR "1-octanesulphonic acid" [Tiab] OR "1-perfluoroctanesulfonic" [Tiab] OR "1-perfluorooctanesulfonic [Tiab]" OR "octanesulfonic acid" [Tiab] OR "octanesulphonic acid" [Tiab] OR 1763-23-1 [rn] OR 2795-39-3 [rn] OR 29081-56-9 [rn] OR 29457-72-5 [rn] OR 4021-47-0 [rn] OR 70225-14-8 [rn] OR 307-35-7 [rn]) OR ("perfluorooctanoic acid"[nm] OR PFOA [Tiab] OR PFAA* [Tiab] OR APFO [Tiab] OR "fluorinated surfactants" [Tiab] OR fluorosurfactant* [Tiab] OR "fluorinated polymer*" [Tiab] OR (fluorinated [Tiab] AND (polymer [Tiab] OR polymers [Tiab])) OR (fluorocarbon [Tiab] AND (polymer [Tiab] OR polymers [Tiab])) OR fluoropolymer* [Tiab] OR (fluorinated [Tiab] AND telomer* [Tiab]) OR fluorotelomer* [Tiab] OR fluoro-telomer* [Tiab] OR fluorotelomer	12,393	PFOA & PFAS concepts

	alcohol*[tiab] OR “telomer alcohol*” [Tiab] OR “polyfluoroalkyl* ” [Tiab] OR “N-ethyl perfluorooctanesulfonamido ethanol” [Tiab] OR “N-ethyl perfluorooctanesulfonamidoethanol” [Tiab] OR “N-EtFOSE” [Tiab] OR perfluoroalkyl* [Tiab] OR perfluorocarbon* [Tiab] OR perfluorocarboxyl* [Tiab] OR perfluorochemical* [Tiab] OR “perfluorinated*” [Tiab] OR (perfluorinated [Tiab] AND (C8 [Tiab] OR carboxylic [Tiab] OR chemical* [Tiab] OR compound* [Tiab] OR octanoic [Tiab])) OR (PFO [Tiab] AND (perfluoroalk* [Tiab] OR perfluorocarb* [Tiab] OR perfluorinat* [Tiab] OR perfluorooc* [Tiab])) OR (C8 [Tiab] AND (perfluoroalk* [Tiab] OR perfluorocarb* [Tiab] OR perfluorinat* [Tiab] OR perfluorooc* [Tiab])) OR perfluoroctanoic [Tiab] OR perfluorooctanoic [Tiab] OR “perfluoro octanoic” [Tiab] OR “perfluoro-n-octanoic” [Tiab] OR “perfluorinated octanoic acid” [Tiab] OR perfluorooctanoate [Tiab] OR perfluoroctanoate [Tiab] OR “perfluoro octanoate” [Tiab] OR perfluoroheptanecarboxylic [Tiab] OR “perfluoro-1-heptanecarboxylic” [Tiab] OR perfluorocaprylic [Tiab] OR perfluorocaprilate [Tiab] OR perfluorocaprylate [Tiab] OR perfluoroacrylate [Tiab] OR “perfluorooctanoyl chloride” [Tiab] OR “pentadecafluoro-1-octanoic” [Tiab] OR “pentadecafluoro-n-octanoic” [Tiab] OR pentadecafluorooctanoate* [Tiab] OR pentadecafluoroctanoate* [Tiab] OR pentadecafluoroctanoic [Tiab] OR pentadecafluorooctanoic [Tiab] OR fluorad [Tiab] OR “FC 143” [Tiab] OR FC143 [Tiab] OR 335-67-1 [rn] OR 3825-26-1 [rn] OR 335-95-5 [rn] OR 2395-00-8 [rn])		
2	#1 AND (tox[sb] OR genotox*[tiab] OR toxicity[tiab])	6,230	Toxicity concept
3	#2 AND ("2016/01/01"[PDat] : "3000/12/31"[PDat])	**2,060**	Limit to 2016-present

The PubMed strategy was then tailored for use in additional databases, listed below, according to the search interface and features unique to each resource. For instance, MeSH terms were replaced with Emtree terms for the Embase search strategy and the PubMed toxicology subset search strategy was translated to Embase syntax.

Data sources and results

The following is a list of the databases searched to find information on PFOA and PFOS, and the number of references retrieved from each.

Table A1.1. Database search results

Source	Results
PubMed (National Library of Medicine) (PubMed)	2,060
Embase (Embase)	1,824
Scopus (Scopus)	1,140
TOXLINE (National Library of Medicine): Toxicology Literature Online, (TOXLINE)	27
TOXNET DART (National Library of Medicine): Toxicology Literature Online, DART Subset (TOXNET DART)	0
SciFinder-n (SciFinder-n)	183

After duplicates were removed, the search yielded a total of 2,766 unique references.

Literature screening

Relavant literature was identified though a multi-step screening process outlined in Figure A1.1. Studies from the database search were imported into a web-based systematic review software, DistillerSR, for title/abstract and full-text screening. Both title/abstract and full-text screening were conducted by two independent reviewers. Studies were screened for inclusion or exlcusion based on the Populations, Exposures, Comparator, and Outcome (PECO) criteria outline in Table A1.2. Studies that met PECO criteria during title and abstract screening were considered for full-text screening. At both the title/abstract and full-text review levels, screening conflicts were resolved by discussion.

Figure A1.1. Literature search: recent studies of PFOA or PFOS and animal toxicity

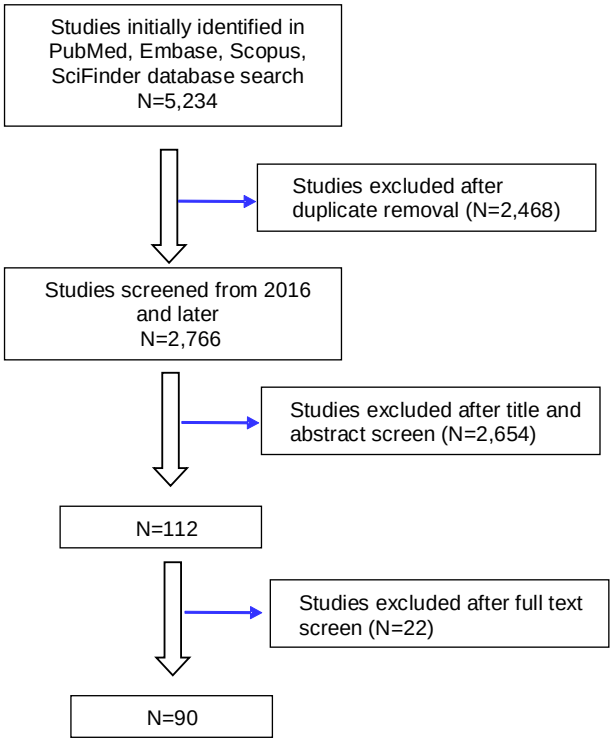

Table A1.2. Populations, exposures, comparators, and outcomes (PECO) criteria

PECO element	Evidence
Populations	**Human:** Studies of any population and lifestage (occupational or general population, including children and other sensitive populations) will be tagged as "potentially relevant supplemental information – human studies". **Exclude**: biomonitoring studies and exposure studies (unless specifically relevant to California). **Animal:** Non-human mammalian animal species of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages). Zebrafish studies will be tagged as "potentially relevant supplemental information". **Mechanistic:** Studies of any human or animal (mammalian and non-mammalian) cell type, and mechanistic/genomic/in silico data with any biological significance will be tagged as "potentially relevant supplemental information".
Exposures	**Relevant forms:** Perfluorooctanoic acid (CAS 335-67-1), and any salt, and any synonyms. If uncertain about chemical identity, please look it up. Perfluorooctane sulfonic acid (CAS 1763-23-1), any salt, and any synonyms. If uncertain about chemical identity, please look it up. **Human:** Any exposure to PFOS or PFOA via any route. **Animal:** Any exposure to PFOA or PFOS via the oral route. Studies involving intraperitoneal or dermal exposures, or exposure to mixtures will be tagged as "potentially relevant supplemental information". **Mechanistic**: Any cell type exposed to PFOS or PFOA alone. Studies involving exposures to mixtures will be tagged as "potentially relevant supplemental information".
Comparators	**Human:** A comparison or referent population exposed to lower levels (or no exposure/exposure below detection limits) or PFOA or PFOS, or exposure to PFOA or PFOS for shorter periods of time. Case reports and case series will be tracked as "potentially relevant supplemental information." **Animal:** A concurrent control group exposed to vehicle-only treatment or untreated control. **Mechanistic:** A concurrent control group of cells exposed to vehicle-only treatment or untreated control.
Outcomes	All health outcomes (both cancer and noncancer) and toxicokinetics. **Exclude**: ecological studies, animal biomonitoring studies, and reviews

During title/abstract or full-text level screening in DistillerSR, studies that did not meet the PECO criteria, but which could provide supporting information were categorized as supplemental material. Studies that were categorized as supplemental material were not necessarily excluded from consideration in the assessment and may be used as supporting evidence. Studies were tagged as potentially relevant supplemental material if they were:

- Human epidemiology studies[13]
- Mechanistic: cancer and noncancer (including in vitro studies)
- Non-mammalian model
- Non-oral routes of administration

A total of 507 studies at the title/abstract screening level and a total of 5 studies at the full text screening level were tagged as supplemental material.

[13] A separate search was conducted specifically for human epidemiology studies as outlined in Appendix 7.

APPENDIX 2. DEFAULT UNCERTAINTY FACTORS

This appendix describes the default uncertainty factors OEHHA generally uses to calculate the Acceptable Daily Dose when deriving PHGs. When scientific evidence is compelling, these defaults are supplanted by alternative factors or modeled results. Table A2.1 below is adapted from OEHHA's "Technical Support Document for the Development of Noncancer Reference Exposure Levels" (OEHHA, 2008).

Table A2.1. Default uncertainty factors for PHG derivation, adapted from OEHHA (2008)

Uncertainty Factor	Value
Interspecies uncertainty factor (UF_A)	
Combined interspecies uncertainty factor (UF_A):	1 human observation √10 animal observation in nonhuman primates 10 where no data are available on toxicokinetic or toxicodynamic differences between humans and a non-primate test species
Toxicokinetic component (UF_{A-k}) of UF_A:	1 where animal and human PBPK models are used to describe interspecies differences √10 nonprimate studies with no chemical or species specific kinetic data
Toxicodynamic component (UF_{A-d}) of UF_A:	1 where animal and human mechanistic data fully describe interspecies differences (*This is unlikely to be the case.)* 2 for residual susceptibility differences where there are some toxicodynamic data √10 nonprimate studies with no data on toxicodynamic interspecies differences
Intraspecies uncertainty factor (UF_H)	
Toxicokinetic component (UF_{H-k}) of UF_H:	1 human study including sensitive subpopulations (e.g., infants and children), or where a PBPK model is used and accounts for measured interindividual variability √10 for residual susceptibility differences where there are some toxicokinetic data (e.g., PBPK models for adults only) 10 to allow for diversity, including infants and children, with no human kinetic data
Toxicodynamic component (UF_{H-d}) of UF_H:	1 human study including sensitive subpopulations (e.g., infants and children) √10 human studies with normal adult subjects only, but no reason to suspect additional susceptibility of children 10 suspect additional susceptibility of children (e.g., exacerbation of asthma, neurotoxicity)

Uncertainty Factor	**Value**
LOAEL uncertainty factor (UF_L)	
Values used:	10 LOAEL, any effect 1 NOAEL or BMDL used
Subchronic uncertainty factor (UF_S)[1]	
Values used:	1 study duration >12% of estimated lifetime √10 study duration 8-12% of estimated lifetime 10 study duration <8% of estimated lifetime
Database deficiency factor (UF_D)	
Values used:	1 no substantial data gaps √10 substantial data gaps including, but not limited to, developmental toxicity

[1] Exposure durations of 13 weeks or less are subchronic regardless of species (OEHHA, 2008)

Reference

OEHHA (2008). Air toxics hot spots risk assessment guidelines: technical support document for the derivation of noncancer reference exposure levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Sacramento, CA.

APPENDIX 3. DRINKING WATER INTAKE RATES

OEHHA's drinking water intake rates were derived from a nationwide survey of food and beverage intake from approximately 20,000 people (US Department of Agriculture's Continuing Survey of Food Intake of Individuals 1994-1996, 1998 dataset), using the 95th percentile values for ages 0<2 (0.196 L/kg-day), 2<16 (0.061 L/kg-day), and 16<70 years (0.045 L/kg-day) as reported in OEHHA (2012). These intake rates were derived from consumers only, using a two-day average for both direct and indirect sources of community water consumption. The lifetime average (0<70 years of age) drinking water intake rate calculated by OEHHA for oral exposure used in the assessment of drinking water contaminants is 0.053 L/kg-day. These rates are discussed in the *Air Toxics Hot Spots Program Risk Assessment Guideline: Technical Support Document for Exposure Assessment and Stochastic Analysis* (OEHHA, 2012).

The US EPA recently updated its chapter on water consumption in the *Exposure Factors Handbook* (EFH) (US EPA, 2019). Their drinking water intake rates, which are lower than those developed by OEHHA in 2012, are based on analyses of the National Health and Nutrition Examination Survey (NHANES) data from 2005 – 2010.

Since then, tap water intake rates have been on the rise. A recent study of trends in tap and bottled water consumption using NHANES 2011 – 2016 data noted, "On the average, about 62% of drinking water came from the tap, a major increase from 56% osbserved in the 2005 – 2010 NHANES database" (Vieux et al., 2020). OEHHA's preliminary analysis of 2015 – 2016 NHANES data (not shown in this document) resulted in estimated drinking water intake rates that are consistent with this increase, and with the intake rates developed previously (OEHHA, 2012).

Furthermore, the two-day average consumer-only 95th percentile intake rate of 0.0439 L/kg-day,[14] derived in the 2019 EFH update for all ages (lifetime), is not different from the value of 0.044 L/kg-day derived for the same parameters in the original EFH (US EPA, 2011). While the weighted average of the 95th percentile lifetime consumption rate (0<70 years old) as calculated from the 2019 EFH intake rates is a bit lower than the value currently used by OEHHA (0.046 L/kg-day[15] compared to 0.053 L/kg-day, for US EPA and OEHHA, respectively), this is partly due to methodological differences in deriving these estimates. The largest difference in water consumption rate was observed in the 0<2 years age range. US EPA's updated water consumption rates for this age group included all infants, while OEHHA's guidance included only infants fed powder formula reconstituted with tap water. The US EPA's inclusion of all infants, particularly those that are exclusively breastfed and drink little or no water, would underestimate drinking water intake rates of infants who are fed reconstituted formula. While OEHHA's approach yielded a higher consumption rate, it is more representative of formula-fed infants. OEHHA is mandated to consider sensitive subgroups, such as children and infants, who may be at greater risk of adverse health effects due to their greater exposure to drinking water contaminants on a body weight basis than the general population.

California-specific consumption rates are likely to be higher than the national average. A variety of other factors, such as geography and resident lifestyles, can contribute to certain subgroups having higher water intake rates. California is diverse in both climate and population and has a

[14] Reported in USEPA (2019) Table 3-21.
[15] The weighted average of the 95th-percentile two-day average values in, and weighted by the number of years in, the 0<2, 2<16, and 16<70 age groups reported in USEPA (2019), Table 3-21.

climate conducive to outdoor activities. California also has a high number of warm, dry days in the warm seasons in much of the state. Extreme heat events in California have increased over the past several years, and are anticipated to continue increasing (OEHHA, 2018). The CDC recommends hydration as a main strategy for combating heat related illnesses,[16] resulting in increased consumption of water during outdoor activities, occupational and recreational, in warm weather.

In 2019, nearly one million Californians were employed in occupations commonly performed outdoors, such as farm work and construction, according to labor statistics released by the US Bureau of Labor Statistics.[17] The California Department of Industrial Relation's Division of Occupational Safety and Health, also known as Cal/OSHA, recommends that farmworkers and other outdoor laborers drink four 8-ounce glasses of water, or a total of one quart, per hour for heat illness prevention.[18] This is equivalent to 7.5 L in an 8 hour shift, twice the 95th percentile consumption rate for adults. A survey of farmworkers in Oregon and Washington found that 78% of participants reported drinking water at least once per hour while working, but did not specify the amount (Bethel, 2018). California has 5.8 times the national average of people employed as farmworkers and laborers.[19] In addition to farmworkers, some construction occupations are two to four times more prevalent in California as well, including solar panel installers, plasterers and stucco masons, and stone masons. Occupational Employment Statistics provided by the US Bureau of Labor Statistics do not specify legal status of workers, so it is possible that undocumented laborers are underrepresented in these numbers, thus the number of workers engaged in outdoor labor in California may be even higher.

Lifestyle and recreation also play a large role in the water consumption habits of Californians. Factors such as the increased use of reusable water bottles, particularly among children, may lead to increased tap water consumption among Californians. Single-use plastic water bottles have been banned in some California cities, and both environmental sustainability and cost effectiveness are possible reasons behind increased sales and use of refillable water bottles state-wide. Water intake in school-aged children is likely higher than current estimates, following the passage of Senate Bill 1413 in 2011, requiring schools to provide access to clean drinking water for children. A survey of 240 California public schools found that 96% allowed student use of reusable water bottles (Altman et al., 2020) which, when coupled with the availability of clean drinking water on school campuses, suggests that water consumption in school-aged children may be higher than estimated by either OEHHA or US EPA, since both values were derived using data collected prior to implementation of SB 1413.

Participation in sports and recreational activities likely also yields increased water consumption in children. In a 2017-2018 survey of California children 6-17 years of age, 58.5% participated in team sports or sports lesson activities after school and on weekends.[20] This was in addition to normal outdoor play and activities on school days. Overall participation in sports and

[16] https://www.cdc.gov/disasters/extremeheat/heattips.html, accessed June 2020.
[17] https://www.bls.gov/oes/current/oes_ca.htm, accessed June 2020.
[18] https://www.dir.ca.gov/dosh/etools/08-006/EWP_water.htm, accessed June 2020.
[19] https://www.bls.gov/oes/current/oes_ca.htm, accessed June 2020.
[20] Child and Adolescent Health Measurement Initiative. 2017-2018 National Survey of Children's Health (NSCH) https://www.childhealthdata.org/browse/survey, accessed June 2020.

exercise is higher in all age groups in western states, with 22.9% of California residents participating in some form of sport or exercise on an average day.[21]

Water consumption during physical activities varies based on environmental conditions and type of activity. A review of athletes' drinking habits during competitive sporting activities evaluated studies involving running (marathon, ultramarathon, triathlon), iron man events, cycling, and indoor and outdoor team sports (soccer, volleyball, and basketball) (Garth, 2013). Mean fluid intake varied considerably, with up to 1.5 L/hour consumed at the high end of the range. The duration of the activity did not seem to relate to the amount of water consumed. Athletes running a half marathon recorded ad libitum fluid intake rate of 0.38 L/hour, while long distance endurance runners consumed between 0.5 and 0.85 L/hour, depending on ambient temperature (Dion, 2013; Hoffman, 2018). A study of endurance athletes running a marathon observed a mean fluid intake rate of 0.5 L/hour (Beis, 2012). Fluid intake for endurance activities may be even higher; for example, a study of ultra-endurance cycling observed fluid intake rates of 0.3 – 1.2 L/hour in a hot climate (Armstrong, 2015). Similar levels of fluid intake were observed for sporting activities with short, intense bursts of activity, such as rugby. Burgh et. al. (2017) recorded 0.88 L intake over a 40-minute period involving six 6-minute games separated by a 2-minute rest period, giving an approximate intake rate of 1.3 L/hour. It is likely that team sports, many of which involve short bursts of intense activity, may last only one hour, whereas endurance sports may last between 4 – 10 hours, occasionally more. Endurance events require extensive training over time, so these elevated water intake rates are not restricted to a single event, but likely persist over longer periods.

Given the scenarios described above, it is unlikely that California's drinking water intake rates have decreased since the publication of OEHHA's guidelines in 2012 (OEHHA, 2012), and the national average presented in US EPA's 2019 Exposure Factors Handbook update may not be representative of this state's water intake rates. Thus, to be protective of all California residents,this assessment uses the age-specific water ingestion estimates based on OEHHA's existing peer-reviewed guidance (OEHHA, 2012).

REFERENCES

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Bargh MJ, King RFGJ, Gray MP, et al. (2017) Why do team-sport athletes drink fluid in excess when exercising in cool conditions? *Appl. Physiol. Nutr. Metab.* 42(3): 271-277.

Beis LY, Wright-Whyte M, Fudge B, et al. (2012) Drinking behaviors of elite male runners during marathon competition. *Clin J Sport Med.* 22(3), 254-261.

Bethel JW, Spector JT, and Krenz J. (2017) Hydration and cooling practices among farmworkers in Oregon and Washington. *J Agromedicine.* 22(3), 22-228.

[21] Average for 2009 -2015 https://www.bls.gov/spotlight/2017/sports-and-exercise/home.htm, accessed June 2020.

Dion T, Savoie FA, Asselin A, et al. (2013) Half-marathon running performance is not improved by a rate of fluid intake above that dictated by thirst sensation in trained distance runners. *Eur. J. Appl. Physiol.* 113, 30-3020.

Garth AK and Burke LM. (2013) What do athletes drink during competitive sporting activities? *Sports Med.* 43, 539-564.

Hoffman MD, Snipe RM, and Costa RJS. (2018) Ad libitum drinking adequately supports hydration during 2 h of running in different ambient temperatures. *Eur. J. Appl. Physiol.* 118, 2687-2697.

OEHHA (2018) Indicators of Climate Change in California. https://oehha.ca.gov/media/downloads/climate-change/report/2018caindicatorsreportmay2018.pdf

US EPA (2019) Update for Chapter 3 of the Exposure Factors Handbook, Ingestion of Water and Other Select Liquids. *EPA/600/R-18/259F*.

Vieux F, Maillot M, Rehm CD, Barrios P, and Drewnowski A. (2020) Trends in tap and bottled water consumption among children and adults in the United States: analyses of NHANES 2011-16 data. *Nutr. J.* 19: 10.

APPENDIX 4. RELATIVE SOURCE CONTRIBUTION

In estimating health-protective levels of chemicals in drinking water for noncancer endpoints, OEHHA considers the relative source contribution (RSC), which is the proportion of exposure to a chemical attributed to tap water as part of total exposure from all sources, including food and ambient air. When developing an appropriate RSC value for a chemical, OEHHA follows US EPA's Exposure Decision Tree Approach (US EPA, 2000). This approach takes into account the availability of exposure data, including the levels and relevant sources of exposure, and any other non-water regulatory standards for the chemical (Figure A4.1). In addition, any specific subpopulations of concern are identified and considered in the process. A chemical-specific RSC value can be calculated when adequate data are available for all sources of exposure, including exposures from drinking water. If data are not adequate for one or more of these non-water exposure sources, then default assumptions may be used to ensure a health-protective RSC value (US EPA, 2000). These default values include a floor (or lower-bound estimate) of 20% and a ceiling (or upper-bound estimate) of 80%. However, US EPA indicates that 50% may be used when information is not available to characterize exposures other than that arising from the source of concern, i.e., drinking water.

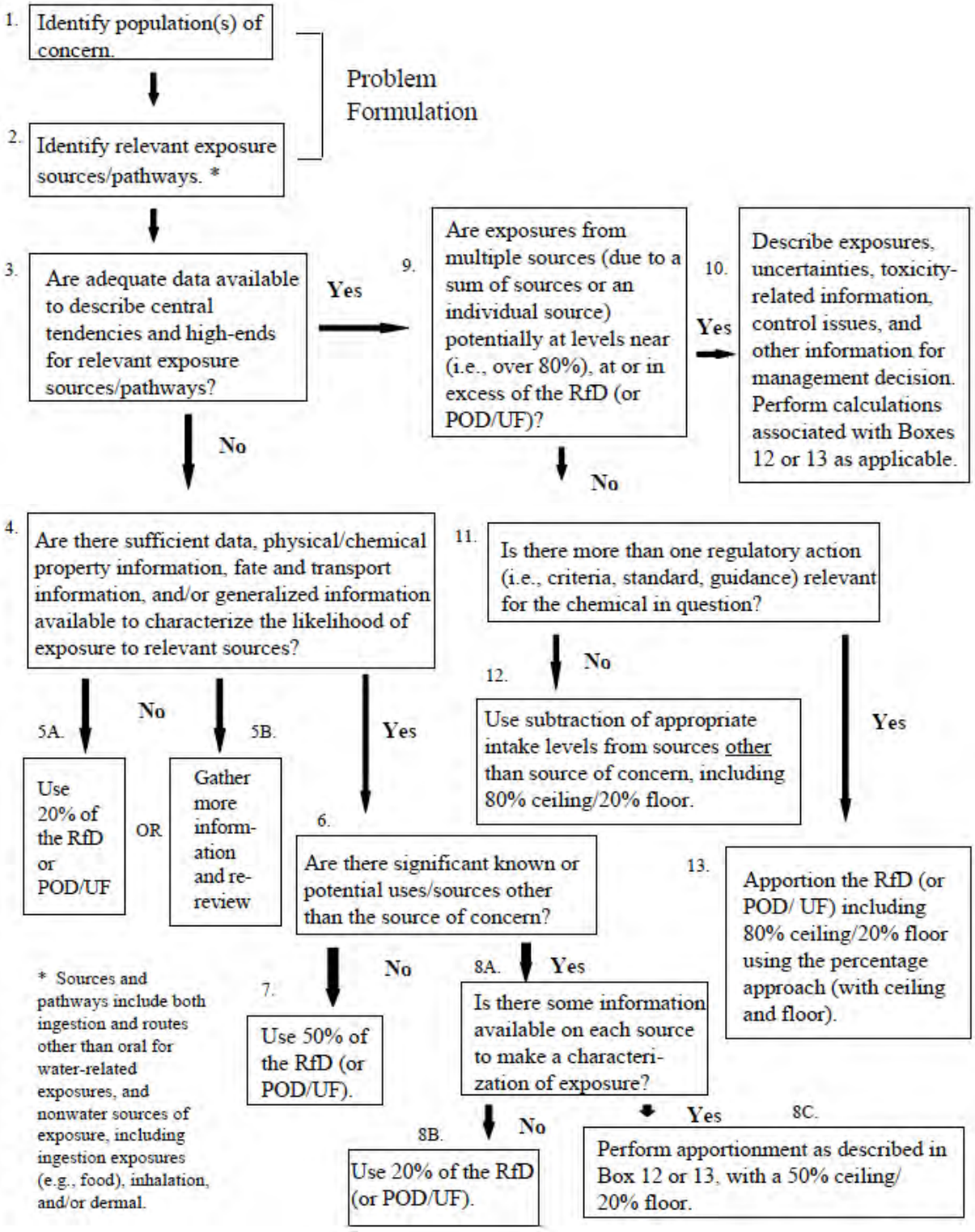
The initial steps in the decision tree, Problem Formulation (Figure A4.1, Boxes 1 and 2), identify populations of concern and relevant sources and pathways of exposure. For PFOA and PFOS, the population of concern is all residents of California. Relevant exposure sources are diet, tap water, household dust, and consumer products (Figure A4.1, Box 2).

Availability of data to describe PFOA and PFOS exposure from these sources is limited. For example, to calculate exposure from dietary sources, a database of PFOA and PFOS levels in individual foods is required, along with dietary consumption rates of the general population. Dietary consumption rates are commonly derived from National Health and Nutrition Examination Survey (NHANES) data; however, a complete dataset for PFOA and PFOS levels in food is lacking. The US Food and Drug Administration (FDA) formed a workgroup to perform ongoing assessments of food contamination related to PFAS.[22] Recently, FDA (2019) released detected levels of 16 PFAS in foods commonly consumed across the US. In the data released thus far, 179 individual food samples from six different food groups were assayed, and PFOS was detected in only two – ground turkey and tilapia (Table A4.1). PFOA was not detected in any of the food samples. The remaining food samples had levels of PFAS below the method detection limit (MDL) for the method used[23] (Table A4.1).

[22] https://www.fda.gov/food/chemicals/and-polyfluoroalkyl-substances-pfas

[23] US FDA Foods Program Compendium of Analytical Laboratory Methods: Chemical Analytical Manual, Method Number: C-010.01. Determination of 16 Perfluoroaklyl and Polyfluoroalkyl Substances (PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). https://www.fda.gov/media/131510/download

Figure A4.1. US EPA exposure decision tree for defining proposed RfD (or POD/UF) Apportionment (US EPA, 2000)



FDA reported MDLs for the following six food groups: lettuce, salmon, bread, cheese, meat products, and milk (Table A4.1). Of these, the European Food Safety Authority (2018) and Christensen et al. (2017) have indicated that meat products and fish may be important contributors to PFOS exposure, and milk, cheese, and fish may be an important contributor to PFOA exposure. However, due to the FDA's limited data on food in the US, dietary exposures to PFOA and PFOS for the US population cannot be quantified using this data set. OEHHA is not aware of any other US-specific or California-specific data sets for PFOA/PFOS in food that can be used for RSC estimation.

Table A4.1. Detected levels of PFOA and PFOS in six food groups, as reported by FDA (2019)

Food	PFOA Residue or *MDL*[1] (ng/g)	PFOS Residue or *MDL* (ng/g)
Ground Turkey	N/A	0.0857
Tilapia	N/A	0.087
Lettuce	*< 0.020*	*< 0.033*
Salmon	*< 0.090*	*< 0.082*
Meat Products	*< 0.090*	*< 0.082*
Cheese	*< 0.419*	*< 0.344*
Milk	*< 0.042*	*< 0.024*
Bread	*< 0.041*	*< 0.033*

[1] Since no residues were detected in any food samples, the respective MDLs are shown.
N/A, not available

EFSA recently released a draft scientific opinion on the risks to human health related to the presence of PFAS in food (EFSA, 2018). Analytical data on PFAS levels from 11,528 samples of food and beverages were used to estimate total dietary intake across multiple age groups. Of the 17 PFAS assayed, four were included in the risk assessment: PFOA, PFOS, PFHxS, and PFNA. The in-depth analysis estimated significant dietary exposures to adults resulting from consumption of fish and seafood, eggs, meat and meat products, and fruit and fruit products. The mean upper-bound chronic dietary exposure to PFOA and PFOS are 4.18 and 4.47 ng/kg bw per day for adults, respectively. Although the EFSA dietary database is extensive, large dietary differences such as eating habits, foods of choice, and food sources, exist between California and the European countries surveyed in this dataset; thus, this dataset is not appropriate for deriving a dietary intake value for the purposes of deriving a RSC value.

Neither the EFSA nor the FDA data account for exposures from food packaging materials that may be in contact with food items. The FDA uses a market basket approach to sampling, where both packaged and non-packaged foods are included. The amount of PFAS chemicals transferred to food products from packaging may be dependent on food type and the time of contact; thus the contribution of food packaging materials to levels detected in food, and to total PFAS exposure, is uncertain. The EFSA draft dietary risk assessment noted that possible exposures can occur from food packaging materials but their contributions were not included in the assessment. A study in California women observed a correlation between consumption of prepared food in cardboard containers with higher serum levels of PFOA and PFOS (Boronow et al., 2019), suggesting migration of the chemicals from food packaging into the food product. While uses of PFAS in packaging and food contact substances is being phased out, they can still be found in food packaging materials (Schaider et al., 2018).

An additional source of PFAS exposure comes from ingestion of household dust (Karaskova et al., 2016; Knobeloch et al., 2012). This exposure pathway may be important for infants and children, who spend more time in contact with flooring and other surfaces which may harbor household dust (i.e., they are closer to the ground), and have increased hand-to-mouth activity. Although neither PFOA nor PFOS are volatile, inhalation of these chemicals bound to fine particulate matter, such as household dust, may contribute to exposure (Trudel et al. 2008). Inhalation of larger particulate matter (e.g., larger than respirable size or >10 um in diameter) in household dust contributes to the ingestion pathway, since large particles would deposit in the upper airway including nose and mouth and be swallowed. The use of PFAS in carpets and rugs is decreasing; however, PFAS-containing carpets and rugs will likely be found in homes for years to come, making household dust and carpet residues a continuing source of exposure. PFOA and PFOS together accounted for a majority of PFAS detected in dust from US homes, and were significantly higher in homes with carpet and wood, versus other types of floor coverings. Of 14 US homes sampled, the mean values for PFOA and PFOS in house dust were 38.6 ng/g and 42.4 ng/g (Karaskova et al., 2016), respectively.

In addition, there are exposures to PFAS from using consumer products, which is a category of exposure that has not been well characterized. PFOA and PFOS can be found in a wide range of products including but not limited to makeup, hygiene products, cookware, and clothing (Fujii et al., 2013; Hu et al., 2018; Gremmel et al., 2016). Hand-to-mouth exposure stemming from the handling of consumer products in the home is of concern, even for adult populations (Poothong et al., 2019). In addition to uncertainty surrounding quantitative levels of PFAS found in consumer products, there is also uncertainty regarding the extent to which the broad category of consumer products contributes to the overall exposure to PFAS.

As discussed, there is evidence to suggest that there are many sources that may contribute significantly to the total exposure to PFOA and PFOS (Figure A4.1, Boxes 4 and 6). However, there are insufficient data to make a characterization of exposure for each source (Figure A4.1, Box 8A). Thus, OEHHA determined it is appropriate to use an RSC of 20% (Figure A4.1, Box 8B) for tap water.

Of note, US EPA's decision tree presents two approaches for deriving the RSC when sufficient data are available: the subtraction approach and the percentage approach (Figure A4.1, Boxes 12 and 13). The subtraction approach may be used when water is considered the most relevant source of exposure and other sources of exposure can be considered background. The RSC is calculated by subtracting the 'background' level of exposure, i.e., the detected serum levels from biomonitoring studies, from the target serum level. This method allows for the maximum possible contaminant levels in water, after subtracting other sources of exposure. The percentage approach is appropriate when exposure from multiple sources are considered relevant, and appropriate data are available to estimate exposure to a chemical for all water and non-water sources of exposure. The RSC is calculated as a percentage based on the exposure due to drinking water divided by the sum of exposures for all sources. Some states with developed health criteria for PFOA and/or PFOS have utilized these approaches in deriving

their RSC values, including Michigan,[24] Minnesota,[25] and New Hampshire.[26] These RSC values varied from 20% to 50% for both PFOA and PFOS, and relied on state-specific and/or NHANES biomonitoring data. In contrast, other regulatory bodies, including New Jersey, US EPA, and Health Canada, considered a default value of 20% to be appropriate due to a lack of information about sources of exposure and inadequate data to develop chemical-specific values.

The subtraction approach is appropriate for use in areas with high levels of PFOA and/or PFOS in drinking water, relative to other sources. However, data are lacking to suggest that this is the case in California. There are California-specific biomonitoring data on PFOA and PFOS (Table A4.2), showing a decreasing trend of PFOA and PFOS serum levels from 2013 to 2018. For comparison, the 2013-2014 dataset for serum levels in adults from NHANES are also shown (CDC, 2018). When using the subtraction approach, serum levels are assumed to represent background exposure levels (i.e., from all sources other than ingestion of water). However, these California-specific serum levels represent exposure from all sources, including tap water, and there are no data to evaluate the contribution of other sources relative to drinking water; thus, they should not be used to determine the RSC.

Table A4.2. Serum levels of PFOA and PFOS detected in Biomonitoring California[27] and NHANES studies

Study	PFOA (ng/ml)		PFOS (ng/ml)	
	Geometric Mean	95th Percentile	Geometric Mean	95th Percentile
CARE-LA (data collected in 2018)	1.04	3.06	2.13	8.33
BEST Expanded (data collected in 2013)	1.49	4.57	5.21	17.6
NHANES (data collected in 2013-2014)	1.98	5.6	5.22	19.5

The percentage approach requires estimations of exposures to all sources. However, there are extremely limited US-specific or California-specific data on PFOA and PFOS in food groups, indoor dust, and consumer products, as discussed above. Thus, OEHHA determined that the percentage approach cannot be used at this time for estimating RSC values for PFOA and PFOS.

[24] Michigan Department of Public Health and Human Services, Division of Environmental Health, Public health drinking water screening levels for PFAS https://www.michigan.gov/documents/pfasresponse/MDHHS_Public_Health_Drinking_Water_Screening_Levels_for_PFAS_651683_7.pdf

[25] Minnesota Department of Health Toxicological Summary for Perfluorooctanoate and Perfluorooctane sulfonate: https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfoa.pdf , https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfos.pdf

[26] Summary Report on the New Hampshire Department of Environmental Services, Development of Maximum Contaminant Levels and Ambient Groundwater Quality Standards for PFOS, PFOA, PFNA, and PFHxS, https://www.des.nh.gov/organization/commissioner/pip/publications/documents/r-wd-19-01.pdf

[27] Biomonitoring California Results Database for PFAS chemicals https://biomonitoring.ca.gov/results/chemical/2183

Due to a lack of information about sources of exposure and inadequate data to develop chemical-specific values, OEHHA is adopting the default value of 20% for PFOA and PFOS in evaluating noncancer endpoints. This determination is consistent with other regulatory bodies, including New Jersey, US EPA, and Health Canada.

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APPENDIX 5. HUMAN EXPOSURE STUDIES AND DATA

Table A5.1. Human exposure studies

Reference	Food	Water	Dust (ingested)	Dermal	Indoor air	Outdoor air	Total
PFOA exposure studies							
Lorber and Egeghy (2011) *young children* % total *adults* % total	*median (range[a])* 8.1 ng/day (1.7-37) 31% 46 ng/day (9.7-208) 72%	 4.8 ng/day (0.72-28) 19% 17 ng/day (2.5-100) 7.5%	 12.6 ng/day (1.0-114) 49% 6.3 ng/day (0.51-55) 19.8%	 0.008 ng/day ($6x10^{-4}$-0.07) <0.1% 0.005 ng/day ($4x10^{-4}$-0.04) <0.1%	 0.26 ng/day (0.12-0.59) 1% 0.61 ng/day (0.26-1.3) 0.4%	 3 pg/day (2-8) <0.1% 4 pg/day (2-9) <0.1%	 *median* 25.8 ng/day *median* 72.2 ng/day
Haug et al. (2011a)[b] *Adult women* % of total *Infants at 6 months* % of total	*median (range[c])* 0.22 ng/kg-day (0.16-0.29) 66% *breastmilk:* 4.1 ng/kg-day (3.5-9.2) 83%	 30 pg/kg-day (27-34) 9.0% -	 49 pg/kg-day (32-81) 14.7% 0.66 ng/kg-day (0.47-1.1) 13%	 - -	 35 pg/kg-day (25-49) 10.5% 0.17 ng/kg-day (0.13-0.20) 3.4%	 - -	*median* *0.33* ng/kg-day *mean* 0.38 ng/kg-day *median* *4.9* ng/kg-day *mean* 14 ng/kg-day
PFOS exposure studies							
Egeghy and Lorber (2011) *young children* % total *adults* % total	*Median (range[a])* 20 ng/day (3.6-97) 42% 112 ng/day (20-550) 71%	 9.3 ng/day (2.7-27) 19% 36 ng/day (11-109) 23%	 17.8 ng/day (0.49-219) 37% 8.6 ng/day (0.25-106) 5.5%	 0.70 ng/day (0.019-8.6) 1.5% 0.29 ng/day (0.008-3.5) 0.2%	 0.10 ng/day (0.047-0.24) 0.2% 0.27 ng/d (0.13-0.6) 0.2%	 2 pg/day (0.9-3) <0.1% 2 pg/day (1-4) <0.1%	 *median* 47.9 ng/day *median* 157 ng/day

Reference	Food	Water	Dust (ingested)	Dermal	Indoor air	Outdoor air	Total
Haug et al. (2011a)[b]	*Median (range[c])*						*median*
Adult women	0. 61 ng/kg-day (0.47-0.88)	4 pg/kg-day (4-5)	11 pg/kg-day (7-22)	-	77 pg/kg-day (36-110)	-	0.70 ng/kg-day *mean* 0.84 ng/kg-day
% of total	87%	0.6%	1.6%		11%		
Infants at 6 months	*breastmilk:* 8.7 ng/kg-day (5.8-9.6)	-	0.09 ng/kg-day (0.07-0.3)	-	0.3 ng/kg-day (0.13-0.43)	-	*median* 9.1 ng/kg-day *mean* 10.0 ng/kg-day
% of total	96%		1%		3.3%		

[a] 5th-95th percentile
[b] Scenario 3 in Haug et al. (2011a) is considered, which included high level of dust exposure (200 mg/kg) and significant contribution of biotransformation of precursors in the indoor air (100% of FOSA/FOSEs to PFOS, and 1.7% of FTOHs to PFOA).
[c] 25th-75th percentile

Table A5.2. PFOA and PFOS trends in serum concentration

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Calafat et al. (2007a)	US, NHANES	General population (≥12 years) (N=2,094)	PFOA	**Total** 1999/2000: 5.2; 2003/04: 3.9 **males** 1999/2000: 5.7; 2003/04: 4.5 **females** 1999/2000: 4.8; 2003/04: 3.6	Slightly **decreased**
			PFOS	**Total** 1999/2000: 30.4; 2003/04: 20.7 **males** 1999/2000: 33.4; 2003/04: 23.3 **females** 1999/2000: 28.0; 2003/04: 18.4	**Decreased**
Kato et al. (2011)	US, NHANES	General population (≥12 years) (N=7,876)	PFOA	1999/00: 5.21 (95th pctl: 11.9) 2003/04: 3.95 (95th pctl: 9.8) 2005/06: 3.92 (95th pctl: 11.3) 2007/08: 4.13 (95th pctl: 9.7)	Slightly **decreased**
			PFOS	1999/00: 30.4 2003/04: 20.7 2005/06: 17.1 2007/08: 13.2	**Decreased**
Wang et al. (2011)	US, Northern California	Pregnant women, archived samples (N=105)	PFOA	1960s[c]: 0.30 1980s: 3.17 2009: 2.21	**Peak** in the 1980s
			PFOS	1960s[c]: 45.90 1980s: 30.60 2009: 9.44	**Decreased**
Olsen et al. (2012)	US, 6 cities (Boston, Charlotte, Hagers-town, Los Angeles, Minnea-polis Saint Paul, Portland)	Red Cross adult plasma samples (N=10/ten-year age interval/sex)	PFOA	**Total** 2000/01: 4.7 (95th pctl: 12) 2006: 3.44 (95th pctl: 7.9) 2010: 2.44 (95th pctl: 6.6) **males** 2000/01: 5.0 (95th pctl: 13.5) 2006: 3.95 (95th pctl: 8.3) 2010: 2.69 (95th pctl: 5.8) **females** 2000/01: 4.4 (95th pctl: 10.7) 2006: 3.0 (95th pctl: 6.7) 2010: 2.22 (95th pctl: 6.8)	Both PFOA and PFOS **decreased**; halving time for PFOS 4.3 years; decreasing trends for both sexes, in all 6 locations
			PFOS	**Total** 2000/01: 34.9 (95th pctl: 75) 2006: 14.5 (95th pctl: 31.5) 2010: 8.3 (95th pctl: 24.9) **males** 2000/01: 37.8 (95th pctl: 80) 2006: 17.1 (95th pctl: 36.5) 2010: 9.7 (95th pctl: 24.9) **females** 2000/01: 32.1 (95th pctl: 74) 2006: 12.3 (95th pctl: 29.7) 2010: 7.2 (95th pctl: 19.6)	

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Gribble et al. (2015)	US, South Carolina	Gullah African Americans (N=71)	PFOA	Median 5.6 at baseline (2010-2013) and decreased by 2.2 (median) over 7.3 years on average	For either PFOA or PFOS, approximately 50% **decrease** over 7 years
			PFOS	Median 41.1 at baseline (2010-2013) and decreased by 21.7 (median) over 7.3 years on average	
Jain (2018)	US, NHANES	Adults (≥20 years), (N=9,650) weighted unadjusted values	PFOA	**Total** 2003/04: 3.99; 2005/06: 4.03 2007/08: 4.17; 2009/10: 3.12 2011/12: 2.13; 2013/14: 2 **males** 2003/04: 4.48; 2005/06: 4.81 2007/08: 4.91; 2009/10: 3.61 2011/12: 2.45; 2013/14: 2.36 **females** 2003/04: 3.57; 2005/06: 3.39 2007/08: 3.56; 2009/10: 2.72 2011/12: 1.88; 2013/14: 1.71	Both PFOA and PFOS **decreased**, on average by 17.8%/2 years and 33.8%/2 years, respectively
			PFOS	**Total** 2003/04: 21.09; 2005/06: 17.7 2007/08: 13.54; 2009/10: 9.7 2011/12: 6.74; 2013/14: 5.27 **males** 2003/04: 23.67; 2005/06: 21.24 2007/08: 17.11; 2009/10: 12.13 2011/12: 8.54; 2013/14: 6.81 **females** 2003/04: 18.81; 2005/06: 14.83 2007/08: 10.79; 2009/10: 7.84 2011/12: 5.4; 2013/14: 4.12	
Kim et al. (2020)	Northern California	Women (mothers) (N=450)	PFOA	2009: 1.66; 2010: 1.37 2011: 1.26; 2012: 1.14 2013: 1.02; 2014: 0.935 2015: 0.801; 2016: 0.675	On average **decreased** by 10.7%/year
			PFOS	2009: 4.86; 2010: 4.1 2011: 3.78; 2012: 3.42 2013: 3.02; 2014: 2.8 2015: 2.42; 2016: 2.12	On average **decreased** by 10.8%/year

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Non-US studies					
Harada et al. (2007a)	Japan (Kyoto)	Adults (N=100)	PFOA	**Males** 1983: 2.52; 1987: 4.4 1991: 5.48; 1995: 6.56 1999: 11.44 **females** 1983: 1.84; 1987: 4.64 1991: 6.36; 1995: 5.52 1999: 8.12	**Increased**
			PFOS	**Males** 1983: 15.1; 1987: 21.9 1991: 15.1; 1995: 18.1 1999: 22.8 **females** 1983: 13.3; 1987: 18.0 1991: 19.1; 1995: 16.4 1999: 18.5	**No trend**
Haug et al. (2009)	Norway	Adult men (N=57, pooled samples)	PFOA	1977: 0.58; 1980: 1.3; 1981: 1.4 1982: 1.4; 1883: 1.5; 1985: 2.2 1986: 2.6; 1988: 2.7; 1989: 3.1 1990: 3.3; 1991: 3.4; 1993: 5.2 1994: 4.1; 1995: 4.4; 1996: 4.0 1997: 4.2; 1999: 4.0; 2000: 4.5 2001: 4.9; 2002: 3.9; 2003: 3.8 2004: 3.4; 2005: 3.5; 2006: 2.7	**Increased** through the 90s, then **decreased**
			PFOS	1977: 3.8; 1980: 6.1; 1981: 9.4 1982: 11; 1983: 10; 1985: 16 1986: 15; 1988: 18; 1989: 22 1990: 20; 1991: 23; 1993: 33 1994: 24; 1995: 31; 1996: 25 1997: 31; 1999: 29; 2000: 30 2001: 27; 2002: 27; 2003: 19 2004: 18; 2005: 21; 2006: 12	**Increased** through the 90s, then **decreased**
Harada et al. (2010)	South Korea	Women, archive samples (N=24-39)	PFOA	**Busan** 1994: 4.1; 2000: 3.7; 2008: 4.5 **Seoul** 1994: 1.1; 2007: 2.7	**No trend to slight increase**
			PFOS	**Busan** 1994: 10.9; 2000: 9.1; 2008: 9.4 **Seoul** 1994: 7.63; 2008: 7.61	**No trend**

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Glynn et al. (2012)	Sweden	Women, 3 weeks after delivery (N= 36, pooled samples)	PFOA	1996: 2.18-2.92; 1997: 2.26-3.07 1998: 2.22-2.66; 1999: 2.38-3.11 2000: 2.50-2.65; 2001: 3.05 2002: 2.17-2.98; 2004: 2.12-2.15 2006: 1.70-2.11; 2007: 1.36-2.42 2008: 1.69-2.01; 2009: 1.54-2.4 2010: 1.39-1.96	**Decreased** an average 3.1 %/year (95% CI: 1.8-4.4 %/year), halving time 22 years
			PFOS	1996: 22.7-27.3; 1997: 20.3-24.8 1998: 20.2-23.1; 1999: 20.0-23.0 2000: 18.7-22.0; 2001: 28.1 2002: 17.0-23.3; 2004: 13.6-16.6 2006: 10.7-16.5; 2007: 8.80-18.3 2008: 9.25-11.1; 2009: 7.14-8.89 2010: 5.11-7.62	**Decreased** an average 8.4 %/year (95% CI: 5.9-11); halving time 8.2 years
Okada et al. (2013)	Japan	Pregnant women (N=150)	PFOA	2003[c]: 2.05; 2005: 1.25 2007: 1.56; 2009: 1.36 2011: 1.42	**Decreased**
			PFOS	2003[c]: 7.76; 2005: 6.20 2007: 6.23; 2009: 4.54 2011: 3.90	**Decreased**
Schröter-Kermani et al. (2013)	Germany	Archived plasma samples (N=260); data also reported separately for men and women	PFOA	1982: 4.0 (range 1.5-7.5) 1986: 7.4 (2.4-15.4) 1989: 6.3 (2.4-11.6) 1992: 5.8 (2.6-9.4) 1995: 5.1 (1.8-8.8) 1998: 5.3 (1.9-10.9) 2001: 5.8 (2.6-10.5) 2003: 4.8 (1.6-9.0) 2005: 5.5 (3.1-12.5) 2006: 4.7 (3.1-9.7) 2007: 5.2 (1.6-10.6) 2008: 4.0 (2.3-6.7) 2010: 3.1 (0.8-8.7)	**Mixed** trend through 2000s
			PFOS	1982: 15.4 (4.6-32.1) 1986: 28.0 (9.5-57.4) 1989: 28.6 (10.3-102.9) 1992: 23.7 (10.4-34.7) 1995: 21.1 (10.1-49.8) 1998: 20.7 (10.3-40.3) 2001: 19.7 (10.3-40.3) 2003: 13.7 (4.9-24.9) 2005: 12.7 (5.7-38.3) 2006: 7.4 (2.4-12.4) 2007: 7.8 (3.4-16.1) 2008: 6.1 (2.6-10.2) 2010: 3.8 (1.9-12.1)	**Peak** in 1989, then **decreased**

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Yeung et al. (2013a)	Germany	Archived serum samples (N=420)	PFOA	**Munster**: concentrations increased from 1981 till 1986 (peak at 10 ng/ml), then steadily decreased through 2010 to approximately 2 ng/ml **Halle**: concentrations decreased 1995-2010	Munster: **peak** (1986), then **decrease** Halle: **decrease**
Yeung et al. (2013b)	Germany	Archived serum samples (N=420)	PFOS	**Munster**: concentrations increased from 1981 till 1986 (peak at approximately 40 ng/ml), then steadily decreased through 2010 to approximately 5 ng/ml **Halle**: concentrations decreased from 1995 through 2010 to approximately 5 ng/ml	Munster: **peak** (1986), then **decrease** Halle: **decrease**
Nøst et al. (2014)	Norway	Adult men (N=53), longitudinal study (same subjects)	PFOA	1979: 0.85; 1986: 2.38 1994: 3.74; 2001: 4.06 2007: 3.00	**Peak** in 2001
			PFOS	1979: 8.94; 1986: 22.7 1994: 36.9; 2001: 43.3 2007: 33.0	**Peak** in 2001
Toms et al. (2014)	Australia	All ages (pooled samples, N=158)	PFOA	2002/03: 10.2; 2006/07: 6.4 2008/09: 5.2; 2010/11: 4.5	**Decreased**
			PFOS	2002/03: 25.9; 2006/07: 15.2 2008/09: 11.9; 2010/11: 10.2	**Decreased**
Bjerregaard-Olesen et al. (2016)	Denmark	Pregnant women (N=1,533)	PFOA	2008[ab]: 0.97; 2009: 0.86 2010: 0.86; 2011: 0.69 2012: 0.57; 2013: 0.51	**Decreased**, adjusted trend: -9.5%/year
			PFOS	2008[ab]: 2.26; 2009: 2.08 2010: 2.01; 2011: 2.00 2012: 1.76; 2013: 1.75	**Decreased**, adjusted trend: -9.3%/year
Eriksson et al. (2017b)	Australia	Adults (N=54, pooled samples)	PFOA	**total[c]** 2002: 8.5; 2004: 6.1; 2006: 6.0 2008: 4.0; 2011: 3.1; 2013: 2.3 **males** 2002: 9.0; 2004: 5.9; 2006: 6.2 2008: 4.3; 2011: 3.4; 2013: 2.3 **females** 2002: 8.0; 2004: 6.4; 2006: 5.7 2008: 3.8; 2011: 2.9; 2013: 2.2	**Decreased** halving time (total): 5.3 years
			PFOS	**total[c]** 2002: 10.9; 2004: 6.9; 2006: 6.6 2008: 3.8; 2011: 2.6; 2013: 1.8 **males** 2002: 11.0; 2004: 7.1; 2006: 6.5 2008: 4.2; 2011: 2.6; 2013: 1.7 **females** 2002: 10.8; 2004: 6.6; 2006: 6.7 2008: 3.3; 2011: 2.3; 2013: 2.1	**Decreased**, halving time (total): 4.2 years

Reference	Area	Participants	PFAS	Year-C_{serum}(ng/ml)[#]	Trend
Seo et al. (2018)	South Korea	Adults (N=786)	PFOA	**Males** 2006: 2.90[c] (range 0.87-8.44) 2007: 5.40 (2.20-9.03) 2008: 4.94 (1.70-11.7) 2009: 5.00 (2.37-9.46) 2011: 4.96 (1.10-13.64) 2013: 4.93 (2.47-9.26) 2014: 12.10 (0.83-31.96) 2015: 4.78 (1.54-8.82) **females** 2006: 2.92[c] (range 0.66-9.99) 2007: 3.96 (1.88-9.34) 2008: 4.68 (1.01-12.37) 2009: 4.25 (1.10-10.46) 2011: 4.51 (0.76-14.17) 2013: 5.50 (1.57-11.71) 2014: 6.25 (0.39-37.79) 2015: 5.48 (0.86-17.18)	**Mixed** trend
			PFOS	**Males** 2006: 11.54 (4.62-29.73) 2007: 14.17 (5.78-29.66) 2008: 16.22 (5.55-50.11) 2009: 15.06 (4.28-31.82) 2011: 16.16 (1.82-40.19) 2013: 32.19 (10.21-70.09) 2014: 9.85 (1.55-29.31) 2015 15.38 (7.37-32.58) **females** 2006: 11.77 (5.90-26.26) 2007: 9.39 (3.35-19.69) 2008: 14.40 (3.51-42.44) 2009: 12.23 (0.69-129.43) 2011: 12.08 (1.17-54.16) 2013: 16.08 (6.07-38.22) 2014: 6.73 (1.04-20.81) 2015: 11.37 (2.04-27.32)	**Mixed** trend
Shu et al. (2019)	Sweden	Pregnant women (N=106-263)	PFOA	2007: 1.81; 2008: 1.65 2009: 1.55; 2010: 1.43	PFOA and PFOS **decreased** by 21% and 31% over 30 months
			PFOS	2007: 6.39; 2008: 5.83 2009: 4.7; 2010: 4.42	

[#]Geometric mean if not indicated otherwise; pctl, percentile
[a] Quantitated from graph using GetData Graph Digitizer 2.26
[b] Median values
[c] Arithmetic mean values

APPENDIX 6. TOXICOKINETICS SUPPLEMENTAL DATA TABLES

Table A6.1. Organ distribution of PFOA and PFOS in rat, mouse, monkey and human

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
Johnson et al. (1979) unpublished[c]	rat (m)	PFOS	not reported in Benskin et al. (2009)	**Liver**>plasma>kidney>lung>spleen>bone marrow>red blood cells>adrenals>testes> skin>fat>eye
Ylinen et al. (1990)	rat (m,f)	PFOA	3,10,30 for 28d (oral gavage)	Low dose (f): serum>**liver(0.75)**>lung, spleen, kidney>>brain(nd), ovary(nd) Mid and high doses (f): serum>kidney(0.6-0.9) >**liver(0.3-0.5)**>spleen>lung>ovary>brain; (m): serum>**liver(0.6-0.96)**>kidney(0.5-0.8)> lung>testes>spleen>brain
Vanden Heuvel et al. (1991)	rat (m,f)	PFOA	3.9; single (i.p.)	2h (m): **liver(1)**,plasma>kidney(0.48)>heart> testes, fat>gastrocnemius 2h (f): plasma>kidney(0.8)>**liver(0.6)**>ovaries
Kudo et al. (2001)	rat (m)	PFOA	25; single (i.p.)	5d: **liver(2.1[d])**>serum
Austin et al. (2003)	rat (f)	PFOS	1,10 for 14d (i.p.)	Low dose: **liver(2.5)**>serum>kidney(0.9)>> ovary>adrenal>heart>>brain>spleen High dose: **liver(2.1)**>serum,kidney(1)>heart> spleen, ovary, hypothalamus>brain
Lau et al. (2003)	pregnant rat	PFOS	1,2,3,5 for 20d (GD2-21) (oral gavage)	F1: **liver(1.3,0.98,1.2,1.5[d])**>serum
Seacat et al. (2003)	rat (m,f)	PFOS	(m) 0.05, 0.18, 0.37, 1.51 for 4w; 0.03, 0.13, 0.34, 1.33 for 14w; (f) 0.05, 0.22, 0.47. 1.77 for 4w; 0.04, 0.15, 0.4, 1.56 for 14w in food	4 weeks: **liver(3.7-12.2)**>serum 14 weeks: **liver(4.3-6.8)**>serum
Luebker et al. (2005a)	pregnant rat	PFOS	0.1-3.2 for about 60d (oral gavage)	GD 21 dams: **liver(3.3-4.9)**>serum; pups: at most doses serum>**liver(0.9)** PND 5 dams: **liver(0.6-1.8)**; pups: **liver(1.8-2)**>serum
Hundley et al. (2006)	rat (m)	PFOA	10; single (oral gavage)	120h: **liver(1.7)**>kidney(1)>blood>>lung> heart>skin>testes>muscle>fat>brain

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
Kudo et al. (2007)	rat (m)	PFOA	0.041,16.56; single (i.v.)	2h low dose: **liver(2.2)**>kidney(1.1), serum> blood>>lung, heart>spleen, testes, intestine> stomach, fat>brain 2h high dose: serum>**liver(0.83)**, kidney(0.75) > blood>lung>heart>spleen>testes>intestine> fat, stomach>brain
Curran et al. (2008)	rat (m,f)	PFOS	0.14-7.58 for 28d (in diet)	**Liver(13-47)**>>spleen>heart>serum
Benskin et al. (2009)	rat (m)	PFOA	0.4; single (oral gavage)	On day 38: **liver**>blood>kidney>lung>heart> testes>spleen>fat>intestine>muscle>brain
		PFOS	0.27; single (oral gavage)	On day 38: **liver**>lung>kidney>blood>spleen> heart>testes>intestine>muscle>brain>fat
Chang et al. (2009)	pregnant rat	PFOS	0.1,0.3,1 for 21d (GD0-20) (oral gavage)	Dams GD 20: **liver(1.8-4.5)**>serum>>brain Pups GD 20: serum>**liver(0.6-0.8)**>>brain Pups PND 4: **liver(2.2-4.2)**>serum>>brain
Cui et al. (2009)	rat (m)	PFOA	5,20 for 28d (oral gavage)	Low dose: kidney(5.8), **liver(5.6)**>lung>blood, heart>testes>spleen>brain[e] High dose: kidney(3.6), **liver(3.3)**>lung> blood>heart>testes>brain>spleen[e]
		PFOS	5,20 for 28d (oral gavage)	Low dose: **liver(4.8)**>heart>kidney(1.3)>blood >testes, spleen>brain[e] High dose (no blood sample available): **liver**>heart>kidney, lung>spleen>brain>testes
Chang et al. (2012)	rat (m)	PFOS	4.2; single (oral gavage)	89d: **liver(9.3)**>plasma>kidney(0.49),lung>> spleen, red blood cells, testes etc.
	rat (m,f)	PFOS	1,15; single (oral gavage)	10w: **liver(m:5.6-17.8; f:1.8-2.6)**>serum
Gao et al. (2015a)	rat (m,f)	PFOA	0.12 (m), 0.15 (f) for 90d[f] (drinking water)	(f): serum>kidney(0.72)>**liver(0.59)**>lung> spleen>heart>brain (m): serum>kidney(0.71)>lung>**liver(0.22)**> heart>spleen>>brain
		PFOS	0.12 (m), 0.15 (f) for 90d[f] (drinking water)	(f): kidney(1), **liver(1)**, serum>lung>spleen> heart>brain (m): **liver(4.3)**>serum, kidney(0.98)>lung> spleen>heart>brain

Kim et al. (2016b)	rat (m,f)	PFOA	1; single (oral gavage, i.v.)	i.v.(m): **liver(2.3)**>kidney(1.2)>plasma>> lung>heart>spleen;
				i.v.(f): kidney>plasma>**liver(0.8)**> lung>heart,spleen;
				results for oral gavage were reported as similar to i.v.

		PFOS	2; single (oral gavage, i.v.)	i.v.: **liver(2-2.6)**>plasma>kidney(0.2)>lung> spleen, heart
Bagley et al. (2017)	rat (m,f)	PFOS	6 (m), 6.6 (f) for 3w (in diet)	23d (m): **liver(1.5)**>serum: (f): **liver**=serum

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
Ishida et al. (2017)	pregnant rat	PFOS	1,2 for 10d (GD11-20) (oral gavage)	PND 4, F0: **liver(4[d])**>serum>>brain PND 4, F1: **liver(2.1-3.2[d])**>serum>>brain
Iwabuchi et al. (2017)	rat (m)	PFOA	0.1 single (oral gavage), or 1,5,25 µg/L[g] for 3 months (drinking water)	28d (single): **liver(1.7)**>>serum>kidney(0.82) >blood>heart>spleen>brain 3 months (high dose): **liver(3.3)**>serum> kidney(0.69)>blood>heart> spleen>>brain
		PFOS	0.1; single (oral gavage), or 1,5,25 µg/L[g] for 3 months (drinking water)	28d (single): **liver(22)**>>kidney(1.6)> serum> blood>spleen>heart,brain 3 months (high dose): **liver(38)**>kidney(1.2)> serum>blood>spleen>heart>brain
Huang et al. (2019a); NTP (2019a)	rat (m,f)	PFOS	2,5; single 2 for 5d (oral gavage)	All doses (single and 5d) (m): **liver(2-29)**>plasma, kidney(~1) >>brain; (f): **liver(1.5-3.7)**>kidney(1.1-1.9)> plasma>>brain
NTP (2019a); Dzierlenga et al. (2020)	rat (m,f)	PFOA	12(m), 80(f); single (oral gavage)	22d (m): **liver(1.3)**>plasma>kidney(0.4)>brain 12h (f): plasma>>**liver**, kidney>>brain
Hundley et al. (2006)	mouse (m)	PFOA	10; single (oral gavage)	120h: **liver(3.1)**>blood>>skin>kidney>fat, lung, heart, muscle>testes>>brain
	mouse (f)	PFOA	10; single (oral gavage)	120h: **liver(4.5)**>blood>kidney>lung, fat> brain>heart>muscle>skin
Chang et al. (2012)	mouse (m,f)	PFOS	1,20; single (oral gavage)	On day 2: **liver(2.2-3.4)**>serum>kidney(0.25-0.30)
Lou et al. (2009)	mouse (m,f)	PFOA	1,10; single (oral gavage)	Males and females: **liver (1.8-2.9)**>plasma> kidney(0.1-0.2) at both doses, at 4h-48d, except low dose (f) at 4h: plasma>liver(0.8)>kidney(0.13)
Minata et al. (2010)	mouse (m)	PFOA	5.2,10.4,21 for 4w (oral gavage)	Low dose: **liver(8.8)**>plasma Mid dose: **liver(4.2)**>plasma High dose: **liver(3.2)**>plasma
Bogdanska et al. (2011)	mouse (m)	PFOS	0.031,23 for 1-5d (oral diet)	Low dose (5d): **liver(5.8)**>lung>bone>blood> kidney(0.9)>pancreas, gut, stomach>skin, spleen, thymus>testes, heart>muscle>brain, fat High dose (5d): **liver(3.6)**>lung>blood> kidney(0.8),skin>bone>pancreas>thyroid>gut, stomach, spleen, thymus>heart, testes>fat, brain, muscle
Macon et al. (2011)	pregnant mouse	PFOA	0.3,1,2 for 17d (GD 1-17) (oral gavage)	Dams PND 7 and PND 14: plasma> **liver (0.25-0.5)**>>brain; Pups, PND 21-84: plasma=**liver**
Fujii et al. (2015)	mouse (m,f)	PFOA	0.13; single (i.v.)	24h (m)[h]: **liver**>serum>>kidney>brain, fat; 24h (f)[h]: serum>**liver**>>kidney>brain, fat

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
			1.3; single (oral gavage)	
Yu et al. (2016)	mouse (m)	PFOA	0.5,2.5 for 28d (oral gavage)	Low dose: **liver(1)**=blood High dose: **liver(1.5)**>blood
Zhang et al. (2016c)	mouse (m)	PFOS	0.003-0.012% (in diet) for 2w	**Liver(2.2-3.5[d])**>serum
Lai et al. (2017b)	pregnant mouse	PFOS	0.3 for 19d (GD 1-18.5) (oral gavage)	Dams **liver(4.4[d])**>F1 liver>serum>placenta
Li et al. (2017b)	mouse (m,f)	PFOA	0.05,0.5,2.5 for 28d (oral gavage)	Low dose: **liver**=serum Mid and high doses: **liver(1.5-2[d])**>serum
Zheng et al. (2017)	mouse (m)	PFOA	1.25 for 28d (presumably oral gavage)	**Liver(2.3[d])**>serum
Lai et al. (2018)	mouse (f)	PFOS	0.0003,0.003 for 7w (oral gavage)	Low dose: **liver**=serum High dose: **liver(4.6)**>serum
Guo et al. (2019)	mouse (m)	PFOA	0.4,2,10 for 28d (oral gavage)	**Liver(2)**>serum
Griffith and Long (1980)	monkey (m,f)	PFOA	3,10 for 90d (oral gavage)	Serum>>**liver(0.06-0.2)**
Seacat et al. (2002)	monkey (m,f)	PFOS	0.03,0.15,0.75 for 6 months (oral, capsules)	Low and high doses: **liver(1.7-2.2)**>serum Mid dose: **liver(1.2f)**>serum; serum> **liver(0.9m)**
Butenhoff et al. (2004a)	monkey (m,f)	PFOA	3,10,20(30)[i] for 6 months (oral, capsules)	Serum>>**liver(0.09-0.28)**
Olsen et al. (2003c)	human (post-mortem)	PFOS	unknown	**Liver(1.4)**>serum
Maestri et al. (2006)	human (post-mortem)[j,k]	PFOA	unknown	Lung>kidney(1.2)>**liver(1)**,blood>thyroid> hypophysis>gonads>fat>pancreas>skeletal muscle>brain>basal ganglia
		PFOS	unknown	**Liver(2.7)**>lung>hypophysis>kidney(1.2)> blood>pancreas, gonads>thyroid>fat>brain, basal ganglia>skeletal muscle
Perez et al. (2013), Fabrega et al. (2014)	Human[j] (post-mortem)	PFOA	unknown	Bone>lung>**liver**>>kidney>>brain, fat
		PFOS	unknown	**Liver**>kidney>lung>>brain>>bone, fat

Reference	Species (sex)	PFAS	Dose (mg/kg[a])	Distribution in organs[b]
Mamsen et al. (2019)	human (post-mortem fetus)	PFOA	unknown	1st trimester: maternal serum>>lung>placenta >**liver(0.12)**>heart>>CNS, fat 2nd trimester: maternal serum>**liver(0.34)**> placenta>lung>heart>fat>CNS 3rd trimester: maternal serum>placenta> **liver(0.38)**>lung>fat>heart>CNS
		PFOS	unknown	1st trimester: maternal serum>lung>placenta> heart>**liver(0.10)**>CNS>fat 2nd trimester: maternal serum>**liver(0.71)**>fat> lung>heart>placenta>CNS 3rd trimester: maternal serum>**liver(0.38)**> lung, fat, heart>placenta>CNS

CNS, central nervous system; d, days; f, female; m, male; w, weeks; GD, gestation day; i.p., intraperitoneal injection; i.v., intravenous injection; nd, non-detected; PND, post-natal day;
[a] mg/kg-day for repeated exposures
[b] for the liver and kidney, when available, concentrations are shown as fold relative to plasma/serum
[c] As reported in Benskin et al. (2009)
[d] values digitized from graphical data prior to conversion
[e] Liver and kidney concentrations relative to blood (different from relative to plasma)
[f] Only lowest dose presented here, data were reported for two more doses (Gao et al., 2015a)
[g] Corresponds to 0.077, 0.38 and 1.8 µg/kg-day, respectively (Iwabuchi et al., 2017)
[h] Absolute levels per organ, as reported (Fujii et al., 2015)
[i] In the high dose group, dose was 30 mg/kg-day for 12 days, at which point dosing was discontinued due to toxicity; dosing was resumed at day 22 at 20 mg/kg-day (Butenhoff et al., 2004a)
[j] No blood or serum samples reported
[k] Blood levels of PFOA and PFOS from a similar population were reported in Ericson et al. (2007) and were 1.80 ng/ml and 7.64 ng/ml, respectively

Table A6.2. Protein binding studies for PFOA and PFOS

Reference	Species	Protein or fraction	PFAS	K_d (M) or % binding	Comment
Klevens and Ellenbogen (1954)	bovine	serum albumin	PFOA	3.1×10^{-3}	
Luebker et al. (2002)	rat	L-FABP	PFOA	40% at 10 µM	Percent inhibition of C11 fatty acid binding to L-FABP
			PFOS	70% at 10 µM	
Han et al. (2003)	rat	albumin	PFOA	4×10^{-4}	>90% of PFOA would be bound to serum albumin (prediction)
	human			4×10^{-4}	
Jones et al. (2003)	bird, fish	SHBP (fish)	PFOA	1.7×10^{-5}[b]	Also reported 100% binding of PFOS to BSA in aqueous solution
			PFOS	2.1×10^{-5}[b]	
		CBG (bird)	PFOA	$1.2\text{-}3.1\times10^{-4}$[c]	
			PFOS	$2.5\text{-}2.7\times10^{-4}$[c]	
Kerstner-Wood et al. (2003)[a]	rat, monkey, human	protein fraction of plasma	PFOA	97-100%	
Messina et al. (2005)	human	serum albumin	PFOA	6.9×10^{-6}, 3.2×10^{-5}	Dissociation constants for a two-step binding reaction
Chen and Guo (2009)	human	albumin	PFOA	3.7×10^{-6}	
			PFOS	4.5×10^{-5}	
Li et al. (2009)	human	serum albumin	PFOS	2×10^{-4}	
Weiss et al. (2009)	human	transthyretin	PFOA	0.95×10^{-6}	IC_{50} values measured at 55 nM T4 in vitro
			PFOS	0.94×10^{-6}	
Wu et al. (2009)	human	albumin	PFOA	2.6×10^{-5} 1.3×10^{-3}	Dissociation constants[d] for a two-step binding reaction
Bischel et al. (2010)	human	albumin	PFOA	$0.7\text{-}5\times10^{-6}$	
MacManus-Spencer et al. (2010)	bovine	serum albumin	PFOA	$0.7\text{-}3\times10^{-5}$; 0.01-0.02	Concentration-dependent K_d values for a two-site model
Qin et al. (2010)	bovine	serum albumin	PFOA	2.1×10^{-5}	Detailed thermodynamic study
Salvalaglio et al. (2010)	human	serum albumin (in silico)	PFOA	95%	Predicted binding rate in human blood over range of concentrations
			PFOS	98%	
Woodcroft et al. (2010)	rat	L-FABP	PFOA	13.1×10^{-6} 23.8×10^{-6}	Two binding sites predicted by displacement
			PFOA	3.1×10^{-6} 26.2×10^{-6}	Three binding sites predicted by

Reference	Species	Protein or fraction	PFAS	K_d (M) or % binding	Comment
				52.6×10^{-6}	isothermal titration calorimetry
Zhang et al. (2013a)	human	L-FABP	PFOA	50.4×10^{-6}	
			PFOS	18.5×10^{-6}	
Beesoon and Martin (2015)	human	serum albumin	n-PFOS	8×10^{-8}	Lower binding affinities for branched PFOA and PFOS
			n-PFOA	1×10^{-4}	
Ren et al. (2015)	human	thyroid receptor	PFOA	42×10^{-6}	IC_{50} at 100 nM T3, in competitive binding
			PFOS	16×10^{-6}	
Ren et al. (2016)	human	transthyretin	PFOA	60×10^{-9}	Detected by competitive binding
			PFOS	20×10^{-9}	
		thyroxine-binding globulin	PFOA	ND	
			PFOS	ND	
Sheng et al. (2016)	human	L-FABP	PFOA	$2.4\text{-}6.5\times10^{-6}$	
EFSA (2018)	human	albumin. low-density lipoproteins	PFOS	Strong binding	Referenced unpublished study
		α-globulin, γ-globulin		Medium to weak binding	

BSA, bovine serum albumin; CBG, corticosteroid-binding globulin; K_d, dissociation constant; L-FABP, liver fatty acid-binding protein; M, mol/L; ND, not detectable; n-PFOA, n-PFOS, linear isomers; SHBP, sex-hormone binding protein; T3, triiodothyronine; T4, thyroxine
[a] as reported in US EPA (2016b)
[b] relative to estrogen (Jones et al., 2003)
[c] relative to cortisol (Jones et al., 2003)
[d] dissociation constants are calculated from reported Gibbs energies (Wu et al., 2009; Qin et al., 2010)

Table A6.3. Experimental evidence[a] for PFOA and PFOS transporters

Reference	Transporter	PFAS	K_m[b] or transport	Comment
Kudo et al. (2002)	rat OAT2, OAT3	PFOA	both active	In vivo study, correlation of expression with PFOA clearance
Katakura et al. (2007)	rat OATP1, OAT3	PFOA	both active	Lack of Mrp2 transport: Mrp2-deficient rats had similar renal clearance of PFOA to wild type rat; no transport by rat Npt2
Nakagawa et al. (2008)	human OAT1	PFOA	48 µM	No transport by human or rat OAT2
	human OAT3		49.1 µM	
	rat OAT1		51 µM	
	rat OAT3		80.2 µM	
Nakagawa et al. (2009)	human OAT4	PFOA	active	Uptake was reported but no kinetic characteristics
Yang et al. (2009a)	rat OATP1a1	PFOA	162 µM	Perfluorocarboxylate inhibition activity profile
	rat OAT1	PFOA	43.2 µM	No transport by rat OAT2, URAT1

Reference	Transporter	PFAS	K_m[b] or transport	Comment
Weaver et al. (2010)	rat OAT3		65.7 µM	
	rat OATP1a1		126.5 µM	
Yang et al. (2010)	human OAT4	PFOA	310 µM	No transport by human OATP1A2
	human URAT1		64.1 µM	
Kummu et al. (2015)	human OAT4	PFOA, PFOS	high correlation	Transport in vitro (perfusion); transporter presumed active if transporter protein expression correlated with PFAS transport efficiency
	human ABCG2		no correlation	
Zhao et al. (2015a)	human NTCP	PFOS	130 µM	Also observed transport by human ASBT, and human OSTα/β for PFOS

ASBT, apical sodium-dependent bile salt transporter; ABCG2, ATP-binding cassette transporter G2; K_m, apparent affinity constant; Mrp2, multidrug resistance-associated protein 2; Npt2, type II sodium-dependent phosphate transporter; NTCP, Na^+/taurocholate co-transporting polypeptide; OAT, organic anion transporter; OATP, organic anion-transporting polypeptide; OST, organic solute transporter; URAT, urate transporter (member of OAT family of transporters).
[a] In vitro studies unless indicated otherwise.
[b] Only mean values are presented.

Table A6.4. Estimate of net renal tubular reabsorption of PFOA in different species (adapted from Han et al. (2012))

Reference	Species /Sex	GFR (ml/day-kg)	CL_R (ml/kg-day)	Net reabsorption (ml/kg-day)[a]	% reabsorbed[b]	% excreted
Kudo and Kawashima (2003)	rabbit female	4,000	670	-	not applicable[c]	-
	rabbit male	4,000	640	-	not applicable[c]	-
Kemper (2003, unpublished study)	rat female	14,400	666	-	not applicable[c]	-
	rat male	14,400	18.2	270	93.7%	6.3%
Ohmori et al. (2003)	rat female	14,400	1,009[d]	-	not applicable[c]	-
	rat male	14,400	21.2[d]	267	92.7%	7.3%
Kudo and Kawashima (2003)	dog female	5,300	50.8	55	52%	48%
	dog male	5,300	43	63	59%	41%
Harada et al. (2005a)	Japanese macaque female	8,500	32	138	81.2%	18.8%
	Japanese macaque male	8,500	15	155	91.2%	8.8%
	mouse female	16,700	16	318	95.2%	4.8%

Reference	Species /Sex	GFR (ml/day-kg)	CL_R (ml/kg-day)	Net reabsorption (ml/kg-day)[a]	% reabsorbed[b]	% excreted
Kudo and Kawashima (2003)	mouse male	16,700	10	324	97%	3%
This document (Table 4.5.1	human	2,570	0.06	51.3	99.8%	0.2%

CL_R, renal clearance; GFR, glomerular filtration rate.
[a] Net tubular reabsorption = $f_u \times GFR - CL_R$, where f_u, the unbound fraction is assumed to be 0.02.
[b] Percent reabsorbed = (net reabsorption)/($f_u \times GFR$) $\times 100$
[c] Overall net tubular secretion, and not reabsorption, is observed ($f_u \times GFR < CL_R$).
[d] Values digitized from graph (Ohmori et al., 2003).

Table A6.5. PBPK models for PFOA and PFOS

Reference	Type	Species	PFAS	Optimization data	Validation data	Comment
Andersen et al. (2006)	3-cmpt	monkey	PFOA PFOS	Noker and Gorman (2003, unpublished); Butenhoff et al. (2004a); Seacat et al. (2002)	high dose (20 mg/kg-day) in Butenhoff et al. (2002); Butenhoff et al. (2004a)	First model to incorporate saturable renal reabsorption; over-predicts PFOS plasma concentrations at high dose
Harris and Barton (2008)	8-cmpt	rat	PFOS	Johnson et al. (1979a, b; unpublished); 3M (2002, unpublished)	Same as optimization studies	Time-dependent urinary elimination rate constant and liver-to-plasma partition coefficient; over-predicts plasma concentrations
Tan et al. (2008)	5-cmpt	rat, monkey	PFOA PFOS	Noker and Gorman (2003, unpublished); Kemper (2003, unpublished); Johnson et al. (1979a, b; unpublished)	Kemper (2003, unpublished); Butenhoff et al. (2002)	Time-dependent descriptors for free fraction and V_d; poor prediction for female rat/PFOA; PFOA data used for validation of monkey/PFOS model; derivation of Andersen et al. (2006) model
Lou et al. (2009)	1-cmpt 2-cmpt	mouse	PFOA	Lou et al. (2009)	high dose in Lou et al. (2009)	Unsatisfactory fit with high doses
Rodriguez et al. (2009)	2-cpmt lactation	mouse	PFOA	Lau et al. (2006); Abbott et al. (2007)	Lau et al. (2006); Wolf et al. (2007)	Gestational and lactational model; tendency to overestimate plasma levels
Loccisano et al. (2011)	9-cmpt	monkey, human	PFOA PFOS	Butenhoff et al. (2004a); Emmett et al. (2006); Kudo et al. (2007); Olsen et al. (2007); Calafat et al. (2007a); Calafat et al. (2007b); Hölzer et al. (2008); (Steenland et al., 2009); Bartell et al. (2010)	Emmett et al. (2006)	Derivation of Andersen et al. (2006) and Tan et al. (2008) models; time-dependent free fraction; model predicted a sharper increase in PFOS plasma concentrations than observed
Loccisano et al. (2012a)	8-cmpt	rat	PFOA PFOS	DePierre (2009, unpublished); Kemper (2003, unpublished); Perkins et al. (2004); Kudo et al. (2007)	Same as optimization studies; additional unpublished data (3M); Seacat et al. (2002)	Time-dependent free fraction (for PFOS)

Reference	Type	Species	PFAS	Optimization data	Validation data	Comment
Loccisano et al. (2012b)	14-17 cmpt	rat	PFOA PFOS	Hinderliter et al. (2005)	Lau et al. (2003); Thibodeaux et al. (2003); Hinderliter et al. (2005); Luebker et al. (2005a); Luebker et al. (2005b); Butenhoff et al. (2009); Chang et al. (2009); Yu et al. (2009)	Gestational and lactational models (separate); derivation of Loccisano et al. (2012a) model, with added dynamic changes in gestation and lactation physiology with minimum PFAS-specific optimization; time-dependent free fraction (for PFOS); over-prediction in pup plasma and tissues
Loccisano et al. (2013)	14-cmpt	human	PFOA PFOS	Kärrman et al. (2007); Midasch et al. (2007); Fromme et al. (2010); Haug et al. (2010)	Inoue et al. (2004); Tittlemier et al. (2004); Fei et al. (2007); Monroy et al. (2008); Fromme et al. (2009); Fromme et al. (2010); Hanssen et al. (2010); Liu et al. (2011); Kim et al. (2011a); Kim et al. (2011b)	Gestational and lactational model with many acknowledged limitations, particularly lack of transporter data; derivation of the rat model (Loccisano et al., 2012b) and human model (Loccisano et al., 2011), with added dynamic changes in gestation and lactation physiology with minimum PFAS-specific optimization; under-prediction for PFOA/milk
Wambaugh et al. (2013)	3-cmpt	rat, mouse, monkey	PFOA PFOS	Kemper (2003, unpublished); Seacat et al. (2002); Butenhoff et al. (2004a); DeWitt et al. (2008); Lou et al. (2009); Chang et al. (2012)	Same as optimization studies	Probabilistic derivation of Andersen et al. (2006) model; wide confidence intervals for optimized parameters
Fabrega et al. (2014); Fabrega et al. (2016)	10-cmpt	human	PFOA PFOS	Maestri et al. (2006); Ericson et al. (2007); Fabrega et al. (2014)	Perez et al. (2013)	Derivation of Loccisano et al. (2011) model; underestimation of steady state serum levels; first human model to be optimized at organ level
Worley and Fisher (2015)	8-cmpt	rat	PFOA	Kemper (2003, unpublished); Kudo et al. (2002)	Kemper (2003, unpublished); Kudo et al. (2002)	First model to parameterize renal transport on individual transporter in vitro data; includes in vitro to in vivo extrapolations; only calibrated for single doses
Tarazona et al. (2016)	1-cmpt 2-cmpt	rabbit	PFOS	Tarazona et al. (2016)	Same as optimization data	1-compartment model provided best fit

Reference	Type	Species	PFAS	Optimization data	Validation data	Comment
Verner et al. (2016)	2-cmpt	human	PFOA PFOS	Published parameters ($T_{1/2}$, V_d, placental transfer, lactational transfer)	Magnus et al. (2006); Fromme et al. (2010); Granum et al. (2013)	Gestational and lactational model, assessed variability in a simple mother-child model; predicts significant peak in child/mother ratio at ~6 months
Cheng and Ng (2017)	19-cmpt	rat	PFOA	Model was built without optimization	Kemper (2003, unpublished); Kudo et al. (2007); Kim et al. (2016b)	Permeability-limited model, only single dose prediction, narrow confidence intervals for predicted plasma concentrations.
Worley et al. (2017a)	8-cmpt	human	PFOA	ATSDR (2016)	Bartell et al. (2010)	Derivation of Worley and Fisher (2015) model
Convertino et al. (2018)	2-cmtp	human	PFOA	Elcombe et al. (2013)	Same as optimization data	Stochastic model for dose-response of adverse effects in a pharmacological trial of PFOA in human cancer patients
Chou and Lin (2019)	7-cmpt	rat, mouse, monkey, human	PFOS	Johnson et al. (1979; unpublished); 3M (2002, unpublished); Haug et al. (2009); Chang et al. (2012); Kim et al. (2016b)	Seacat et al. (2002); Olsen et al. (2003b); Olsen et al. (2003c); Olsen et al. (2008); Fabrega et al. (2014)	Derivation of Worley et al. (2017a) model; Bayesian-MCMC simulation; some parameter values sourced from other models such as Loccisano et al. (2011)
Goeden et al. (2019)	2-cmpt	human	PFOA	Summary statistics of published cord-maternal serum and breastmilk transfer studies	Fromme et al. (2010); Mogensen et al. (2015a)	Gestational and lactational model in Excel; predicts a 6-fold increase (relative to mother's serum) in infant PFOA over the first year of life

cmpt, compartment; MCMC, Markov chain Monte Carlo; $T_{1/2}$, half-life; V_d, volume of distribution

Table A6.6. Comparison of reported PFOA serum concentrations (C_{serum}) in subchronic mouse studies to concentrations obtained from PBPK models

Study	Route/duration	Dose (mg/kg-day)	Reported C_{serum} (mg/L)	Calculated[#] C_{serum} Wambaugh et al. (2013) model (mg/L)	Calculated[#] C_{serum} Rodriguez et al. (2009) model (mg/L)
Lau et al. (2006)	Oral gavage 17 days	1 3 5 10 20 40	21.9 40.5 71.9 116 181 271	50.7 52.2 53.8 57.6 65.4 80.8	23.8 57.8 84 136 227 396
Li et al. (2017b)	Oral gavage 28 days	 0.05 0.5 2.5	male/female 1.2/0.97 5.9/2.7 13.5/9.5	not sex-specific 49.9 50.3 53.2	not sex-specific 1.4 12.8 50.4

[#] Value calculated by OEHHA, based on model codes and parameter values obtained from corresponding publications.

APPENDIX 7. HUMAN EPIDEMIOLOGY STUDIES – GENERAL METHODS AND SUMMARY TABLES

General Methods

Literature search

OEHHA attempted to identify all published human epidemiologic literature on the possible health effects of PFOS and PFOA. For most health outcomes, the most recent reviews by the US Environmental Protection Agency (US EPA) were used to identify literature published prior to December 1, 2015, the last day of the literature search performed for these documents. OEHHA searched two electronic databases, PubMed and Embase to identify literature published from December 1, 2015 until January 2, 2020, the last date of the initial full literature review. The exposure portion of the search string included the key words shown below. These keywords are essentially the same as those used by NTP in its recent review of PFOA and PFOS and immunotoxicity (NTP, 2016). The outcome portions of the search string used are given in the sections describing each health outcome.

(perfluoroalkyl*[tiab] OR perfluorocaprylic[tiab] OR perfluorocarbon*[tiab] OR perfluorocarboxyl*[tiab] OR perfluorochemical*[tiab] OR (perfluorinated[tiab] AND (C8[tiab] OR carboxylic[tiab] OR chemical*[tiab] OR compound*[tiab] OR octanoic[tiab])) OR PFAA*[tiab] OR “fluorinated polymer”[tiab] OR “fluorinated polymers”[tiab] OR (fluorinated[tiab] AND (polymer[tiab] OR polymers[tiab])) OR (fluorocarbon[tiab] AND (polymer[tiab] OR polymers[tiab])) OR Fluoropolymer*[tiab] OR (fluorinated[tiab] AND telomer*[tiab]) OR fluorotelomer*[tiab] OR fluoro-telomer*[tiab] OR fluorosurfactant*[tiab] OR “FC 143”[tiab] OR FC143[tiab] OR 335-67-1 [rn] OR Pentadecafluorooctanoate*[tiab] OR Pentadecafluorooctanoate*[tiab] OR pentadecafluoroctanoic[tiab] OR pentadecafluorooctanoic[tiab] OR “pentadecafluoro-1-octanoic”[tiab] OR “pentadecafluoro-n-octanoic”[tiab] OR “perfluoro-1-heptanecarboxylic”[tiab] OR perfluorocaprylic[tiab] OR perfluoroheptanecarboxylic[tiab] OR perfluoroctanoate[tiab] OR perfluorooctanoate[tiab] OR “perfluoro octanoate”[tiab] OR “perfluorooctanoic acid”[nm] OR perfluoroctanoic[tiab] OR perfluorooctanoic[tiab] OR “perfluoro octanoic”[tiab] OR “perfluoro-n-octanoic”[tiab] OR “perfluorooctanoyl chloride”[tiab] OR PFOA[tiab] OR APFO[tiab] OR 1763-23-1[rn] OR 307-35-7[rn] OR “1-octanesulfonic acid”[tiab] OR “1-perfluorooctanesulfonic”[tiab] OR “1-perfluoroctanesulfonic”[tiab] OR “heptadecafluoro-1-octanesulfonic”[tiab] OR “heptadecafluoro-1-octane sulfonic”[tiab] OR “heptadecafluorooctanesulfonic”[tiab] OR “heptadecafluorooctane sulfonic”[tiab] OR “heptadecafluoroctane sulfonic”[tiab] OR “perfluoroalkyl sulphonate”[tiab] OR perfluoroctanesulfonate[tiab] OR perfluorooctanesulfonate[tiab] OR “perfluoroctane sulfonate”[tiab] OR “perfluorooctane sulfonate”[tiab] OR “perfluoro-n-octanesulfonic”[tiab] OR perfluoroctanesulfonic[tiab] OR perfluorooctanesulfonic[tiab] OR “perfluorooctane sulfonic acid”[nm] OR “perfluoroctane sulfonic”[tiab] OR “perfluorooctane sulfonic”[tiab] OR perfluoroctanesulphonic[tiab] OR perfluorooctanesulphonic[tiab] OR “perfluoroctane sulphonic”[tiab] OR “perfluorooctane sulphonic”[tiab] OR perfluoroctylsulfonic[tiab] OR PFOS [tiab])

PubMed and Embase searches were supplemented by also searching the bibliographies of all studies meeting OEHHA’s inclusion and exclusion criteria (described below) and all recent relevant review articles. For some outcomes (e.g., immune toxicity), other sources were searched (e.g., the NTP’s most recent review of this outcome (NTP, 2016)), and these are noted in the sections below.

OEHHA used the following inclusion and exclusion criteria to identify studies for this review. All human epidemiologic studies of PFOS or PFOA and an adverse human health effect presenting

results as mean differences, regression or correlation coefficients, relative risks, or any other appropriate outcome metrics were eligible for inclusion. No restrictions were placed on the methods used to evaluate PFOA or PFOS exposure levels although almost all studies identified assessed exposure using blood or drinking water concentrations. Studies using modeled intakes of PFOS or PFOA based on drinking water or blood levels, industrial hygiene records, or similar factors were also included.

Studies that involved cohort, case-control, and cross-sectional designs were included. Case-reports were excluded because of the lack of a comparison group. Abstracts and studies without original data (e.g., reviews or editorials) were also excluded. Ecologic studies were considered, although the potential for ecologic fallacy (bias that may occur because an association between variables at an aggregate level may not exist at an individual level) and confounding was evaluated for studies using this design. Cross-sectional studies were initially included although the potential for reverse causation or exposure misclassification (e.g., latency effects) were evaluated. There were no exclusions based on study location, language, or statistical adjustments. Several studies reported results only for multiple PFAS combined. Although the results of these studies were considered in this review, they were not included in OEHHA's detailed evaluations when results were not specifically given for PFOS or PFOA. Studies were initially excluded based on title search, and if needed, then by abstract and full article review.

With the rapid expansion of the PFOA/PFOS database, OEHHA expanded its literature review to a cutoff date of September 20, 2020. This review used the same criteria discussed above. Studies identified after January 2, 2020 that influenced OEHHA's conclusions regarding the adverse health effects of PFOA or PFOS are reviewed here but not included in some of the review tables presented in this document.

Assessment of study methods and quality

OEHHA evaluated the quality of each study identified and the major aspects of causal inference using an updated version of the Hill criteria (Hill, 1965). The criteria used in these evaluations are described below, and are similar to those described in the NTP Risk of Bias Tool (NTP, 2019a).

Study design: When assessing causal inference, evidence from cross-sectional and ecologic studies is sometimes considered to be weaker than evidence from studies using cohort, case-control, or randomized clinical trial designs. For example, in order for an exposure to cause an outcome, the exposure must come before the outcome. However, this temporal relationship can be difficult to establish in some cross-sectional studies. Importantly though, there are many exceptions to this broad generalization. In some cross-sectional studies, such as when a clear mechanism leading from the exposure to outcome has been well established, or when the exposure has been shown to be stable over time, one can sometimes be reasonably confident that the exposure preceded the outcome (Shahar and Shahar, 2013). Common criticisms of ecologic studies include ecologic fallacy (bias that may occur because an association between variables at the aggrefate level may not exist at an individual level) and confounding. However, in some ecologic studies, major errors caused by these factors can be ruled out with good confidence (Smith et al., 2018). OEHHA did not automatically rate cross-sectional and ecologic studies as having weaker study designs because of these issues, but rather evaluated each study on its own merits.

Factors related to bias:
Selection: After reviewing the eligibility criteria for each study (if provided), an attempt was made to evaluate whether all eligible people, or a random selection of all eligible people, were invited to participate in the study. If this did not occur, the possibility of selection bias may be increased.
Participation: Of those who were invited to participate, an attempt was made to evaluate the percentage of people who actually agreed to participate and the percentage of people for whom there were sufficient data to be included in the final study analyses. If this percentage is low (e.g., below 60-70%), this may also increase the possibility of selection bias.
Equal groups: Studies were evaluated for whether there were any major socioeconomic status (SES) or other relevant differences between people with higher or lower PFOA or PFOS levels or between people with or without the outcome of interest. If there were, OEHHA evaluated whether the researchers attempted to account for these differences in the statistical analyses. If major differences occurred, the risk of important selection bias or confounding may be increased.
Blinding: Studies were evaluated for whether the researchers measuring the exposure were blinded to the outcome status of the participants and whether the researchers measuring the outcome were blinded to the exposure status of the participants. If not, the risk of exposure or outcome misclassification may be increased.
Above detection: When this review began, an attempt was made to extract information on the percentage of participants who had detectable levels of PFOA or PFOS. A low percentage could limit the precision of the study findings. However, as this review proceeded, it became clear that the very large majority of participants in all of the studies reviewed had detectable levels of PFOA and PFOS, even in studies that involved participants with no obvious high exposure source. As such, detailed information on this factor is not presented for all health outcomes.
Levels: When possible, data on the distribution of PFOA or PFOS levels among the study participants were extracted. In general, true effects are easier to identify when the contrast in exposure within the study population is large. This criterion was also used to identify a possible source of heterogeneity among studies and to identify studies in which exposure levels may be too low to identify true associations.

Exposure and outcome methods: For each study, the methods used to assess PFOA and PFOS exposure levels and the methods used to evaluate the outcome of interest were evaluated. In general, whether each of these were validated, generally accepted, or otherwise reasonable methods for assessing exposure and outcome was examined. Inaccurate or imprecise methods could lead to exposure or outcome misclassification.

Confounding: Factors most likely to cause confounding were identified. This evaluation considered the following four criteria:

1. What factors are strongly related to the exposure of interest?
2. What factors are strongly related to the outcome of interest?
3. Are these factors in the causal chain linking the exposure and the outcome?
4. Which of the factors are prevalent enough to cause important confounding?

To begin this process OEHHA attempted to identify those factors most commonly associated with higher PFOA and PFOS exposures. Studies from a variety of different countries were included based on the idea that the major sources of PFOA and PFOS exposure are likely to be

different across different study populations. A brief review of the results of a few of the more informative studies is shown in Table A7.1.

Table A7.1. Factors associated with higher PFAS exposure: a review of selected studies

Reference	Where/Who	Factors	
Calafat et al., 2007	US NHANES; age ≥12	+males +non-Hispanics +education	?smoker ?BMI
Coakley et al., 2018	New Zealand; adults	+males +age	
Eriksen et al., 2011	Denmark; men	+never smoker +frying food -BMI	-alcohol +eggs not fish
Harris et al., 2017	Boston; children	+fast food consumption -BMI	-Black -higher income
Jain, 2014	US NHANES; age ≥12	PFOA: -caffeine -milk -dry beans +non-alcohol beverages	PFOS: -caffeine +fish +/-fat +meat -milk +alcohol -cheese
Kang et al., 2018	Korea; children	+breast-feeding duration +fish +non-stick frying pan use*	+waterproof clothing* *(effect sizes are small)
Kato et al., 2009	NHANES; ages 3-11	+non-Mexican Americans	
Kato et al., 2016	Japan; children	-parity	not education, income, smoking, fish
Lee et al., 2017	Korea; adults	+males +plastic wrap +disposable cups	+BMI +exercise
Lee et al., 2018	Korean; women (breast milk samples)	+age +BMI +parity +snacks	+eating out +organochlorines, heptachlor, PCBs, DDE
Manzano-Salgado et al., 2016	Spain; pregnant women	-parity +age	+fish
Nelson et al., 2010	NHANES; ages 12-80	+males +non-Hispanic whites	+SES +/-BMI (depends on age)
Olsen et al., 2017	US Red Cross donors; ages 20-69	+male	
Richterová et al., 2018	Slovakia; pregnant women	+fish +age	+higher education -parity
Skuladottir et al., 2015	Denmark; pregnant women	+meat -vegetables	
Tian et al., 2018	China; pregnant women	+age +parity +education +passive smoking -household income +red meat	+poultry intake +fish +pastries +fried food +tap water consumption
Ye et al., 2018	NHANES; ages 3-11	+males +age	+non-Hispanics

Studies are ordered alphabetically; ages are in years unless otherwise stated
Most studies examined PFOA, PFOS, or combined PFAS serum concentrations where PFOA and PFOS were the predominant agents.
Abbreviations: "+," positively associated with higher PFOS, PFOA, or all PFAS combined concentrations; "-," negatively associated with higher PFOS, PFOA, or all PFAS combined concentrations; "?," data are mixed or unclear; BMI, body mass index; DDE, dichlorodiphenyldichloroethylene; NHANES, US National Health and Nutrition Examination Survey; PCB, polychlorinated biphenyls; SES, socioeconomic status

Interpretation of study results

***Statistical significance*:** Each study was evaluated for whether it found evidence for an association between PFOA or PFOS and the outcome of interest and whether the relevant result was statistically significant. Statistical significance was defined as a p-value <0.05 or 95% confidence intervals (CI) that excluded 1.0 for relative risk estimates or 0 for mean differences or correlation or regression coefficients. OEHHA acknowledges that these definitions are somewhat arbitrary, that some results representing true effects may not meet these definitions, and that some results meeting these definitions may not represent true effects. As such, no conclusions were based solely on statistical significance.

***Magnitude of the association*:** The relative risk estimate (e.g., odds ratio (OR)) was evaluated to determine whether it was greater than 1.2 (or <0.83 if less than 1.0); mean differences were greater than 10%; correlation coefficients were >0.10; or regression coefficients seemed to indicate an effect size >10% between high and low exposure groups. Studies meeting these criteria were labeled as "Large magnitude: yes" in the review tables. This criteron is similar to the "Large magnitude" criterion used by NTP (2019b) and the "Strength of the association" criterion used by AB Hill (1965). In general, small effect sizes (e.g., relative risks close to 1.0 or mean differences close to 0) are more likely to be due to confounding or bias than larger effect sizes (Axelson, 1978; Schlesselman, 1978). Use of these criteria is not meant to imply that all small effect sizes are due to confounding or bias or that all large effect sizes are real. OEHHA also acknowledges that the specific criteria used here are somewhat arbitrary and were sometimes difficult to quantify. This was especially true for linear regression coefficients, where effect sizes were oftentimes difficult to express as a simple single percentage. Importantly though, these criteria were not used as sole determinants of causality, but rather only used to help identify some results that might be especially prone to important bias or confounding.

***Dose-response*:** If an association was identified, the shape of the dose-response curve was evaluated. For many toxic chemical exposures, as the level of exposure increases the degree of toxicity or the numbers of people with the toxic endpoint also increases. Importantly though, dose-response relationships are not always mono-tonic or linear. Here, when statistically significant effects were seen, OEHHA evaluated whether or not a dose-response relationship was seen and attempted to describe the pattern of this relationship. In general, dose-response patterns that are similar across different studies may be more likely to represent real effects. When patterns were not mono-tonic or were not consistent from one study to the next, OEHHA explored whether there might be a potential reason for this.

***Temporal assessment*:** Studies were evaluated as to whether the exposure that was assessed likely occurred before the outcome occurred. Further discussion of this issue is presented under "Study design" above.

***Subgroup only*:** Studies were evaluated for whether statistically significant results were only identified in a specific subgroup of participants. Some studies presented results for all participants combined and separate results for certain subgroups (e.g., males and females). This criterion was considered because increasing the number of subgroups assessed could be helpful for identifying a particularly susceptible group, and evaluating whether a study may have missed an important subgroup. However, examining multiple subgroups in a single study could also increase the chance of false positive results (i.e., the issue of "multiple comparisons").

***Adjustments*:** Studies were evaluated for whether large changes in results were seen following statistical adjustments. These large changes could indicate that a major confounder was present in the original study design. However, they could also be the result of a statistical artifact (e.g., co-variance). In addition, if adjustments move the effect measure closer to the null (e.g., a relative risk of 1.0), a large difference between the adjusted and unadjusted result would raise concerns that any remaining association in the adjusted results might be due to residual confounding. If a large difference between adjusted and unadjusted results was seen, OEHHA evaluated whether the authors provided information that could be used to identify which particular confounder caused this difference. Generally, this involved evaluating information on which potential confounders were strongly associated with both the exposure and the outcome of interest, and information showing that these associations were in the proper direction to cause the potential confounding observed. Overall, unexplained large differences between adjusted and unadjusted results were considered a weakness of the study.

Immunotoxicity

Literature search and methods

OEHHA reviewed a number of previously published review documents on the possible immunotoxicity of PFOA and PFOS, including those published by the NTP (NTP, 2016), US EPA (US EPA, 2016b; US EPA, 2016d) and Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2018a). OEHHA also identifed and reviewed the human epidemiologic evidence published since the 2016 NTP report (NTP, 2016). To do this OEHHA attempted to identify all human epidemiologic evidence on the immunotoxicological effects of PFOS and PFOA published between May 18, 2016 (the end of the NTP literature review) and September 20, 2020 (the end of this literature review) using the methods described above. The exposure portion of the search string OEHHA used is given in the *General Methods* section above. The outcome portion of the search string used is shown below. This is the same search string used by NTP (2016b).

(immunology[sh] OR immune[tiab] OR immunocomp*[tiab] OR immunogen*[tiab] OR immunolog*[tiab] OR immunotox*[tiab] OR immunotoxins[mh] OR immunity[tiab] OR autoimmun*[tiab] OR "host resistance"[tiab] OR immunocompetence[mh] OR "immune system"[mh] OR spleen[tiab] OR splenic[tiab] OR splenocyt*[tiab] OR thymus[tiab] OR thymic[tiab] OR thymocyt*[tiab] OR leukocyt*[tiab] OR granulocyt*[tiab] OR basophil*[tiab] OR eosinophil*[tiab] OR neutrophil*[tiab] OR lymph[tiab] OR lymphoid*[tiab] OR lymphocyt*[tiab] OR "b-lymphocyte"[tiab] OR "b-lymphocytes"[tiab] OR "t-lymphocyte"[tiab] OR "t-lymphocytes"[tiab] OR "killer cell"[tiab] OR "killer cells"[tiab] OR "NK cell"[tiab] OR "NK-cell"[tiab] OR "NK-cells"[tiab] OR macrophag*[tiab] OR "mast cell"[tiab] OR "mast cells"[tiab] OR monocyt*[tiab] OR phagocyt*[tiab] OR dendrit*[tiab] OR "t-cell"[tiab] OR "t cell"[tiab] OR "t cells"[tiab] OR "t-cells"[tiab] OR "T helper"[tiab] OR "T-helper"[tiab] OR "b-cell"[tiab] OR "b cell"[tiab] OR "b cells"[tiab] OR "b-cells"[tiab] OR antibod*[tiab] OR histamine*[tiab] OR histocompatib*[tiab] OR immunoglobulins[mh] OR immunoglobulin*[tiab] OR "immunoglobulin A"[tiab] OR IgA[tiab] OR "immunoglobulin D"[tiab] OR IgD[tiab] OR "immunoglobulin E"[tiab] OR IgE[tiab] OR "immunoglobulin G"[tiab] OR IgG[tiab] OR "immunoglobulin M"[tiab] OR IgM[tiab] OR "antigens, CD"[mh] OR CD3 [tiab] OR CD4 [tiab] OR CD8 [tiab] OR CD25 [tiab] OR CD27 [tiab] OR CD28 [tiab] OR CD29 [tiab] OR CD45*[tiab] OR cytokines[mh] OR cytokine*[tiab] OR chemokine*[tiab] OR inteferon*[tiab] OR interleukin*[tiab] OR "IL-6"[tiab] OR "IL-8"[tiab] OR lymphokine*[tiab] OR monokine*[tiab] OR ("tumor necrosis"[tiab] AND (factor[tiab] OR factors[tiab])) OR "TNF alpha"[tiab] OR "TNFalpha"[tiab] OR "immune system diseases"[mh] OR autoimmun*[tiab] OR addison[tiab] OR rheumatoid[tiab] OR glomerulonephritis[tiab] OR diabetes[tiab] OR graves[tiab] OR lupus[tiab] OR thyroiditis[tiab] OR hypersensitiv*[tiab] OR sensitization OR hyperresponsiv*[tiab] OR allergy[mh] OR allerg*[tiab] OR atopy[tiab] OR atopic[tiab] OR dermatitis[tiab] OR eczema[tiab] OR otitis[tiab] OR “ear infection”[tiab]

OR “ear inflammation”[tiab] OR Respiratory tract infections[mh] OR (respiratory[tiab] AND infection*[tiab]) OR asthma[tiab] OR bronchitis[tiab] OR pneumonia[tiab] OR bronchiolitis[tiab] OR rhinitis[tiab] OR sinusitis[tiab] OR wheez*[tiab] OR crackle*[tiab] OR cough[mh] OR cough*[tiab] OR dyspnea[tiab] OR gastroenteritis[tiab] OR inflammation[mh] OR inflammat*[tiab] OR pro-inflammat*[tiab] OR anti-inflamm*[tiab] OR "inflammation mediators"[mh] OR autacoid*[tiab] OR eicosanoid*[tiab] OR prostaglandin*[tiab] OR immunomodulation[mh] OR immunomodul*[tiab] OR immunotherap*[tiab] OR vaccin*[tiab] OR immuniz*[tiab] OR immunosuppress*[tiab] OR desensitiz*[tiab] OR immunoproteins[mh] OR immunoprotein*[tiab] OR "c-reactive protein"[tiab] OR CRP[tiab] OR "complement component" [tiab] OR (complement[tiab] AND (C1 OR C2 OR C3 OR C4 OR C5 OR C6 OR C7 OR C8 OR C9)))

OEHHA’s literature search was done as described in the *General Methods* section above. In their review, NTP (2016) divided study results into five major outcome categories. These were antibody response, infectious disease or disease resistance, natural killer cell activity, hypersensitivity, and autoimmunity. Some of the specific diseases, conditions, or effects assessed within each of these categories are shown in Table A7.2. OEHHA classified studies based on these same five categories, plus an additional category labeled “Other,” which includes immune related outcomes not covered under the other five categories. OEHHA excluded studies in which the outcome was telomere length, metabolomics, or Clara cell protein levels. Because the strongest evidence linking PFOA or PFOS to immunotoxicity identified by NTP (2016) was for antibody responses, a number of additional detailed evaluations for this particular outcome are presented.

Table A7.2. Categories of immune toxicity and the specific outcomes in each

Major category	Specific outcomes
Antibody response	IgG levels to vaccines
Infectious disease or disease resistance	Hospitalizations for infectious disease Gastroenteritis Otitis media Colds Influenza
Natural killer cell activity	Multiple
Hypersensitivity	IgE Asthma or related symptoms Eczema Allergy Eosinophils Rhinitis COPD
Autoimmunity	Crohn’s disease Ulcerative colitis Lupus Type 1 diabetes Multiple sclerosis Rheumatoid arthritis IgG Autoantibodies
Other	CRP White blood cell counts Cytokine levels

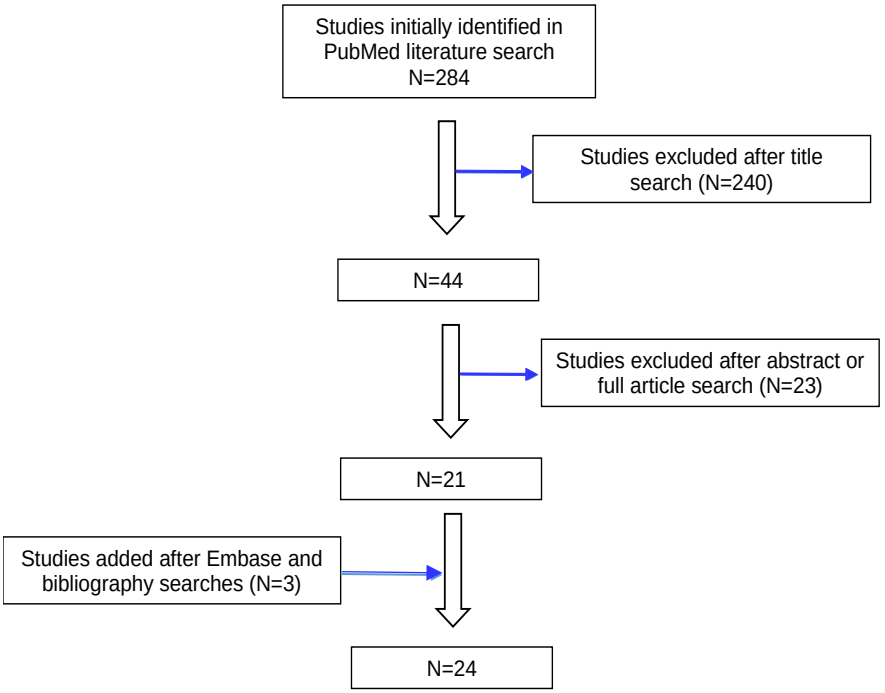
OEHHA evaluated study quality using the factors described above. In the overall evaluation of the evidence, OEHHA considered the possibility that cross-sectional studies might be a weaker

design since certain immunologic outcomes or diseases (or the products associated with their treatment) could potentially lead to increased PFOA or PFOS exposure. In addition, the following factors were identified as those that are especially likely to be potential confounders, that is, prevalent factors that appear to be related both to PFOA or PFOS exposure and immune function (see Table A7.1; de Bruyn 2010; Patin et al., 2018; Van Loveren et al., 2001): age, sex, race/ethnicity, socioeconomic determinants, body mass index (BMI), breast-feeding, and other chemical exposures like polychlorinated biphenyls (PCBs), other PFAS, or organochlorines. These are similar to the potential confounders identified by NTP (2016) in their immunotoxicity review.

Results

A general description of the literature search is provided in Figure A7.1. A list of studies excluded based on OEHHA's abstract or full article review is provided in Table A7.28.

Figure A7.1. Literature search: recent epidemiologic studies of PFOA or PFOS and immunotoxicity*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

Twenty-four publications published since the 2016 NTP review (NTP, 2016) met the inclusion criteria described above. For each, study descriptions and factors related to OEHHA's evaluations of bias and causality are presented in Tables A7.3 (for PFOA) and A7.4 (for PFOS). A number of publications presented results for several different outcomes, and results for each outcome are presented separately in these tables.

Table A7.3. Recent epidemiologic studies on PFOA and immunotoxicity

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Grandjean et al., 2017a	Faroe Islands 1997-2000 and 2007-09 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤5 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 2.8 (2.0-4.5) ng/ml at age 18 months	Serum Near birth, 18 months, age 5	Antibody response: diphtheria	Diphtheria IgG Age 5	**2007-09 cohort:** PFOA IgG %change Birth -18.9 (p=0.03) 18 mo. 4.1 (p=0.63) 5 yr. 18.3 (p=0.24) **Combined cohort:** PFOA IgG %change Birth -17.8 (p=0.009) 18 mo. 5.4 (p=0.52) 5 yr. 3.4 (p=0.73) No major differences between 1997-2000 and 2007-09 cohorts	Percent change for a 2-fold increase in PFOA	Adjusted for age and sex Additional adjustments for PCB concentrations and Cesarean section had little effect	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	PFAS concentrations highly correlated with breast-feeding duration Correlation coefficients for PFAS levels up to 0.7 for age 18 months and age 5 years 44% and 36% had IgG below protective levels at age 5 for diphtheria and tetanus, respectively Combined: 2007-09 and 1997-2000 Faroe Islands cohorts combined
Grandjean et al., 2017b	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤13 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 4.4 (3.6-5.7) ng/ml	Serum Ages 7 and 13	Antibody response: diphtheria	Diphtheria IgG Age 13	PFOA IgG %change Age 7 -9.2 (p=0.48) Age 13 -25.3 (p=0.03)	Percent change for a 2-fold increase in PFOA	Adjusted for age, sex, booster type at age 5 Additional adjustment for PCBs had little effect	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: see notes Subgroup only: no Adjustments: unclear	PFAS concentrations at age 13 correlated with those at age 7 but not with maternal levels Excludes those with likely booster after age 5 Decline seen prospectively but not statistically significant
Stein et al., 2016a	New York 2010-11	Prospective cohort	Who: adults Ages: 18-49 N: 75	Selection: unclear Participation: unclear Equal groups: yes although analyses limited Blinded: yes Above detection: 100% Levels: geometric mean = 2.28, upper tertile range = 2.8-8.1 ng/ml	Serum Day 0	Antibody response: FluMist	Anti-A H1N1 antibody response by histochem-ical staining (IHC) and hemagglut-ination-inhibition (HAI) Day 30	OR = 6.8 (1.0-48.1) p-trend = 0.07 for HAI OR = 1.8 (0.7-48.1) p-trend=0.27 for IHC	Upper vs. lower tertile of PFOA	Adjusted for age, sex, and race/ethnicity "No covariates… associated with both exposure and outcome"	Large magnitude: yes Statistical significance: borderline Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	Less than 20 subjects seroconverted
Pilkerton et al., 2018	US NHANES 1999-2000, 2003-04	Cross-sectional	Who: children and adults Ages: ≥12 N: 2,389	Selection: multi-stage cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Above detection: unclear but low LOD Levels: mean = 4.3-6.0 ng/ml	Serum	Antibody response: rubella	Serum IgG	4th vs. 1st quartile: Men: β = -0.4450 (p=0.03) Women: β = -0.1658 (p=0.68) Per quartile increase: For all adults combined: F-value = 6.60 (p=0.002) Children (0-18): no association (p=0.80)	Fourth vs. first quartile of PFOA; linear regression coefficients for increasing PFOA quartile	Adjusted for age, BMI, education, sex, and ethnicity, and parity	Large magnitude: unclear Statistical significance: yes Dose-response: step-shaped Temporal association: no Subgroup only: yes, men Adjustments: unclear	Results for children only and all adults presented only as F-values Gender specific analyses are for adults only

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Grandjean et al., 2017a	Faroe Islands 1997-2000 and 2007-09 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤5 N: 349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 2.8 (2.0-4.5) ng/ml at age 18 months	Serum Near birth, 18 months, age 5	Antibody response: tetanus	Tetanus IgG Age 5	**2007-09 cohort:** PFOA IgG %change Birth -22.2 (p=0.007) 18 mo. -16.3 (p=0.03) 5 yr. -25.3 (p=0.03) **Combined cohort:** PFOA IgG %change Birth -17.6 (p=0.007) 18 mo. -16.5 (p=0.03) 5 yr. -18.7 (p=0.02) No major differences between 1997-2000 and 2007-09 cohorts	Percent change for a 2-fold increase in PFOA	Adjusted for age and sex Additional adjustments for PCB concentrations and Cesarean section had little effect	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	PFAS concentrations highly correlated with breast-feeding duration Correlation coefficients up to 0.7 for age 18 and age 5 PFAS levels 44% and 36% had IgG below protective levels at age 5 for diphtheria and tetanus, respectively Combined: 2007-09 and 1997-2000 Faroe Islands cohorts combined
Grandjean et al., 2017b	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤13 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 4.4 (3.6-5.7) ng/ml	Serum Ages 7 and 13	Antibody response: tetanus	Tetanus IgG Age 13	PFOA IgG %change Age 7 2.9 (p=0.86) Age 13 -5.6 (p=0.71)	Percent change for a 2-fold increase in PFOA	Adjusted for age, sex, booster type at age 5 Additional adjustment for PCBs had little effect	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS concentrations at age 13 correlated with those at age 7 but not with maternal levels Excludes those with likely booster after age 5
Hammer et al., 2019	Faroe Islands 1986-2009	Prospective cohort	Who: children and adults Ages: all N: 5,698	Selection: involved in previous cohort studies Participation: 75% of all Faroese births during the recruitment periods Equal groups: unclear Blinded: unclear Above detection: not given Levels: unclear	Serum	Autoimmunity: inflammatory bowel disease	Nationwide Inflammatory Disease database	OR = 0.60 (0.23-1.56)	Upper vs. lower tertile of PFOA	Adjusted for age and calendar period	Large magnitude: yes (below 1.0) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Includes six different cohorts and a wide range of follow-up periods 37 cases
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Ages: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Hypersensitivity: allergy (all)	ISAAC questionnaire Age 4	No association Similar results in males and females	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	All allergic diseases and related symptoms Combines eczema, wheezing and rhinoconjunctivitis

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: allergy	Question-naire and skin prick test Ages 5 and 13	**PFOA in maternal serum:** No association for outcome at ages 5 or 13 **PFOA at age 5:** No association for outcome at ages 5 or 13 **PFOA at age 13 and outcome at age 13:** No association	OR for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at ages 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all first year students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: allergy (food)	Self-reported or FX5 food panel IgE Ages 16 (self-reported) and 18 (IgE)	OR = 0.27 (0.12-0.65) (food sensitization, IgE) No association with self-reported food allergies	Above vs. below the median PFOA	Adjusted for sex, age, BMI, physical activity, SES, diet, and allergy medications	Large magnitude: yes Statistical significance: yes Dose-response: not assessed Temporal association: yes Subgroup only: no Adjustments: unclear	Few details provided F/U: 3 years
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: allergy (food)	Self-reports	ORs = 1.32 (0.92-1.90) for current allergy (higher in boys), near 1.0 for "ever" allergy	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Averina et al., 2019	Northern Norway 2010-13	Cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: allergy (nickel)	Self-reported	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided OR not given
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: asthma	MeDALL question-naire Ages 16 and 18	OR = 2.07 (1.01-4.23) cross-sectional study Prospective results not provided	Fourth vs. first quartiles of PFOA	Unclear	Large magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: unclear	F/U: 3 years Few details provided

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Beck et al., 2019	Odense, Denmark 2010-12	Prospective cohort	Who: children Ages: 5 N: 981	Selection: all pregnant women in the municipality Participation: <43% Equal groups: asthma or wheeze greater in boys, smoking parents, and family history of asthma Blinded: unclear Levels: median (IQR) = 1.68 (1.13-2.35) ng/ml	Maternal serum, weeks 8-16	Hyper-sensitivity: asthma	ISSAC question-naire age 5	Doctor diagnosed asthma: Girls: OR = 1.70 (0.63-4.56) Boys: OR = 0.72 (0.46-1.12) Elevated OR for self-reported asthma in boys Wheeze: all ORs near 1.0	OR for a doubling of PFOA concentratio n	Adjusted for parity, education, BMI, asthma predisposition, and sex Also stratified by sex	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: inconsistent by sex Adjustments: small changes only	Includes asthma and wheezing Samples stored at -80º C Self-reported asthma: only those who did not also report doctor diagnosed asthma Inconsistent results across sexes and self-reports vs. doctor diagnosed asthma
Gaylord et al., 2019	US 2014-16	Cross-sectional	Who: children and adults Ages: 13-22 N: 287	Selection: exposed to the World Trade Center disaster as children and controls Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: mean (SD) = 1.53 (0.65) ng/ml	Serum	Hyper-sensitivity: asthma	Question-naire: similar to NHANES	OR = 1.34 (0.55-3.29)	OR per unit change in log PFOA	Adjusted for sex, race/ethnicity, BMI, and tobacco smoke exposure	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: yes, unadjusted OR = 1.00	
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: asthma	ISAAC question-naire Age 4	OR = 1.25 (0.71-2.22) in males for wheezing	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: yes, males and wheezing Statistical significance: no Dose-response: no Temporal association: no Subgroup only: males Adjustments: little change	Includes asthma and wheezing Crude OR = 1.37 (0.824-2.28) in males
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Hyper-sensitivity: asthma	Parent interview, clinical exam, and spirometry Ages 2-10	No association for asthma OR = 1.27 (0.91-1.78) for wheeze	OR for a doubling of PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: no, for asthma; yes for wheeze Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: asthma	Doctor diagnosed (asthma), self-reports (wheeze)	ORs for asthma and wheeze near 1.0, similar results in boys and girls	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Includes asthma and wheezing Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Hyper-sensitivity: asthma	Questionnaire: based on symptoms, doctor's diagnosis or medication use, up to age 10 (cross-sectional), between ages 10-16, or at age 16 (prospective)	<u>Cross-sectional:</u> OR = 1.29 (lower confidence interval is 0.99, upper is 0.17) in girls Unadjusted OR = 1.39 (1.09-1.77) All other ORs near 1.0	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: unknown Dose-response: no Temporal association: no Subgroup only: girls Adjustments: large change	Includes asthma and wheezing F/U: 6 years Environment and Childhood Asthma study Incorrect confidence interval Large change with adjustments Multiple comparisons: many different outcomes and PFAS assessed
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	Maternal plasma, 1st trimester	Hyper-sensitivity: asthma	ISAAC question-naire at ages 1.5, 4 and 7	No association overall and in boys and girls, some decrease at age 7 for wheeze and by breast-feeding status (see notes)	Change in the outcome for a doubling of PFOA	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: inconsistent Statistical significance: no Dose-response: no Temporal association: no Subgroup only: see notes Adjustments: little change	Includes asthma and wheezing Spanish INMA birth cohort Samples stored at -80º C Inconsistent results by breast-feeding status (low ORs with never and longest category of breast-feeding)
Qin et al., 2017	Taiwan 2009-10	Case-control (cross-sectional)	Who: children Ages: 10-15 N: 300	Selection: unclear Participation: <50% Equal groups: similar except higher ETS in non-asthmatics Blinded: unclear Above detection: >96% Levels: median (IQR) = 0.50 (0.43-0.69) ng/ml	Serum	Hyper-sensitivity: asthma	Physician diagnosed	OR = 2.76 (1.82-4.17)	OR for each IQR increase in PFOA	Adjusted for age, sex, BMI, education, exercise, ETS, month of survey Matched on age and sex	Large magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: little change	Cases from two hospitals in northern Taiwan, controls randomly selected from seven public schools in northern Taiwan

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: asthma	Question-naire Ages 5 and 15	**PFOA in maternal serum:** Age OR 5 1.37 (0.81-2.32) 13 1.12 (0.67-188) **PFOA at age 5:** Age MMR OR 5 no 10.4 (1.06-102) 5 yes 0.76 (0.41-1.39) 13 no 9.92 (1.06-93) 13 yes 0.65 (0.35-1.20) **PFOA at age 13 and outcome at age 13:** No association Age column refers to outcome age	OR for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5, birth weight, and family history of chronic bronchitis/asthma	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: yes Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption Stratified by MMR vaccination if p-interaction <0.2
Zeng et al., 2019	Shanghai 2012-15	Prospective cohort	Who: children Ages: 0-5 N: 358	Selection: pregnancies at two participating hospitals Participation: <50% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median and IQR = 7.13 (5.15-9.97) ng/ml boys and 6.51 (4.57-8.73) ng/ml girls	Cord plasma	Hyper-sensitivity: asthma	Pediatrician diagnosis and spirometry	Some ORs above and some below 1.0 based on gender but with very wide confidence intervals	Unclear	Adjusted for child weight, gestational age, breast-feeding, maternal education and BMI, and income	Large magnitude: mixed above and below 1.0 – see results Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Includes asthma and wheezing F/U: 5 years Samples stored at -80º C
Averina et al., 2019	Northern Norway 2010-13	Cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: eczema	Self-reported doctor diagnosed	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided
Chen et al., 2018	Shangha i 2012-15	Prospective cohort	Who: children Ages: 0-24 months N: 687	Selection: unclear Participation: 65.1% among those enrolled Equal groups: Eczema and non-eczema cases similar except allergic family history, nulliparous, and breast-feeding greater in eczema cases Blinded: unclear Above detection: >90% Levels: fourth quartile ≥9.42 ng/ml	Cord blood	Hyper-sensitivity: eczema	ISAAC question-naire Ages 6, 12, and 24 months	Females: OR = 2.52 (1.12-5.68) Males: no association	Fourth vs. first quartile of PFOA	Adjusted for maternal age, maternal BMI, gestation week at delivery, birth weight, maternal and paternal education, mode of delivery, family history, family income, ethnicity, paternal smoking, and breast-feeding Less than 2% of mothers drank alcohol or smoked	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: females Adjustments: little change	25.2% of children developed atopic dermatitis by 24 months of age 75.4% delivered by Caesarian section

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: eczema	ISAAC question-naire Age 4	OR = 0.59 (0.32-1.08, p-trend = 0.02) in males OR = 1.21 (0.68-2.17, p-trend = 0.36) in females	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, number of siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: yes Dose-response: non-linear Temporal association: yes Subgroup only: yes, see results Adjustments: little change	Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Hyper-sensitivity: eczema	Parent interview and clinical exam Ages 2-10	No association	OR for a doubling of PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: eczema	Doctor diagnosed	ORs near 1.0, similar results in boys and girls	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Hyper-sensitivity: eczema	Questionnaire: based on parent reported eczema up to age 10 (cross-sectional), between ages 10-16, or at age 16 (prospective)	OR = 1.24 (0.86-1.78) for girls ages 10 and16 (prospective) All other ORs closer to 1.0	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: girls Adjustments: unadjusted OR = 1.18	F/U: 6 years Environment and Childhood Asthma study Some change with adjustments Multiple comparisons: many different outcomes and PFAS assessed

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	Maternal plasma, 1st trimester	Hyper-sensitivity: eczema	ISAAC question-naire at ages 1.5, 4 and 7	No association overall and in boys and girls OR = 0.59 (0.37-0.96) for children of "never" breast-feeding mothers	Change in the outcome for a doubling of PFOA	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education in overall analysis	Large magnitude: yes (decreased OR) Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: never breast-feeding mother Adjustments: not given for this subgroup	Spanish INMA birth cohort Samples stored at -80º C
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: eczema	ISAAC question-naire Age 13	Prenatal exposure and outcome at age 13: OR = 1.36 (0.85-2.19) No clear association at other ages	OR for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: yes Adjustments: unclear	Higher PFAS concentrations near birth and at ages 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Wen et al., 2019a	Taiwan 2001-5	Prospective cohort	Who: children Ages: 5 N: 836	Selection: prenatal exams at participating hospital Participation: unclear Equal groups: atopic dermatitis was associated with breast-feeding, parental education, and atopy Blinded: unclear Above detection: 50.75% Levels: median (IQR) = 0.65 (0.23-1.96) ng/ml	Cord plasma	Hyper-sensitivity: eczema	ISAAC question-naire	Hazard ratio= 1.89 (1.10-3.16) Higher risks with GSTT1-null phenotype (Wen et al., 2019b)	Above vs. below the upper quartile PFOA	Adjusted for sex, parental education and atopy, breast-feeding, and maternal age at childbirth	Large magnitude: yes Statistical significance: yes Dose-response: risks increased at upper quartile Temporal association: yes Subgroup only: no Adjustments: little change	F/U: 5 years Median age of atopic dermatitis development was 6 months in the exposed group Prevalence of atopic dermatitis was 7.1% Low exposure levels
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: IgE	Serum Age 18	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided OR not given

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: IgE	Cord blood Serum at age 7	No association	Percent difference for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at ages 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Zeng et al., 2019	Shanghai 2012-15	Prospective cohort	Who: children Ages: 0-5 N: 358	Selection: pregnancies at two participating hospitals Participation: <50% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median and IQR = 7.13 (5.15-9.97) boys and 6.51 (4.57-8.73) ng/ml girls	Cord plasma	Hyper-sensitivity: IgE Age 5	Serum	Some ORs above and some below 1.0 based on gender and exposure level	Unclear	Adjusted for child weight, gestational age, breast-feeding, maternal education and BMI, and income	Large magnitude: mixed above and below 1.0 – see results Statistical significance: no Dose-response: U-shaped Temporal association: no Subgroup only: no Adjustments: not given	F/U: 5 years Samples stored at -80º C
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 2.1 (1.2) ng/ml females, 1.9 (0.7) ng/ml males	Serum Age 16	Hyper-sensitivity: rhinitis	Self-reported doctor diagnosed Ages 16 and 18	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Allergic rhinitis Few details provided ORs not given
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOA higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 2.04-10.12 ng/ml	Maternal serum Gestation weeks 10-16	Hyper-sensitivity: rhinitis	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	OR = 1.37 (0.75-2.51)	Upper vs. lower tertile of PFOA	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: rhinitis	ISAAC question-naire Age 4	OR = 1.27 (0.62-2.61) overall OR higher in females	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, number of siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Outcome is rhinoconjunctivitis Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Hyper-sensitivity: rhinitis	Parent interview and clinical exam Ages 2-10	OR = 1.30 (0.97-1.74)	OR for a doubling of PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: rhinitis	Self-reports	OR = 1.32 (0.86-2.03), similar in boys and girls	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Hyper-sensitivity: rhinitis	Questionnaire: based on parent reported symptoms	<u>Cross-sectional:</u> All ORs near 1.0 <u>Prospective:</u> OR = 1.08 (1.01-1.14), higher in girls	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: no Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: mostly in girls Adjustments: little change	F/U: 6 years Environment and Childhood Asthma study Multiple comparisons: many different outcomes and PFAS assessed Small OR

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.0 (1.6-2.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: rhinitis	ISAAC question-naire Age 13	No association for exposure prenatally or ages 5 or 13	OR for each 2-fold increase in PFOA	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Outcome is rhinoconjunctivitis Higher PFAS concentrations near birth and at ages 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Goudarzi et al., 2017	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 2.013 (1.314-3.346) ng/ml	Maternal plasma Gestation weeks 28-32	Infection: any	Self-reported doctor diagnosed otitis media, pneumonia, varicella, or respiratory syncytial virus Age 4	No association in males OR = 1.37 (0.85-2.21) in females	Fourth vs. first quartiles of PFOA	Adjusted for maternal age, number of siblings, maternal education, breast-feeding, and smoking Additional adjustment or stratification for day-care attendance, ETS, pets, carpets, heating/cooling systems, presence of mold or dew condensation in home had little effect	Large magnitude: yes, females Statistical significance: no Dose-response: no Temporal association: no Subgroup only: females Adjustments: unclear	
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Infection: colds	Parent interview, clinical exam, and spirometry Ages 0-2	β = -0.04 (-0.08-0.01, p=0.089)	Number of colds age 0-10 per log2 PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-3 N: 1,207	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Infection: colds	Self-reports	OR below 1.0	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no (below 1.0) Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Infection: colds	Questionnaire: parent reported symptoms at ages 10 and 16	ORs above 1.4 in both boys and girls but with wide confidence intervals	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: some moderate changes	F/U: 6 years Environment and Childhood Asthma study Some change with adjustments Multiple comparisons: many different outcomes and PFAS assessed

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOA higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 2.04-10.12 ng/ml	Maternal serum Gestation weeks 10-16	Infection: cough	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOA	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOA higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 2.04-10.12 ng/ml	Maternal serum Gestation weeks 10-16	Infection: fever	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	OR = 1.97 (1.07-3.62)	Upper vs. lower tertile of PFOA	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOA higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 2.04-10.12 ng/ml	Maternal serum Gestation weeks 10-16	Infection: gastro-enteritis (diarrhea, vomiting)	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOA	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Infection: gastro-enteritis (diarrhea, gastric flu)	Self-reports	OR = 1.48 (1.31-1.67) for ages 6-7, near 1.0 for ages 0-3	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: older age group Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.54 (1.86-3.30) ng/ml	Maternal serum, median 18 weeks	Infection: lower respiratory tract infections	Self-reports	OR = 1.27 (1.12-1.43) for ages 0-3, near 1.0 for ages 6-7	OR for one IQR increase in PFOA	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: younger age group Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 1.6 (1.2-2.1) ng/ml	Cord blood	Infection: lower respiratory tract infections	Parent interview, clinical exam, and spirometry Ages 0-10	β = 0.28 (0.22-0.35, p <0.001)	Number of infections age 0-10 per log2 PFOA	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: unclear Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.36 (1.77) ng/ml	Serum, age 10	Infection: lower respiratory tract infections	Questionnaire: parent reported bronchitis or pneumonia at ages 10 and 16	ORs = 1.49 (1.15-1.92) in girls for ages 10 and 16 ORs closer to 1.0 in boys	Per IQR increase in PFOA	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: girls Adjustments: little change	F/U: 6 years Environment and Childhood Asthma study Multiple comparisons: many different outcomes and PFAS assessed
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	Maternal plasma, 1st trimester	Infection: lower respiratory tract infections	ISAAC question-naire at ages 1.5, 4 and 7	No association overall and in boys and girls, some decrease at age 7	Change in the outcome for a doubling of PFOA	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: yes, age 7 (OR ≈ 0.65) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Spanish INMA birth cohort Samples stored at -80º C

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Honda-Kohmo et al., 2019	West Virginia and Ohio 2005-6	Cross-sectional	Who: adults Ages: ≥20 N: 5,270	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >90% Levels: mean (SD) = 29.0 (12.7-72.8) ng/ml	Serum	Other: CRP	Serum	OR = 1.05 (1.01-1.09) in those without diabetes Similar result for those with diabetes but wider confidence interval	Per lognormal increase	Unclear	Large magnitude: no Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	C8 Health Project
Matilla-Santander et al., 2017	Spain 2003-08	Cross-sectional	Who: pregnant women Ages: first trimester N: 651	Selection: "population-based" Participation: 55% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	Plasma	Other: CRP	Serum	No association	Quartiles of PFOA and continuous	Adjusted for sub-cohort, country, BMI, breast-feeding, parity, gestation week, physical activity, and diet	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	
Stein et al., 2016a	New York 2010-11	Cross-sectional	Who: adults Ages: 18-49 N: 75	Selection: unclear Participation: unclear Equal groups: yes although analyses limited Blinded: yes Above detection: 100% Levels: geometric mean = 2.28, upper tertile range = 2.8-8.1 ng/ml	Serum	Other: cytokines	Serum or nasal secretion	No association with IFN-α2, IFN-gamma, TNF-α, IP1-), MCP-1, MIP-1a, G-CSF, IP-10, or mIgA	Upper vs. lower tertile of PFOA	Adjusted for age, sex, and race/ethnicity "No covariates… associated with both exposure and outcome"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	See original article for cytokine abbreviations

Rows are sorted by outcome then by first author
Data in parentheses are 95% confidence intervals unless otherwise stated
Ages are in years unless otherwise noted
Abraham et al., 2020 is reviewed below
Abbreviations: β, regression coefficient; BMI, body mass index; CRP, C-reactive protein; ETS, environmental tobacco smoke; HAI, hemagglutination-inhibition; IQR, interquartile range; IHC, histochemical staining; ISAAC, International Study of Asthma and Allergies in Childhood; LOD, limit of detection; MeDALL, Mechanism of Development of Asthma; N, number of participants; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PCBs, polychlorinated biphenyls; SES, socioeconomic status

Table A7.4. Recent epidemiologic studies of PFOS and immunotoxicity

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Grandjean et al., 2017a	Faroe Islands 2007-09 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤5 N: 349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 7.1 (4.5-10.0) ng/ml at age 18 months	Serum Near birth, 18 months, age 5	Antibody response: diphtheria	Diphtheria IgG Age 5	**2007-09 cohort:** PFOS IgG %change Birth -14.0 (p=0.20) 18 mo. 17.6 (p=0.06) 5 yr. 17.2 (p=0.21) **Combined cohort:** PFOS IgG %change Birth -24.5 (p=0.002) 18 mo. 15.1 (p=0.10) 5 yr. -1.3 (p=0.88)	Percent change for a 2-fold increase in PFOS	Adjusted for age and sex Additional adjustments for PCBs and Cesarean section had little effect	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	PFAS concentrations highly correlated with breast-feeding duration Correlation coefficients up to 0.7 for age 18 and age 5 PFAS levels 44% and 36% had IgG below protective levels for diphtheria and tetanus at age 5, respectively Combined: 2007-09 and 1997-2000 Faroe Islands cohorts combined
Grandjean et al., 2017b	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤13 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 15.3 (12.4-19.0) ng/ml	Serum Ages 7 and 13	Antibody response: diphtheria	Diphtheria IgG Age 13	PFOS IgG %change Age 7 -25.7 (p=0.06) Age 13 -10.5 (p=0.37)	Percent change for a 2-fold increase in PFOS	Adjusted for age, sex, booster type at age 5 Additional adjustment for PCBs had little effect	Large magnitude: yes Statistical significance: borderline Dose-response: linear Temporal association: no Subgroup only: no Adjustments: unclear	PFAS concentrations at age 13 correlated with those at age 7 but not with maternal levels Excludes those with likely booster after age 5
Stein et al., 2016a	New York 2010-11	Prospective cohort	Who: adults Ages: 18-49 N: 75	Selection: unclear Participation: unclear Equal groups: yes although analyses limited Blinded: yes Above detection: 100% Levels: geometric mean = 5.22 ng/ml, upper tertile range = 7.2-21.4 ng/ml	Serum Day 0	Antibody response: FluMist	Anti-A H1N1 antibody response by histochemical staining (IHC) and hemagglutination-inhibition (HAI) Day 30	OR = 1.3 (0.2-7.3) p-trend=0.81 for HAI OR = 2.4 (0.9-6.6) p-trend=0.12 for IHC	Upper vs. lower tertile of PFOS	Adjusted for age, sex, and race/ethnicity "No covariates… associated with both exposure and outcome"	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Less than 20 subjects seroconverted
Pilkerton et al., 2018	US NHANES 1999-2000, 2003-04	Cross-sectional	Who: children and adults Ages: ≥12 N: 2,389	Selection: multi-stage cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Above detection: unclear but low LOD Levels: mean = 22-28 ng/ml	Serum	Antibody response: rubella	Serum IgG	4th vs. 1st quartile: Men: β = 0.0086 (p=0.97) (β is negative in quartiles 2 and 3) Women: -0.1664 (p=0.73) Per quartile increase: All adults combined: F value = 3.44, p=0.03 Children: no association	Fourth vs. first quartile of PFOS; regression coefficient for per quartile increase in PFOS	Adjusted for age, BMI, education, sex, and ethnicity, and parity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Results for children only and all adults combined presented only as F-values Gender specific analysis includes only adults

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Grandjean et al., 2017a	Faroe Islands 2007-09 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤5 N: 349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 7.1 (4.5-10.0) ng/ml at age 18 months	Serum Near birth, 18 months, age 5	Antibody response: tetanus	Tetanus IgG Age 5	**2007-09 cohort:** PFOS IgG %change Birth -10.8 (p=0.30) 18 mo. -7.0 (p=0.40) 5 yr. -9.1 (p=0.43) **Combined cohort:** PFOS IgG %change Birth -10.6 (p=0.20) 18 mo. -7.1 (p=0.39) 5 yr. -10.5 (p=0.18) No major differences between 1997-2000 and 2007-09 cohorts	Percent change for a 2-fold increase in PFOS	Adjusted for age and sex Additional adjustments for PCB concentrations and Cesarean section had little effect	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS concentrations highly correlated with breast-feeding duration Correlation coefficients up to 0.7 for age 18 and age 5 PFAS levels 44% and 36% had IgG below protective levels for diphtheria and tetanus at age 5, respectively Combined: 2007-09 and 1997-2000 Faroe Islands cohorts combined
Grandjean et al., 2017b	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: ≤13 N: 275-349	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median (IQR) = 15.3 (12.4-19.0) ng/ml	Serum Ages 7 and 13	Antibody response: tetanus	Tetanus IgG Age 13	PFOS IgG %change Age 7 2.7 (p=0.85) Age 13 14.8 (p=0.24)	Percent change for a 2-fold increase in PFOS	Adjusted for age, sex, booster type at age 5 Additional adjustment for PCBs had little effect	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS concentrations at age 13 correlated with those at age 7 but not with maternal levels Excludes those with likely booster after age 5
Hammer et al., 2019	Faroe Islands 1986-2009	Prospective cohort	Who: all children and adults Ages: all N: 5,698	Selection: involved in previous cohort studies Participation: 75% of all Faroese births during the recruitment periods Equal groups: unclear Blinded: unclear Above detection: not given Levels: unclear	Serum	Autoimmunity: inflammatory bowel disease	Nationwide Inflammatory Disease database	OR = 0.30 (0.08-1.07) N=37 cases	Upper vs. lower tertile of PFOS	Adjusted for age and calendar period	Large magnitude: yes (OR below 1.0) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Includes six different cohorts and a wide range of follow-up periods
Xu et al., 2020	Ronneby, Sweden 1980-2013	Prospective cohort	Who: children and adults Ages: >10 N: 63,074	Selection: all residents of Ronneby and a nearby unexposed town from 1980-2013 Participation: likely close to 100% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: serum median (IQR) = 216 (118-300) ng/ml in a subsample	Exposure to contaminated water based on address and water records	Autoimmunity: inflammatory bowel disease	Swedish National Patient Registry and death records	HR for Crohn's disease = 1.58 (1.00-2.49) for 1985-94 but not for the later years; near 1.0 for ulcerative colitis	Ever exposed to contaminate water source	Adjusted for calendar year, age, and gender	Large magnitude: yes Statistical significance: yes Dose-response: no – see notes Temporal association: yes Subgroup only: no Adjustments: not given	High environmental contamination of PFOS and PFHxS, moderate for PFOA; PFOS, PFOA, and PFHxS highly correlated

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: allergy (all)	ISAAC question-naire Age 4	No association Similar results in males and females	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Includes all allergic diseases and related symptoms Combines eczema, wheezing and rhinoconjunctivitis
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: allergy	Question-naire and skin prick test Ages 5 and 13	**PFOS in maternal serum:** Age OR 5 0.73 (0.38-1.41) 13 1.25 (0.75-2.11) **PFOS at age 5:** Age MMR OR 5 no 6.15 (0.77-49.2) 5 yes 0.80 (0.43-1.49) 13 NA 0.76 (0.49-1.18) **PFOS at age 13 and outcome at age 13:** OR = 0.90 (0.63-1.29) Age column refers to outcome age	OR for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption Stratified by MMR vaccination if p-interaction <0.2
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: allergy (food)	Self-reported or FX5 food panel IgE Ages 16 (self-reported) and 18 (IgE)	No association	Unclear	Adjusted for sex, age, BMI, physical activity, SES, diet, and allergy medications	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Authors report "no statistically significant association" but actual OR not given
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: allergy (food)	Self-reports	ORs near 1.0, similar results in boys and girls	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Averina et al., 2019	Northern Norway 2010-13	Cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: allergy (nickel)	Self-reported	OR = 2.23 (1.14-4.35)	Fourth vs. first quartiles of PFOS	Sex and age	Large magnitude: yes Statistical significance: yes Dose-response: step-shaped Temporal association: no Subgroup only: no Adjustments: large change (see notes)	Unadjusted OR = 1.44 (0.76-2.71)
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: asthma	MeDALL question-naire Ages 16 and 18	OR = 2.11 (1.02-4.37) cross-sectional OR = 1.23 (0.53-2.83) prospective	Fourth vs. first quartiles of PFOS	Adjusted for sex, age, BMI, physical activity, SES, and fish intake	Large magnitude: yes Statistical significance: yes Dose-response: inverted U Temporal association: no Subgroup only: no Adjustments: small change	Unadjusted OR = 2.37 cross-sectional
Beck et al., 2019	Odense, Denmark 2010-12	Prospective cohort	Who: children Ages: 5 N: 981	Selection: all pregnant women in the municipality Participation: <43% Equal groups: asthma or wheeze greater in boys, smoking parents, and family asthma Blinded: unclear Levels: median (IQR) = 7.73 (5.68-10.44) ng/ml	Maternal serum, weeks 8-16	Hyper-sensitivity: asthma	ISSAC question-naire Age 5	Doctor diagnosed asthma: Girls: 1.60 (0.46-5.59) Boys: 0.74 (0.46-1.20) Elevated OR for self-reported asthma in boys Wheeze: all ORs near 1.0	OR for a doubling of PFOS	Adjusted for parity, education, BMI, asthma predisposition, and sex Also stratified by sex	Large magnitude: yes Statistical significance: unclear Dose-response: unclear Temporal association: yes Subgroup only: inconsistent Adjustments: small changes only	Includes asthma and wheezing Samples stored at -80º C Self-reported asthma: only those who did not also report doctor diagnosed asthma Inconsistent results across genders and self-reports with doctor diagnosed
Gaylord et al., 2019	US 2014-16	Cross-sectional	Who: children and adults Ages: 13-22 N: 287	Selection: exposed to the World Trade Center disaster as children and controls Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: mean (SD) = 3.45 (3.30) ng/ml	Serum	Hyper-sensitivity: asthma	Question-naire: similar to NHANES	No association	OR per unit change in log PFOS	Adjusted for sex, race/ethnicity, BMI, tobacco smoke exposure	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: asthma	ISAAC question-naire Age 4	No association Similar results in males and females	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, number of siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Includes asthma and wheezing Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Hyper-sensitivity: asthma	Parent interview, clinical exam, and spirometry Ages 2 and 10	No association	OR for a doubling of PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values Similar results for wheeze
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: asthma	Doctor diagnosed (asthma), self-reports (wheeze)	ORs for asthma and wheeze near 1.0, similar results in boys and girls	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Hyper-sensitivity: asthma	Questionnaire: based on symptoms, doctor’s diagnosis or medication use, up to age 10 (cross-sectional), between ages 10-16, or at age 16 (prospective)	All ORs near 1.0, similar results in boys and girls	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Includes asthma and wheezing F/U: 6 years Environment and Childhood Asthma study

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.06 (4.52-7.82) ng/ml	Maternal plasma, 1st trimester	Hyper-sensitivity: asthma	ISAAC question-naire at ages 1.5, 4 and 7	No clear associations, some ORs below 1.0 but inconsistent by outcome, gender, breast-feeding status, and age	Change in the outcome for a doubling of PFOS	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: inconsistent Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Includes asthma and wheezing Spanish INMA birth cohort Samples stored at -80º C
Qin et al., 2017	Taiwan 2009-10	Case-control (cross-sectional)	Who: children Ages: 10-15 N: 300	Selection: unclear Participation: <50% Equal groups: similar except higher ETS in non-asthmatics Blinded: unclear Above detection: >96% Levels: median (IQR) = 28.8 (12.4-42.0) ng/ml	Serum	Hyper-sensitivity: asthma	Physician diagnosed	OR = 1.30 (1.00-1.69)	For each IQR increase in PFOS	Adjusted for age, sex, BMI, education, exercise, ETS, and month of survey Matched on age and sex	Large magnitude: yes Statistical significance: borderline Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: little change	Cases from two hospitals in northern Taiwan, controls randomly selected from seven public schools in northern Taiwan Some evidence of interaction with testosterone and estradiol levels (Zhou et al., 2017)
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: asthma	Question-naire Age 5	**PFOS in maternal serum:** Age OR 5 1.21 (0.64-2.29) 13 1.61 (0.84-3.08) **PFOS at age 5:** Age MMR OR 5 no 3.96 (0.55-28.4) 5 yes 0.98 (0.55-1.76) 13 no 5.41 (0.62-47.2) 13 yes 0.94 (0.51-1.74) **PFOS at age 13 and outcome at age 13:** OR = 0.63 (0.41-0.97) Age column refers to outcome age	OR for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5, birth weight, and family history of chronic bronchitis/asthma	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption Stratified by MMR vaccination if p-interaction <0.2
Zeng et al., 2019	Shanghai 2012-15	Prospective cohort	Who: children Ages: 0-5 N: 358	Selection: pregnancies at two participating hospitals Participation: <50% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median and IQR = 2.49 (1.81-3.51) boys and 2.38 (1.73-3.13) ng/ml girls	Cord plasma	Hyper-sensitivity: asthma	Pediatrician diagnosis and spirometry	Some ORs above and some below 1.0 based on gender but with very wide confidence intervals	Unclear	Adjusted for child weight, gestational age, breast-feeding, maternal education and BMI, and income	Large magnitude: see results Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	F/U: 5 years Includes asthma and wheezing Samples stored at -80º C PFOA levels higher than PFOS levels

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Averina et al., 2019	Northern Norway 2010-13	Cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum	Hyper-sensitivity: eczema	Self-reported doctor diagnosed	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided Authors report “no statistically significant association” but actual OR not given
Chen et al., 2018	Shanghai 2012-15	Prospective cohort	Who: children Ages: 0-24 months N: 687	Selection: unclear Participation: 65.1% among those enrolled Equal groups: Similar except allergic family history, nulliparous, and breast-feeding greater in eczema cases vs. non-cases Blinded: unclear Above detection: 100% Levels: fourth quartile ≥3.22 ng/ml	Cord blood	Hyper-sensitivity: eczema	ISAAC question-naire Ages 6, 12, and 24 months	Females: no association Males: no association	Fourth vs. first quartile of PFOS	Adjusted for maternal age, maternal BMI, gestation week at delivery, birth weight, maternal and paternal education, mode of delivery, family medical history, family income, ethnicity, paternal smoking, and breast-feeding Less than 2% of mothers drank alcohol or smoked	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	25.2% of children developed atopic dermatitis by 24 months of age 75.4% delivered by Caesarian section
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: eczema	ISAAC question-naire Age 4	No association	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Similar results in males and females Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Hyper-sensitivity: eczema	Parent interview and clinical exam Ages 2 and 10	No association	OR for a doubling of PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: eczema	Doctor diagnosed	ORs near 1.0, similar results in boys and girls	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Hyper-sensitivity: eczema	Questionnaire: based on symptoms, doctor's diagnosis or medication use, up to age 10 (cross-sectional), between ages 10-16, or at age 16 (prospective)	All ORs near or below 1.0, similar results in boys and girls	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: ORs lower after adjustments	F/U: 6 years Environment and Childhood Asthma study
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.06 (4.52-7.82) ng/ml	Maternal plasma, 1st trimester	Hyper-sensitivity: eczema	ISAAC question-naire at ages 1.5, 4 and 7	OR = 0.86 (0.75-0.98); greatest effects at ages 4 and 7, and in girls (OR = 0.77 (0.64-0.94)) and with never breast-feeding (OR = 0.44 (0.25-0.78))	Change in the outcome for a doubling of PFOS	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: yes (ORs below 1.0) Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: yes Adjustments: little change	Spanish INMA birth cohort Samples stored at -80º C
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: eczema	ISAAC question-naire Age 13	No association for exposure prenatally or age 5 Age 13 exposure without MMR vaccine: OR = 8.94 (0.27-299)	OR for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption Very wide confidence intervals

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wen et al., 2019a	Taiwan 2001-5	Prospective cohort	Who: children Ages: 5 N: 836	Selection: prenatal exams at participating hospital Participation: unclear Equal groups: atopic dermatitis was associated with breast-feeding, parental education, and atopy Blinded: unclear Above detection: 89.57% Levels: median (IQR) = 2.49 (2.18-5.05) ng/ml	Cord plasma	Hyper-sensitivity: eczema	ISAAC question-naire	HR = 1.43 (0.82-2.43)	Above vs. below the upper quartile of PFOS	Adjusted for sex, parental education and atopy, breast-feeding, and maternal age at childbirth	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: little change	F/U: 5 years Prevalence of atopic dermatitis was 7.1%
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: IgE	Serum Age 18	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Few details provided Authors report “no statistically significant association” but actual OR not given
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: IgE	Serum Cord blood and age 7	No association	Percent difference for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Zeng et al., 2019	Shangha i 2012-15	Prospective cohort	Who: children Ages: 5 N: 358	Selection: pregnancies at two participating hospitals Participation: <50% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: median and IQR = 2.49 (1.81-3.51) ng/ml boys, 2.38 (1.73-3.13) ng/ml girls	Cord plasma	Hyper-sensitivity: IgE Age 5	Serum	Some ORs above and some below 1.0 based on gender and exposure levels but with very wide confidence intervals	Unclear	Adjusted for child weight, gestational age, breast-feeding, maternal education and BMI, and income	Large magnitude: see results Statistical significance: no Dose-response: U-shaped Temporal association: no Subgroup only: see results Adjustments: not given	F/U: 5 years Samples stored at -80º C PFOA levels higher than PFOS levels

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Averina et al., 2019	Northern Norway 2010-13	Prospective cohort and cross-sectional	Who: children Ages: first year high school students N: 675	Selection: all students in eight high schools invited Participation: 60.4% Equal groups: unclear Blinded: unclear Above detection: unclear Levels: geometric mean (IQR) = 5.8 (2.7) ng/ml females, 6.8 (3.0) ng/ml males	Serum Age 16	Hyper-sensitivity: rhinitis	Self-reported doctor diagnosed Ages 16 and 18	No association	Unclear	Unclear	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Outcome is allergic rhinitis Few details provided OR not given
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOS higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 10.19-25.10 ng/ml	Maternal serum Gestation weeks 10-16	Hyper-sensitivity: rhinitis	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOS	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	
Goudarzi et al., 2016	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Hyper-sensitivity: rhinitis	ISAAC question-naire Age 4	No association Similar results in males and females	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, siblings, maternal education, parental allergies, infant gender, breast-feeding, day-care attendance, and ETS Additional adjustments for household income, smoking, alcohol, BMI, pets, carpets, heating/cooling systems, and mold or dew condensation in homes had little effect 6.2% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Outcome is rhinoconjunctivitis Participants had higher maternal education, lower maternal smoking rates, and lower postnatal ETS rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Hyper-sensitivity: rhinitis	Parent interview and clinical exam Ages 2 and 10	No association	OR for a doubling of PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied to p-values For reported symptoms and IgE

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 39% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Hyper-sensitivity: rhinitis	Self-reports	ORs near 1.0, similar results in boys and girls	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Hyper-sensitivity: rhinitis	Questionnaire: based on parent reported symptoms at age 10 or 16	All ORs near 1.0, similar results in boys and girls	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	F/U: 6 years Environment and Childhood Asthma study
Timmer-mann et al., 2017	Faroe Islands 1997-2000 (year of birth)	Prospective cohort and cross-sectional	Who: children Ages: 0-13 N: 559	Selection: unclear Participation: unclear Equal groups: see notes Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.7 (5.2-8.5) ng/ml at age 13	Serum Third trimester, and ages 5 and 13	Hyper-sensitivity: rhinitis	ISAAC question-naire Age 13	No association for exposure prenatally or age 5 or 13	OR for each 2-fold increase in PFOS	Adjusted for family history of eczema in children, allergic eczema, hay fever, maternal pre-pregnancy BMI, maternal smoking during pregnancy, sex, duration of breast-feeding, fish intake at age 5, number of siblings, and day-care attendance at age 5	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Outcome is rhinoconjunctivitis Higher PFAS concentrations near birth and at age 5-7 PFAS concentrations related to breast-feeding, maternal BMI, maternal and paternal smoking, and fish consumption
Goudarzi et al., 2017	Hokkaido, Japan 2003-09	Prospective cohort	Who: children Age: 4 N: 1,558	Selection: appears to be all pregnant women in 37 hospitals and clinics in Hokkaido, Japan Participation: <18.9% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 4.925 (3.667-6.654) ng/ml	Maternal plasma Gestation weeks 28-32	Infection: any	Self-reported doctor diagnosed otitis media, pneumonia, varicella, or respiratory syncytial virus Age 4	OR = 1.61 (1.18-2.21)	Fourth vs. first quartiles of PFOS	Adjusted for maternal age, siblings, maternal education, breast-feeding, and smoking Additional adjustment or stratification for day-care attendance, ETS, pets, carpets, heating/cooling systems, presence of mold or dew condensation in home had little effect	Large magnitude: yes Statistical significance: yes Dose-response: step-shaped Temporal association: yes Subgroup only: no Adjustments: unclear	Similar results in males and females Participants had higher maternal education and lower maternal smoking rates than original cohort
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Infection: colds	Parent interview, clinical exam, and spirometry Ages 0-2	No association	Number of colds ages 0-10 per log2 PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not “statistically significant”	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-3 N: 1,207	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Infection: colds	Self-reports	OR below 1.0	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: no (below 1.0) Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Infection: colds	Questionnaire: based on parent reported symptoms at age 10 or 16	Some ORs above and some below 1.0 but very inconsistent by age and gender or very wide confidence intervals	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: see results Statistical significance: see results Dose-response: no Temporal association: see results Subgroup only: see results Adjustments: some moderate changes	F/U: 6 years Environment and Childhood Asthma study Multiple comparisons: many different outcomes and PFAS assessed
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOS higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 10.19-25.10 ng/ml	Maternal serum Gestation weeks 10-16	Infection: cough	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOS	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOS higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 10.19-25.10 ng/ml	Maternal serum Gestation weeks 10-16	Infection: fever	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	OR = 2.35 (1.34-4.11)	Upper vs. lower tertile of PFOS	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: yes Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustment: little change	Data reported for an average of 86% of days in the year Similar results in those reporting at least 25 of 26 weeks

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Dalsager et al., 2016	Denmark 2010-12	Prospective cohort	Who: children Ages: 0-3 N: 359	Selection: all pregnant women living in Odense from 2010-12 were invited Participation: <43% Equal groups: PFOS higher in nulliparous, younger maternal age, decreased education, higher BMI, older child and male child Blinded: unclear Above detection: unclear, but LOD was low (0.03 ng/ml) Levels: upper tertile range = 10.19-25.10 ng/ml	Maternal serum Gestation weeks 10-16	Infection: gastro-enteritis (diarrhea, vomiting)	Mobile phone question-naires on symptoms every 2 weeks for one year Ages 1-3 Proportion of days with symptoms of infectious disease	No association	Upper vs. lower tertile of PFOS	Adjusted for maternal age, maternal education, parity, and child's age Additional adjustments for duration of breast-feeding, day-care, maternal smoking, and child's sex had little effect 3% of mothers smoked during pregnancy	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustment: little change	
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Infection: gastro-enteritis (diarrhea, gastric flu)	Self-reports	OR = 1.12 (1.01-1.24) at ages 6-7, near 1.0 at ages 0-3	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: no Statistical significance: see notes Dose-response: no Temporal association: yes Subgroup only: older age group Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up Not statistically significant when adjusted for multiple comparisons
Impinen et al., 2018	Oslo 1992-93	Prospective cohort	Who: children Ages: 0-10 N: 641	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >96% Levels: median (IQR) = 5.2 (4.0-6.6) ng/ml	Cord blood	Infection: lower respiratory tract infections	Parent interview, clinical exam, and spirometry Ages 0-10	β = 0.50 (0.42-0.57, p <0.001)	Number of infections ages 0-10 per log2 PFOS	Adjusted for sex Birth weight, birth month, breast-feeding, maternal smoking, other smoking, parental asthma and allergies, parental education, and household income not "statistically significant"	Large magnitude: unclear Statistical significance: yes Dose-response: linear Temporal association: yes Subgroup only: no Adjustments: unclear	At age 2, subjects included cases with bronchial obstruction and controls; details of selection and recruitment not provided Details of selection and recruitment of subjects at age 10 also not provided Bonferroni correction applied
Impinen et al., 2019	Norway 1999-2008	Prospective cohort	Who: children Ages: 0-7 N: 1,207 at ages 0-3 and 921 at ages 6-7 (same children)	Selection: all pregnant women scheduled for ultrasound Participation: 63% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 12.87 (9.92-16.63) ng/ml	Maternal serum, median 18 weeks	Infection: lower respiratory tract infections	Self-reports	OR = 1.20 (1.07-1.34) for ages 0-3, near 1.0 for ages 6-7	OR for one IQR increase in PFOS	Adjusted for maternal age, BMI, education, and smoking Adjusting for nursey school attendance had little impact Stratified by gender	Large magnitude: borderline Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: younger age group Adjustments: not given	Samples shipped at ambient temperature Cohort members were participants in pre-eclampsia and subfecundity case-control studies Multiple comparisons: many different outcomes assessed Some loss to follow-up
Kvalem et al., 2020	Oslo, Norway	Prospective cohort and cross-sectional	Who: children Ages: 10 and 16 (age at interview) N: 378	Selection: newborns 1992-3 in Oslo recruited into the original study Participation: unclear but less than 10-15% Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 19.4 (9.23) ng/ml	Serum, age 10	Infection: lower respiratory tract infections	Questionnaire: based on parent reported bronchitis or pneumonia at age 10 or 16	OR = 1.34 (1.17-1.55) overall at ages 10 to 16 mostly in boys OR near 1.0 at age 16 (last 12 months)	Per IQR increase in PFOS	Adjusted for BMI, puberty, maternal education, and activity	Large magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: higher in boys Adjustments: little change	F/U: 6 years Environment and Childhood Asthma study Inconsistent results by age Multiple comparisons: many different outcomes and PFAS assessed

Author year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Manzano-Salgado et al., 2019	Spain 2003-8	Prospective cohort	Who: children Ages: 1.5-7 N: 1,071-1,188	Selection: giving birth in a participating hospital Participation: unclear Equal groups: unclear Blinded: unclear Above detection: 100% Levels: median (IQR) = 6.06 (4.52-7.82) ng/ml	Maternal plasma, 1st trimester	Infection: lower respiratory tract infections	ISAAC question-naire at ages 1.5, 4 and 7	No association overall and by gender, some decrease with increasing age (OR = 0.83 (0.57-1.20) at age 7) and with never breast-feeding (OR = 0.66 (0.42-1.05))	Change in the outcome for a doubling of PFOS	Adjusted for maternal age, parity, breast-feeding, pre-pregnancy BMI, region, and country Little change when adjusted for other PFAS, fish consumption, smoking and maternal education	Large magnitude: yes (ORs below 1.0) Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: yes Adjustments: little change	Spanish INMA birth cohort Samples stored at -80º C
Ammitz-boll et al., 2019	Denmark Years unknown	Cross-sectional	Who: adults Ages: unclear N: 79	Selection: unclear Participation: unclear Equal groups: PFOS increased with age and in men, decreased with years menstruation, number of children, and breast-feeding Blinded: unclear Levels: median (IQR) = 9.41 (6.41-13.05) ng/ml	Serum PFOS	Multiple immune cell types	Serum	Associations with CD27 and plasmablasts. In the CD4- CD8+ T cell category: associations with T central memory cells, T naïve, T effector memory, and CXCR3hi cells (all p <0.05)	Spearman correlation coefficients	Appears unadjusted	Large magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	May have included roughly 50% multiple sclerosis patients Results for PFOA not given Clinical relevance is unknown Multiple comparisons issues: 50 cell types tested
Honda-Kohmo et al., 2019	West Virginia and Ohio 2005-6	Cross-sectional	Who: adults Ages: ≥20 N: 5,270	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: >90% Levels: median (IQR) = 21.1 (13.7-31.3) ng/ml	Serum	Other: CRP	Serum	OR = 1.05 (1.01-1.09) in those without diabetes Similar results in those with diabetes but with wider confidence interval	Per lognormal increase	Unclear	Large magnitude: no Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	C8 Health Project Same result as for PFOA
Matilla-Santan-der et al., 2017	Spain 2003-08	Cross-sectional	Who: pregnant women Ages: first trimester N: 1,240	Selection: "population-based" Participation: <55% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: median (IQR) = 6.05 (4.51-7.81) ng/ml	Plasma	Other: CRP	Serum	No association	Quartiles of PFOS and continuous	Adjusted for sub-cohort, country, BMI, breast-feeding, parity, gestation week, physical activity, and diet	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	
Stein et al., 2016a	New York 2010-11	Cross-sectional	Who: adults Ages 18-49 N: 75	Selection: unclear Participation: unclear Equal groups: yes although analyses limited Blinded: yes Above detection: 100% Levels: geometric mean = 5.22 ng/ml, upper tertile range = 7.2-21.4 ng/ml	Serum	Other: cytokines	Serum or nasal secretion	No association with IFN-α2, IFN-gamma, TNF-α, IP1-), MCP-1, MIP-1a, G-CSF, IP-10, or mIgA	Upper vs. lower tertile of PFOS	Adjusted for age, sex, and race/ethnicity "No covariates… associated with both exposure and outcome"	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Less than 20 subjects seroconverted Please see original article for cytokine abbreviations

Rows are sorted by outcome then by first author
Data in parentheses are 95% confidence intervals unless otherwise stated
Ages are in years unless otherwise noted
Abraham et al., 2020 is reviewed below
Abbreviations: β, regression coefficient; BMI, body mass index; CRP, C-reactive protein; ETS, environmental tobacco smoke; HAI, hemagglutination-inhibition; IQR, interquartile range; IHC, histochemical staining; ISAAC, International Study of Asthma and Allergies in Childhood; LOD, limit of detection; MeDALL, Mechanism of Development of Asthma; N, number of participants; NA, given available; OR, odds ratio; PCBs, polychlorinated biphenyls; SES, socioeconomic status

Liver Toxicity

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018), and others, OEHHA searched for all new human epidemiologic studies on PFOA or PFOS and liver toxicity published since the 2016 US EPA reviews. Briefly, PubMed and Embase were searched for all human studies on PFOA or PFOS and liver toxicity published between December 2015 (the end of the US EPA literature searches) and September 20, 2020 (the end of OEHHA's literature search). The outcome portion of the search string used is shown below.

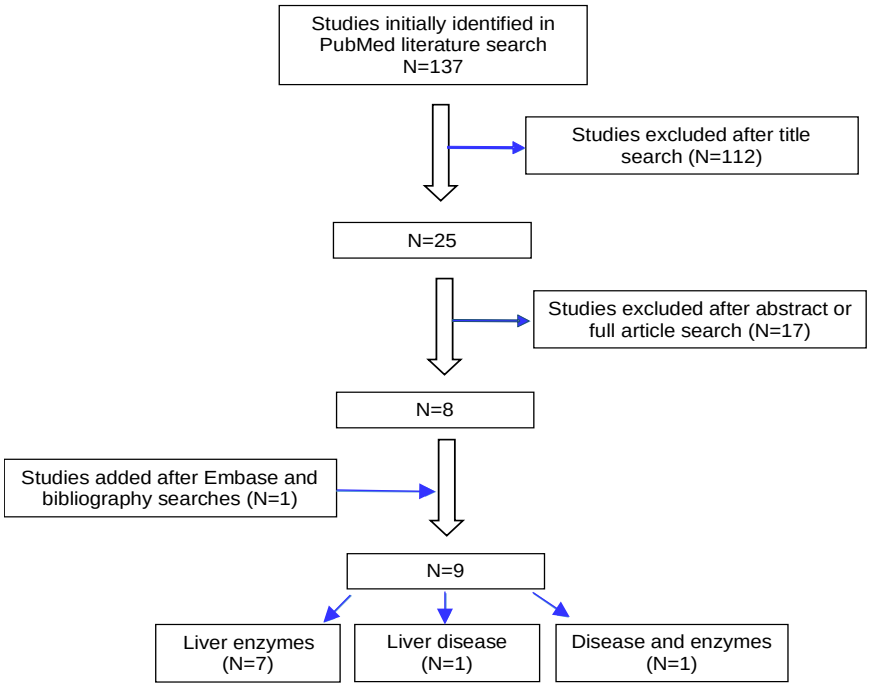
(liver[tiab] OR hepati[tiab] OR cirrhosis OR alanine transaminase OR ALT OR aspartate transaminase OR AST OR alkaline phosphatase OR ALP OR gamma-glutamyl transpeptidase OR GGT OR bilirubin*) NOT (mice[tiab] OR mouse[tiab] OR rat[tiab] OR rats[tiab] OR zebrafish[tiab])*

These key words were based on the outcomes that were assessed in the studies identified by US EPA (2016b and 2016b) and on the key words of articles OEHHA identified in a preliminary literature search.

Results

A general description of OEHHA's literature search is shown in Figure A7.2. A list of studies excluded based on OEHHA's abstract or full article review is provided in Table A7.28.

Figure A7.2. Literature search: recent epidemiologic studies of PFOA or PFOS and liver toxicity*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

OEHHA identified nine studies of PFOA or PFOS and liver toxicity published since December 2015 or that were otherwise not included in the most recent US EPA (2016a; 2016b) reviews (Tables A7.5 and A7.6). Two of these provided only results for PFOA while the others provided information on both PFOA and PFOS. Five studies used a cross-sectional design, two used prospective cohort designs, one used a retrospective cohort design, and one involved both prospective and cross-sectional analyses. Six of these studies were done in the US, two were done in Europe, and one was done in Asia. One study involved highly exposed workers, two involved residents in highly exposed communities, and the remainder were general population studies with no known high exposure source. Seven studies reported information only on liver enzymes, one on liver disease mortality, and one study reported information on both liver enzymes and liver disease. Further details of the methods and results of these studies are shown in Tables A7.5 and A7.6.

Table A7.5. Recent epidemiologic studies on PFOA and liver toxicity

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Girardi and Merler, 2019	Where: Trissino, Veneto, Italy Years: 1960-2018	Occupation-al Retro-spective cohort	Who: worked at least 1 day from 1948-2002 Ages: adults N: 462 chemical or office workers, 1,383 railroad workers	Selection: all male workers, at least 6 months, from 1960-2008 Participation: unclear Equal groups: unclear Blinded: unclear Above detection: not given Levels: geometric mean = 4.048 µg/ml	Job activities and tasks based on records and some interviews used to classify workers as ever, never exposed, or office workers. Jobs, years worked, and serum levels in a subsample (N=120 workers) used to model cumulative exposure	Liver cirrhosis mortality	Local and national death records	SMR = 1.71 (0.77-3.81) RR = 3.87 (1.18-12.7) N=6 exposed cases	Regional mortality rates (for SMRs), and local railroad workers (for RRs); tertiles of cumulative exposure	Adjusted for age Males only	Large magnitude: yes Statistical significance: yes Dose-response: plateau Temporal association: yes Subgroup only: no Adjustments: not given	F/U 1970 to 2018, average 31.7 years Average length of employment = 17.1 years Other chemicals produced at the plant include fluoroaromatics, benzotrofluorides, and PFOS (mean serum PFOS = 0.148 µg/ml, correlation with PFOA = 0.59) Elevated SMRs for suicides
Darrow et al., 2016	Parkers-burg, West Virginia 2005-06	Prospective cohort	Who: adults Ages: ≥20 N: 30,723	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear Levels: medians ranged from 11.9 to 25.7 ng/ml depending on age group	Environmental fate and transport model used to produce yearly and cumulative serum levels - see notes	ALT, GGT, direct bilirubin, liver disease	Serum (liver enzymes) and self-reported and adjudicated (liver disease)	<u>Regression coefficients:</u> ALT: 0.012 (0.008 to 0.016) GGT: 0.003 (-0.003 to 0.008) Direct bilirubin: -0.005 (-0.008 to -0.002) An estimated 6 percent (95% CI, 4-8 percent) increase in ALT level was seen between the fifth and first quartiles of PFOA exposure Liver disease: all hazard ratios near 1.0 Also associated with CK18 M30, a marker of liver cell apoptosis (see Bassler et al., 2019)	Linear regression (liver enzymes) Unknown for liver disease	Adjusted for age, sex, BMI, alcohol consumption, regular exercise, smoking, education, insulin resistance, fasting, occupational exposure, and race	Large magnitude: no Statistical significance: yes Dose-response: inverted U Temporal association: yes Subgroup only: no Adjustments: yes (increased)	Groundwater contamination of PFOA from a nearby chemical manufacturing plant Exposure models included information on PFOA water concentrations, residential history, water sources, water consumption rates and job, department, and work histories if occupationally exposed. Correlation coefficient between modeled serum estimates and measured serum concentrations in was 0.71 The percentage change in liver function biomarkers for a given change in PFOA can be calculated as [exp(β)–1] × 100, where β is the linear regression coefficient. For continuous ln PFOA, this would represent the percent change in the biomarker for a 1 ln unit increase in PFOA

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Nian et al., 2019	Shen-yang, China	Cross-sectional 2015-16	Who: adults Ages: ≥35 N: 1,605	Selection: government workers, retirees, and others who were long-term residents of the city Participation: 93% Equal groups: unclear Blinded: unclear Above detection: 99.9% Levels: median (IQR) = 6.19 (4.08-9.31) ng/ml	Serum	AST, ALT, ALP, TB, GGT	Serum	Regression coefficients: ALT: 7.4 (3.9-11.0) AST: 2.9 (0.7-5.2) ALP: -1.1 (-2.9-0.8) GGT: 8.6 (4.9-12.3) TB: 1.6 (-1.1-4.2) Results are for total PFOA; associations less strong for iso-PFOA than n-PFOA	Percent change in linear regression coefficients of lognormal liver biomarkers per each one lognormal increase in PFOA	Adjusted for age, sex, career, income, education, alcohol, smoking, giblet and seafood consumption, exercise, and BMI Similar results when those taking relevant medications, smokers, and alcohol drinkers are excluded	Large magnitude: no Statistical significance: yes Dose-response: linear or plateau Temporal association: no Subgroup only: no Adjustments: not given	The city is one of the largest fluoropolymer manufacturing centers in China Fasting samples PFAS highly correlated
Attanasio, 2019	US NHANES 2013-16	Cross-sectional	Who: adolescents Ages: 12-19 N: 354 males and 305 females	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Levels: geometric mean (SE) = 1.50 (0.06) and 1.22 (0.06) in males and females, respectively	Serum	ALT, AST, GGT, TB	Serum	Regression coefficients: Males: ALT: -0.11 (-0.21 to -0.01) (p-trend = 0.09) AST: no association GGT: -0.13 (-0.25-0.003) (p-trend = 0.08) TB: 0.19 (0.08-0.30) (p-trend <0.01) ORs for an elevated ALT and AST are below 0.5 Females: ALT: 0.20 (0.07-0.34) (p-trend <0.01)* AST: 0.11 (0.00-0.21) (p-trend = 0.03)* GGT: 0.19 (0.02-0.35) (p-trend = 0.02)* TB: 0.10 (-0.01-0.20) (p-trend = 0.09)* ORs for an elevated ALT, AST, and GGT are above 1.5.	Difference in log liver outcome between the 4th and 1st quartiles of PFOA; logistic regression	Adjusted for age, race, BMI, education, poverty index, smoking, and menarche	Large magnitude: yes Statistical significance: yes Dose-response: linear or plateau Temporal association: no Subgroup only: mostly in women Adjustments: *large changes with adjustments	Opposite effects in males and females
Gleason et al., 2015	US NHANES 2007-10	Cross-sectional	Who: children and adults Ages: ≥12 N: 4,333	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Levels: median (IQR) = 3.7 (2.5-5.2)	Serum	ALT, AST, GGT, , ALP, TB	Serum	Regression coefficients: ALT: 0.038 (0.014-0.062) GGT: 0.058 (0.021-0.096) AST: 0.025 (0.007-0.043) ALP: 0.003 (-0.023-0.016) TB: 0.048 (0.016-0.081)	Linear regression for ln PFOA and ln liver enzyme	Adjusted for age, gender, race/ethnicity, BMI, poverty, and alcohol	Large magnitude: unclear Statistical significance: yes Dose-response: mixed, see their Figure 1 Temporal association: no Subgroup only: no Adjustments: reduced magnitude with adjustments	SAS surveyreg used Not in US EPA 2016a or 2016b

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Jain and Ducatman, 2018a	US NHANES 2011-14	Cross-sectional	Who: adults Ages: ≥20 N: 3,573	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Above detection: 99.4% Levels: geometric means and 95% CIs = 2.2 (2.0 - 2.3) and 2.0 (1.8 - 2.1) ng/ml in non-obese and obese participants, respectively	Serum	AST, ALT, GGT, ALP, and TB	Serum	Regression coefficients: **For obese participants:** ALT: 0.07065, p <0.01 AST: 0.0744, p=0.03 GGT: 0.07422, p=0.03 TB: 0.06023, p=0.03 **Non-obese subjects:** no associations A 10 percent increase in PFOA was associated with a 0.68 percent increase in ALT, a 0.71 percent increase in GGT, a 0.49 percent increase in AST, a 0.13 percent increase in ALP, and a 0.58 percent increase in TB among obese participants	Linear regression, see notes	Adjusted for gender, race/ethnicity, age, poverty income ratio, physical activity, BMI, and serum cotinine	Large magnitude: unclear Statistical significance: yes Dose-response: yes Temporal association: no Subgroup only: yes, obese Adjustments: unclear	Regression coefficients appear to represent linear associations between log10 PFAS and log10 liver enzyme levels Associations varied by GFR but not clearly consistent with confounding (Jain, 2019)
Khalil et al., 2018	Dayton, Ohio 2016	Cross-sectional	Who: obese children Ages: 8-12 N: 48	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear but low detection limits Levels: median (IQR) = 0.99 (0.45) ng/ml	Serum	ALT, AST	Serum	ALT: no association AST: no association	Linear regression	Adjusted for age, sex, and ethnicity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	
Mora et al., 2018	Boston, Massa-chusetts 1999-2002	Prospective cohort and cross-sectional	Who: maternal offspring pairs Ages: 6-11 (children) N: 653	Selection: unclear Participation: 38% Equal groups: unclear Blinded: unclear Above detection: >99% Levels: median (IQR) = 5.4 (3.9-7.6) ng/ml	Plasma Maternal (gestation week 7) and child average age 7 years	ALT	Plasma Child Average age 7 years	Cohort: no associations Cross-sectional: β = -0.7 (-1.4 to 0.0) Similar results in boys and girls	Change per interquartile increase in PFOA	Adjusted for maternal education, prenatal smoking, gestational age at blood draw, child's sex, race/ethnicity, and age at ALT measurement Additional adjustments for household income, marital status, maternal plasma albumin concentrations and GFR during pregnancy, breast-feeding duration; and child's physical activity, screen time, and fast food and soda consumption did not materially change PFAS coefficients	Large magnitude: unclear Statistical significance: borderline Dose-response: yes Temporal association: no Subgroup only: no Adjustments: unclear	Difference between prospective vs. cross-sectional results

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Salihovic et al., 2018	Sweden 2001-14	Prospective cohort	Who: adults Ages: 75-80 N: 1,002	Selection: randomly selected from general population registers Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear but low detection limits Levels: median (IQR) = 3.31 (2.52-4.39) ng/ml	Serum or plasma	ALT, ALP, GGT, TB	Serum	Regression coefficients: TB: -1.39 (p <0.001) ALT: 0.04 (p <0.001) ALP: 0.11 (p <0.001) GGT: 0.07 (p=0.01) No interaction with BMI	Change in lipid level per ln increase in PFOA	Adjusted for sex, LDL and HDL cholesterol, serum triglycerides, BMI, fasting glucose levels, statin use, and smoking Similar results following adjustment for only sex or only sex and GFR	Large magnitude: unclear Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: no	Ten year follow-up Correlation between PFOS and PFOA = 0.45

Data in parentheses are 95% confidence intervals unless otherwise stated
High exposure occupational and community studies are listed first, then rows are sorted alphabetically by first author
Ages are in years unless otherwise noted
Abbreviations: β, regression coefficient; ALT, alanine aminotransferase, ALP, alkaline phosphatase; AST, aspartate; BMI, body mass index; F/U, follow-up; GFR, glomerular filtration rate; GGT, γ-glutamyl transferase; HDL, high density lipoprotein; IQR, interquartile range; LDL, low density lipoprotein; N, number of participants; OR, odds ratio; RR, rate ratio; SE, standard error; SMR, standardized mortality ratio; TB, total bilirubin

Table A7.6. Recent epidemiologic studies on PFOS and liver toxicity

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Nian et al., 2019	Shen-yang, China	Cross-sectional 2015-16	Who: adults Ages: ≥35 N: 1,605	Selection: government workers, retirees, and others who were long-term residents of the city Participation: 93% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: median (IQR) = 27.39 (18.05-40.62) ng/ml	Serum	AST, ALT, ALP, TB, GGT	Serum	Regression coefficients: ALT: 4.1 (0.6-7.7) AST: 2.0 (-0.3-4.3) ALP: no association GGT: 2.8 (-0.8-6.5) TB: 2.4 (-0.3-6.5) Results are for total PFOS; associations less strong for branched chain PFOS than n-PFOS except for TB (β = 2.5 (0.1-4.9))	Percent change in linear regression coefficients of lognormal liver biomarkers per each one lognormal increase in PFOS	Adjusted for age, sex, career, income, education, alcohol, smoking, giblet and seafood consumption, exercise, and BMI Mixed results when those taking relevant medications, smokers, and alcohol drinkers are excluded, some associations still seen	Large magnitude: no Statistical significance: yes Dose-response: plateau Temporal association: no Subgroup only: no Adjustments: not given	The city is one of the largest fluoropolymer manufacturing centers in China Fasting samples PFAS highly correlated
Attanasio, 2019	US NHANES 2013-16	Cross-sectional	Who: adolescents Ages: 12-19 N: 354 males and 305 females	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Levels: geometric mean (SE) = 3.68 (0.12) and 2.76 (0.14) in males and females, respectively	Serum	ALT, AST, GGT, TB	Serum	Regression coefficients: Males: ALT: no association AST: no association GGT: no associationa TB: 0.14 (0.02-0.27) (p-trend <0.01) ORs for an elevated ALT, AST, and GGT are near 1.0 Females: ALT: 0.15 (0.02-0.29) (p-trend = 0.13)* AST: 0.14 (0.04-0.23) (p-trend = 0.04)* GGT: 0.11 (-0.02-0.24) (p-trend = 0.34) TB: 0.10 (0.02-0.19) (p-trend <0.01)* ORs for an elevated ALT, AST, and GGT are above 1.5.	Difference in log liver outcome between the 4th and 1st quartiles of PFOS; logistic regression	Adjusted for age, race, BMI, education, poverty index, smoking, and menarche	Large magnitude: yes Statistical significance: yes Dose-response: linear or plateau Temporal association: no Subgroup only: mostly in women Adjustments: *large changes with adjustments	Some different effects in males and females

Author Year	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confounding	Other aspects of causal inference	Notes
Gleason et al., 2015	US NHANES 2007-10	Cross-sectional	Who: children and adults Ages: ≥12 N: 4,333	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Levels: median (IQR) = 11.3 (7.0-18.0) ng/ml	Serum	ALT, AST, GGT, , ALP, TB	Serum	ALT: no association GGT: no association AST: no association ALP: no association TB: no association	Linear regression for ln PFOS and ln liver enzyme	Adjusted for age, gender, race/ethnicity, BMI, poverty, and alcohol	Large magnitude: unclear Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: reduced magnitude with adjustments	SAS surveyreg used Not in US EPA 2016a or b
Jain and Ducatman, 2018a	US NHANES 2011-14	Cross-sectional	Who: adults Ages: ≥20 N: 3,573	Selection: random cluster sampling Participation: unclear Equal groups: unclear Blinded: likely Above detection: 99.4% Levels: geometric means and 95% CIs = 6.3 (5.8 - 6.8) and 5.5 (5.0 - 6.0) ng/ml in non-obese and obese participants, respectively	Serum	AST, ALT, GGT, ALP, and TB	Serum	No associations in obese or non-obese participants	Linear regression	Adjusted for gender, race/ethnicity, age, poverty income ratio, physical activity, BMI, and serum cotinine	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Associations varied by GFR but not clearly consistent with confounding (Jain, 2019)
Khalil et al., 2018	Dayton, Ohio 2016	Cross-sectional	Who: obese children Ages: 8-12 N: 48	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear but low detection limits Levels: median (IQR) = 2.79 (2.10) ng/ml	Serum	ALT, AST	Serum	ALT: no association AST: no association	Linear regression	Adjusted for age, sex, and ethnicity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	
Mora et al., 2018	Boston, Massa-chusetts 1999-2002	Prospective cohort and cross-sectional	Who: maternal offspring pairs Ages: 6-11 (children) N: 653	Selection: unclear Participation: 38% Equal groups: unclear Blinded: unclear Above detection: >99% Levels: median (IQR) = 24.6 (17.9–34) ng/ml	Plasma Maternal (gestation week 7) and child average age 7 years	ALT	Plasma Child Average age 7 years	Prospective cohort: no association Cross-sectional: no association Similar results in boys and girls	Change per interquartile increase in PFOS	Adjusted for maternal education, prenatal smoking, gestational age at blood draw, child's sex, race/ethnicity, and age at ALT measurement Additional adjustments for household income, marital status, maternal plasma albumin concentrations and GFR during pregnancy, breast-feeding duration; and child's physical activity, screen time, and fast food and soda consumption did not materially change coefficients	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	
Salihovic et al., 2018	Sweden 2001-14	Prospective cohort	Who: adults Ages: 75-80 N: 1,002	Selection: randomly selected from general population registers Participation: unclear Equal groups: unclear Blinded: unclear Above detection: unclear but low detection limits Levels: median (IQR) = 13.2 (9.95-17.8) ng/ml	Serum or plasma	ALT, ALP, GGT, TB	Serum	<u>Regression coefficients:</u> TB: -0.58 (p <0.001) ALT: 0.03 (p <0.001) ALP: 0.02 (p=0.37) GGT: 0.02 (p=0.40)	Change in lipid level per ln increase in PFOS	Adjusted for sex, LDL and HDL cholesterol, serum triglycerides, BMI, fasting glucose levels, statin use, and smoking	Large magnitude: unclear Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: unclear	Ten year follow-up Correlation between PFOS and PFOA = 0.45

Thyroid Toxicity

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018a), and others, OEHHA searched for all new human epidemiologic studies on PFOA or PFOS and thyroid toxicity published since the 2016 US EPA reviews. PubMed and Embase were searched for all human studies on PFOA or PFOS and thyroid disease or thyroid hormones published between December 2015 (the end of the US EPA literature search) and Septmber 20, 2020 (the end of OEHHA's literature search). The outcome portion of the search string used is shown below.

(thyro OR goiter OR Grave* OR hyperthyro* OR hypothyro* OR diiodotyrosine OR triiodothyronine OR TSH OR T4 OR TT4 OR FT4 OR T3 OR Hashimoto*)*

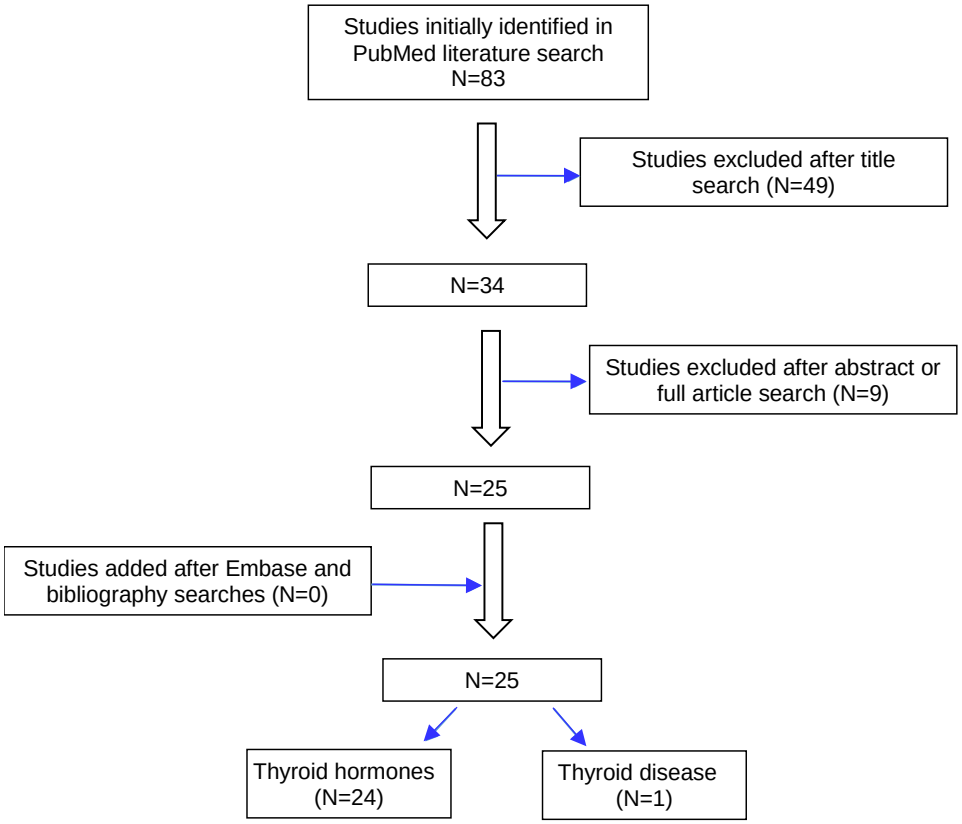
The key words in this search string were based on the outcomes identified by US EPA and on preliminary searches OEHHA performed in PubMed.

In the large majority of studies OEHHA identified, and in the large majority of studies identified by US EPA (2016a, b), the outcomes assessed were thyroid hormone levels and not outcomes involving more overt thyroid disease. In order to identify trends in the literature overall, the results across all studies were compared regardless of the date published. This assessment included the studies identified by three sources: 1. those identified by US EPA (2016a, b); 2. those identified in a 2017 meta-analysis of PFOA or PFOS and thyroid hormones in children and pregnant women (Ballesteros et al., 2017); and 3. the more recent studies that OEHHA identified in this literature search. The studies OEHHA identified from these three sources did not present results using the same scales for exposure and outcome, which made it difficult to directly and quantitatively compare results across all studies. For example, some studies reported only linear regression coefficients while others reported only correlation coefficients. In some studies, regression coefficients were calculated for the change in thyroid hormone levels associated with each log-normal increase in PFOA or PFOS, while in others this was for each log10 increase or each interquartile increase. Because of this heterogeneity, OEHHA initially sought to identify trends across studies by simply evaluating whether or not study results were consistent with either a positive association (e.g., a positive regression or correlation coefficient) or an inverse association (e.g., a negative regression or correlation coefficient) between PFOA or PFOS and thyroid hormone levels, and whether or not these associations were statistically significant. Since clear trends were not seen when OEHHA examined all studies together, the studies were then sub-categorized based on various aspects of study quality or study design and were evaluated for whether trends might be seen within these subcategories. OEHHA also attempted to identify trends in study results across various sex and age groups by categorizing results as being in only men or only women, or only in newborns, young children (generally 0-5 years old), older children (generally 10-19 years old), or adults (most subjects ≥19 years old). Because clear trends could not be identified in any of these categories, studies and their results and key design features are shown in summary form only.

Results

A general description of OEHHA's literature search is shown in Figure A7.3. A list of studies excluded after abstract and title search is provided in Table A7.28.

Figure A7.3. Literature search: recent epidemiologic studies of PFOA or PFOS and thyroid toxicity*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

Overall, OEHHA found 25 publications that examined associations between PFOA or PFOS and thyroid hormone levels (Table A7.7). A number of these studies presented results for different demographic groups, and overall there were a total of 67 sets of results for PFOA or PFOS. Twenty of these were in non-pregnant adults, 11 were in pregnant females, 20 were in newborns, six were in young children, nine were in older children, and two involved children with a wide range of ages. The studies took place in a wide variety of locations throughout Europe, Asia, and the US. Of the 67 sets of results, 16 involved prospective analyses and 51 were cross-sectional. Sample sizes ranged from 29 to 7,020 participants. In all but one study (Lopez-Espinosa et al., 2012), the outcome and exposure were assessed using PFOA or PFOS and thyroid hormone concentrations in serum or plasma. In Lopez-Espinosa et al. (2012), in utero PFOA serum concentrations were estimated using models that included information on PFOA releases from a nearby chemical plant, environmental characteristics of the region, and participant information on residential history, water sources, water intake, and TK data. Few studies presented detailed information on subject recruitment or participation rates and it appears that most studies involved convenience samples. Studies were adjusted for a variety of different potential confounding variables and these are shown in Table A7.7.

Table A7.7 Studies reporting regression or correlation coefficients between PFOA or PFOS and thyroid hormone levels: all years[a]

Study	Source[b]	Location	Design	N	Exp	Group	Sex	PFAS	TH	PFOA[c]			PFOS[c]			Adjustments	Notes
										TSH	T4	fT4	TSH	T4	fT4		
Blake et al., 2018	New	Fernald, OH	Pros	122	Low	Adults	All	Adult	Adult	↓	↓	0	↑↑	↓	0	Age, year, sex, educ, income, marital, BMI	Living near a uranium processing facility
Bloom et al., 2010	USEPA	New York	CS	31	Low	Adults	All	Adult	Adult	↓	0	↓	↑	0	↑	Unknown	In anglers
Byrne et al., 2018	New	Alaska	CS	85	Low	Adults	All	Adult	Adult	↑↑	↑	↓	↓	↓	↑	Age, sex, smok, other PFAS	Native Alaskans
Dallaire et al., 2009	USEPA	Canada	CS	623	High	Adults	All	Adult	Adult	0	0	0	↓↓	0	↑↑	Age, sex, BMI, educ, lipids, smok	Inuit population
Emmett et al., 2006	USEPA	Southeast Ohio	CS	371	High	Adults	All	Adult	Adult	↑	0	0	0	0	0	Unclear	High environmental exposure
Jain &Ducatman 2019a	New	US	CS	7,020	Low	Adults	All	Adult	Adult	--	--	--	--	--	--	Gender, race, age, BMI, fasting, poverty, smok, calories, others	NHANES 2007-12, inconsistent by GFR
Li et al., 2017	New	China	CS	202	Low	Adults	All	Adult	Adult	↑	0	↓	↑↑	0	↓↓	Unadjusted	
Liu et al., 2018a	New	Boston	CS	621	Low	Adults	All	Adult	Adult	↓	↑	↑	↓	↑↑	↑	Age, race, sex, educ, smok, ETOH, exercise, meno, diet	Overweight and obese adults
Olsen et al., 2007	USEPA	Antwerp, Belgium and Decatur, AL	CS	506	High	Adults	All	Adult	Adult	↑	↓	↓↓	ns	ns	0	Age, BMI, ETOH	Occupational exposure
Shrestha et al., 2015	USEPA	Hudson River Valley	CS	87	Low	Adults	All	Adult	Adult	↑	↑	↑	↑	↑↑	↑↑	Age, sex, educ, PCBs	
Webster et al., 2016	USEPA	US	CS	1,525	Low	Adults	All	Adult	Adult	↑	↓↑	↑	↓	↑	↑	Age, race, cotinine, sex, parity, pregnancy, meno	NHANES 2007-8, assoc. with anti-TPO
Crawford et al., 2017	New	North Carolina	CS	99	Low	Adults	Female	Adult	Adult	↑	↑	↑	↓	↑	↑	Age	
Heffernan et al., 2018	New	UK	CS	30	Low	Adults	Female	Adult	Adult	↓	0	↓	↑	0	↓	Age, BMI, GFR, albumin, meds	POS case control study: controls
Heffernan et al., 2018	New	UK	CS	29	Low	Adults	Female	Adult	Adult	↑↑	0	↑	↑	0	↓	Age, BMI, GFR, albumin, meds	POS case control study: cases
Seo et al., 2018	New	S Korea	CS	357	Low	Adults	Female	Adult	Adult	ns	0	↑	ns	0	↑↑	Unk	
Wen et al., 2013	USEPA	US	CS	509	Low	Adults	Female	Adult	Adult	↓	↑	↓	↑	↑	↑	Age, race, ETOH, smok, iodine	NHANES 2007-8, 2009-10
Zhang et al., 2018	New	China	CS	120	Low	Adults	Female	Adult	Adult	↑↑	0	↓↓	↑↑	0	↓↓	Age, BMI, educ, income, sleep, parity	Premature ovarian insufficiency study
Seo et al., 2018	New	S Korea	CS	Unk	Low	Adults	Male	Adult	Adult	ns	0	ns	ns	0	↑↑	Unk	
Wen et al., 2013	USEPA	US	CS	672	Low	Adults	Male	Adult	Adult	↑	↑↓	↓	↓	↓	↓	Age, race, ETOH, smok, iodine	NHANES 2007-8, 2009-10
Caron-Beaudoin et al., 2019	New	Quebec	CS	89	Low	Child: all	Female	Child	Child	↑↓	0	↑↓	↑↓	0	↑↓	Age, BMI, ethnicity	Some differences by tribe and antibody status
Caron-Beaudoin et al., 2019	New	Quebec	CS	95	Low	Child: all	Male	Child	Child	↑↓	0	↑↓	↑↓	0	↑↓	Age, BMI, ethnicity	
Kim et al., 2011	Balles	S Korea	CS	31	Low	Child: newborn	All	Cord	Cord	↑	↓	0	↓	↓	0	Age, BMI, gest	
Kim et al., 2011	Balles	S Korea	Pros	29	Low	Child: newborn	All	Preg	Cord	↑↑	↓	0	↑	↓	0	Age, BMI, gest	
Wang et al., 2014	Balles	Taiwan	Pros	114	Low	Child: newborn	All	Preg	Cord	↓	↑	↓	↓	↑	↑	Age, educ, parity, neonatal sex, cesarean	

Study	Source[b]	Location	Design	N	Exp	Group	Sex	PFAS	TH	PFOA[c]			PFOS[c]			Adjustments	Notes
										TSH	T4	fT4	TSH	T4	fT4		
Yang et al., 2016	New	China	CS	157	Low	Child: newborn	All	Preg	Cord	↓	↓	↓	↓↓	↓	↓	Age, BMI, income, parity, delivery	
Aimuzi et al., 2019	New	Shanghai	CS	262	Low	Child: newborn	Female	Cord	Cord	ns	0	ns	↓↓	0	ns	Age, fish, parity, gest, BMI	All had Cesarean sections
de Cock et al., 2014	Balles	Netherlands	Pros	31	Low	Child: newborn	Female	Cord	Newborn	0	↑↑	0	0	↑	0	Age, weight, gest, parity, smok, ETOH, BMI	
Dufour et al., 2018	New	Belgium	CS	101	Low	Child: newborn	Female	Cord	Newborn	↓	0	0	↓	0	0	Parity, gest	
Shah-Kulkarni et al., 2016	New	S Korea	CS	147	Low	Child: newborn	Female	Cord	Cord	↓	↑	0	↓	↑	0	Age, educ, BMI, ETOH, parity, gest	
Tsai et al., 2017	New	Taiwan	CS	54	Low	Child: newborn	Female	Cord	Cord	↑	↑	0	↑	↑	0	Age, BMI, educ, gest, cesarean	
Preston et al., 2018	New	Boston	Pros	229	Low	Child: newborn	Female	Preg	Newborn	0	↓	0	0	↓	0	Age, race, smok, parity, gest, cesarean	
Xiao et al., 2019	New	Faroe Islands	Pros	172	Low	Child: newborn	Female	Preg	Cord	↑	↑	↑↓	↑	↑	↑	Gest, educ, ETOH, BMI, parity, smok, Hg, PCBs	
Aimuzi et al., 2019	New	Shanghai	CS	305	Low	Child: newborn	Male	Cord	Cord	ns	0	↑↑	ns	0	ns	Age, fish, parity, gest, BMI	All had Cesarean sections
de Cock et al., 2014	Balles	Netherlands	Pros	52	Low	Child: newborn	Male	Cord	Newborn	0	↑	0	0	↓	0	Age, weight, gest, parity, smok, ETOH, BMI	
Dufour et al., 2018	New	Belgium	CS	113	Low	Child: newborn	Male	Cord	Newborn	↓	0	0	↓	0	0	Parity, gest	
Shah-Kulkarni et al., 2016	New	S Korea	CS	132	Low	Child: newborn	Male	Cord	Cord	↓	↓	0	↑	↑	0	Age, educ, BMI, ETOH, parity, gest	
Tsai et al., 2017	New	Taiwan	CS	64	Low	Child: newborn	Male	Cord	Cord	↑	↓	0	↑↑	↓↓	0	Age, BMI, educ, gest, cesarean	
Itoh et al., 2019	New	Japan	Pros	259	Low	Child: newborn	Male	Preg	Cord	↑↓	0	↑↓	↑↑	0	↑↓	Age, parity, educ, ETOH, smok, BMI	
Preston et al., 2018	New	Boston	Pros	236	Low	Child: newborn	Male	Preg	Newborn	0	↓	0	0	↓↓	0	Age, race, smok, parity, gest, cesarean	
Xiao et al., 2019	New	Faroe Islands	Pros	172	Low	Child: newborn	Male	Preg	Cord	↑	↓	↑↓	↑	↑↓	↑	Gest, educ, ETOH, BMI, parity, smok, Hg, PCBs	
Kang et al., 2018	New	S Korea	CS	147	Low	Child: older	All	Child	Child	↓	0	↑	↓	0	↑↓	Age, sex, BMI, income, ETS	
Khalil et al., 2018	New	Ohio	CS	48	Low	Child: older	All	Child	Child	↓	0	↓	↓	0	↓	Age, sex, race	Obese children
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	Pros	2,741	High	Child: older	All	Preg	Child	↑	↓	0	0	0	0	Age, sex, and month of sampling	High environmental exposure, modeled
Lewis et al., 2015	Balles	US	CS	145	Low	Child: older	Female	Child	Child	↓↓	↑	↑	↓	↓	↑	Age, BMI, income, race, cotinine	NHANES
Lin et al., 2013	Balles	Taipei	CS	144	Low	Child: older	Female	Child	Child	↓	0	↓	↓	0	↑	Age, sex, smok, ETOH	
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	CS	3,062	High	Child: older	Female	Child	Child	↑	↓	0	↑	↑↑	0	Age, sex, and month of sampling	High environmental exposure
Lewis et al., 2015	Balles	US	CS	158	Low	Child: older	Male	Child	Child	↑	↓	↓	↑↑	↓	↑	Age, BMI, income, race, cotinine	NHANES
Lin et al., 2013	Balles	Taipei	CS	65	Low	Child: older	Male	Child	Child	↓	0	↓	↑↓	0	↓	Age, sex, smok, ETOH	
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	CS	3,328	High	Child: older	Male	Child	Child	↑	↑	0	↑	↑↑	0	Age, sex, and month of sampling	High environmental exposure
Kim et al., 2016	New	S Korea	CS	40	Low	Child: young	All	Child	Child	ns	0	ns	ns	0	ns	Unadjusted	

Study	Source[b]	Location	Design	N	Exp	Group	Sex	PFAS	TH	PFOA[c]			PFOS[c]			Adjustments	Notes
										TSH	T4	fT4	TSH	T4	fT4		
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	Pros	476	High	Child: young	All	Preg	Child	↓	↑↑	0	0	0	0	Age, sex, and month of sampling	High environmental exposure, modeled
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	CS	500	High	Child: young	Female	Child	Child	↓↓	↓	0	↑	↑	0	Age, sex, and month of sampling	High environmental exposure
Kato et al., 2016	New	Japan	Pros	212	Low	Child: young	Female	Preg	Child	↑	0	↓	↑↑	0	↓	Age, parity, anti-TPO, sampling, gest, weight, cesarean	
Lopez-Espinoza et al., 2012	Balles	Mid-Ohio Valley	CS	471	High	Child: young	Male	Child	Child	↓	↑	0	↑	↑	0	Age, sex, and month of sampling	High environmental exposure
Kato et al., 2016	New	Japan	Pros	180	Low	Child: young	Male	Preg	Child	↓	0	↑	↑↑	0	↓	Age, parity, TPO, sampling, gest, weight, cesarean	
Itoh et al., 2019	New	Japan	Pros	240	Low	Child: newborn	Female	Preg	Cord	↑↓	0	↑↓	↑↓	0	↑↓	Age, parity, educ, ETOH, smok, BMI	Similar results by thyroid antibody status
Berg et al., 2015	Balles	Norway	CS	375	Low	Pregnant	Female	Preg	Preg	↑	0	0	↑↑	0	0	Age, parity, BMI, thyroxine binding capacity	
Inoue et al., 2019	New	Denmark	CS	1,366	Low	Pregnant	Female	Preg	Preg	↑	0	↑↓	↑	0	↓	Age, SES, BMI, parity, birth year	Inconsistent by gestational week
Itoh et al., 2019	New	Japan	CS	701	Low	Pregnant	Female	Preg	Preg	↑↓	0	↑↓	↓	0	↑	Age, parity, BMI, educ, ETOH, smok	Small differences by thyroid antibody status
Kato et al., 2016	New	Japan	CS	392	Low	Pregnant	Female	Preg	Preg	↑	0	↑	↓↓	0	↑	Age, parity, educ, anti-TPO, diet, sampling	
Preston et al., 2018	New	Boston	CS	718	Low	Pregnant	Female	Preg	Preg	↑	↑	↓↓	↓	↑	↓	Age, race, smok, diet, parity, gest	
Reardon et al., 2019	New	Calgary	Pros	494	Low	Pregnant	Female	Preg	Preg	↑↓	0	0	↑↓	0	0	Age, ethnicity, smok, thyroid condition, ETOH, drug use	No interaction with gestational age or TPO
Wang et al., 2013	Balles	Norway	CS	903	Low	Pregnant	Female	Preg	Preg	↓	0	0	↑↑	0	0	Age, HDL chol, diet, parity, gest	
Wang et al., 2014	Balles	Taiwan	CS	283	Low	Pregnant	Female	Preg	Preg	↑	↑	↓	↓	↑	↑	Age, educ, parity, neonatal sex, cesarean	
Webster et al., 2014	Balles	Vancouver	CS	151	Low	Pregnant	Female	Preg	Preg	↑	↓	↓	↑	↓	↑	Age, sampling time, anti-TPO	Association seen with anti-TPO
Yang et al., 2016	New	China	CS	157	Low	Pregnant	Female	Preg	Preg	↓	↑	↑↓	↓↓	↑	↓	Age, BMI, income, delivery	
Xiao et al., 2019	New	Faroe Islands	CS	172	Low	Pregnant	Female	Preg	Preg	↑	↑↓	↑↓	↑	↑↓	↑↓	Gest, educ, ETOH, BMI, parity, smok, Hg, PCBs	

[a] Rows are sorted by Group then by Sex
[b] Source: results are from US EPA (2016a and b), Ballesteros et al. (2017)("Balles"), or OEHHA's updated literature review ("New")
[c] Codes to the results:
↑, positive association, not statistically significant
↑↑, positive association, statistically significant
↓, inverse association, not statistically significant
↓↓, inverse association, statistically significant
↑↓, regression or correlation coefficient is zero
0, data not provided
ns, result not statistically significant but actual coefficient not given

Abbreviations: anti-TPO, anti-thyroid peroxidase antibodies; BMI, body mass index; chol, cholesterol; CS, cross sectional study design; educ, education; ETOH, alcohol use; ETS, environmental tobacco smoke; exp, high or low exposure scenario; gest, gestational age; GFR, glomerular filtration rate; Hg, mercury; marital, marital status; meds, medication use; meno, menopause; N, number of participants; NHANES, US National Health and Nutrition Examination Survey; PCBs, polychlorinated biphenyls; PFAS, period when PFOA or PFOS were measured; POS, polycystic ovarian syndrome; Preg, pregnant; Pros, prospective study design; smok, smoking; TH, period when thyroid hormone levels were measured; TPO, thyroid peroxidase antibody; Unk, unknown

Serum Lipid Concentrations

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018a), and others, OEHHA searched for all human epidemiologic studies on PFOA or PFOS and lipid levels or lipid specific disorders (e.g., hypercholesterolemia) published since the 2016 US EPA reviews, and up to September 20, 2020. The general methods described above were used. Because the studies identified by US EPA mostly involved total cholesterol, LDL, HDL, and TG levels, OEHHA's focus was also on these particular outcomes, although other lipid-related outcomes were also considered. The outcome portion of the search string used is shown below. The key words in this string were based on the outcomes in the studies identified by US EPA and on the key words and medical subject headings listed in articles identified in a preliminary search OEHHA performed in PubMed.

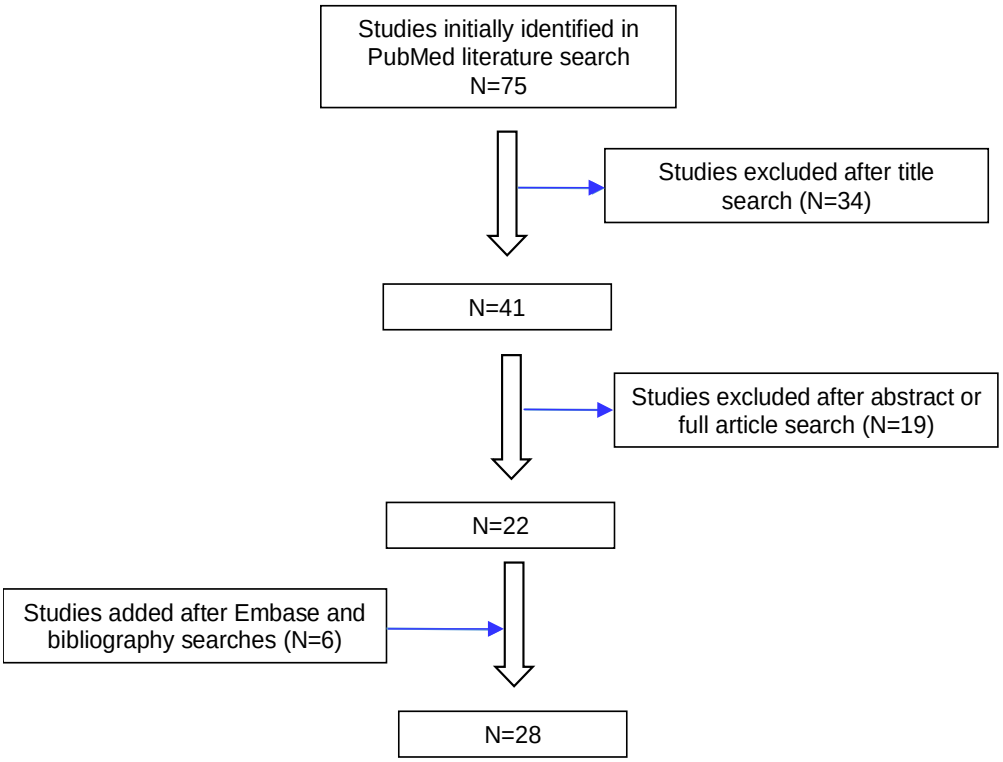
(cholesterol[tiab] OR lipid[tiab] OR LDL[tiab] HDL[tiab] OR VLDL[tiab] OR lipoprotein*[tiab] OR apolipoprotein*[tiab] OR triglyceride*[tiab] OR hypercholesterol*[tiab] OR hypocholesterol*[tiab] OR hyperlipid*[tiab] OR hypolipid*[tiab] OR dyslipidemia*[tiab] OR hyertriglyceridemia[tiab])*

Results

OEHHA identified 28 human epidemiologic studies of PFOA and PFOS and lipid levels published since (or otherwise not included in) the 2016 US EPA reviews. Details for each study are provided in Tables A7.8 and A7.9. A general description of the literature search is provided in Figure A7.4. A list of studies excluded after abstract and title search is provided in Table A7.28. A brief summary of the results of each study and quality ratings given to each study are shown in Tables A7.11 and A7.12.

Twenty-seven of 28 studies provided results for both PFOA and PFOS, with one only providing results for PFOA (Starling et al., 2017). Eighteen studies were done primarily in adults, 10 were done primarily in children. Most studies were done in the US, but several included study populations from Asia and Europe. Seven studies were based on information provided as part of US NHANES. All used blood concentrations to assess exposure and outcome. In the large majority of studies, fasting blood samples were used. Five of the studies used prospective cohort designs, the rest were cross-sectional studies. The overall quality of the studies done in adults appeared to be fairly high, with overall quality scores ≥8 in most studies (see Tables A7.11 and A7.12). Study quality tended to be lower in studies done in children.

Figure A7.4. Literature search: recent epidemiologic studies of PFOA or PFOS and lipid levels*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

Table A7.8. Recent epidemiologic studies of PFOA and lipid levels

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Christen-sen et al., 2019	Location: US NHANES Years: 2007-14	Cross-sectional	Who: adults Ages: ≥20 N: 2,975	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 2.8 ng/ml	Serum, fasting	Blood, fasting, and use of lipid lowering medication	TC: NA LDL: NA HDL: OR for low HDL = 1.26 (0.73-2.16) TG: OR for high TG = 1.27 (0.73-2.22) Other: NA	OR for 4th vs. 1st quartile of PFOA	Adjusted for survey year, age, sex, race/ethnicity, income, alcohol, and smoking	Large magnitude: yes for HDL and TG Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Presents results adjusted and unadjusted for NHANES sampling weights "High triglycerides" if TG >150 mg/dl or medication use; "Low HDL" if HDL <30 mg/dl or medication use Potential weaknesses: Low statistical power Cross-sectional: reverse causation
Dong et al., 2019	Location: US NHANES Years: 2003-14	Cross-sectional	Who: adults Ages: 20-80 N: 8,948	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 3.0 ng/ml	Serum, fasting	Blood, fasting	TC: β = 1.48 (0.2-2.8), means for each quintile are given in their Figure S4 LDL: small increase (figure form only) HDL: no association TG: NA Other: NA See separate entry for results in adolescents	Change in lipid level (mg/dl) per ng/ml increase in PFOA (most data in figure form)	Adjusted for age, gender, race, BMI, income, waist circumference, activity, diabetes, smoking, and alcohol	Large magnitude: no Statistical significance: yes for TC Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies PFOA and PFOS levels decreased over time PFOA and PFOS correlation = 0.69 Reference doses and benchmark doses also calculated Potential weaknesses: Cross-sectional: reverse causation Unclear if adequately adjusted for survey year
He et al., 2018	Location: US NHANES Years: 2003-12	Cross-sectional	Who: adults Ages: ≥20 N: 7,904	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: mean = 3.46-4.50 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: 1.43% (0.62-2.34%) increase in men and 1.07% (0.27-1.97%) increase in women LDL: 1.25% increase in women, p=0.128 for quartile 4 vs. 1, no increase in men HDL: no association TG: 1.61 to 2.06% increase in men and women for quartile 4 vs. 1, p >0.05 Other: NA	Percent change in lipid level per interquartile increase in PFOA	Adjusted for age, race, BMI, education, alcohol, income, and activity Stratified by gender	Large magnitude: no Statistical significance: yes for TC Dose-response: inverse U in women, linear in men Temporal association: no Subgroup only: for TC strongest associations in men ages 40-60 and women ages 60-70 Adjustments: not given	Overlap with other NHANES studies Potential weaknesses: Small effect size Cross-sectional: reverse causation Not adjusted for survey year
Huang et al., 2018	Location: US NHANES Years: 1999-2014	Cross-sectional	Who: adults Ages: ≥20 N: 10,859	Selection: randomized cluster sample Participation: unclear Equal groups: data only for total PFAS Blinded: yes Levels: median = 3.17 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: R = 0.092 LDL: R = 0.086 HDL: R = -0.037 TG: R = 0.086 Other: NA All p-values <0.01	Spearman correlation coefficients (R)	Results appear to be unadjusted	Large magnitude: no Statistical significance: yes for all Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Correlation with PFOS = 0.69 PFOA and PFOS levels declined over time Potential weaknesses: Relatively small effect sizes Limited co-variates including survey year Some lipid levels have also declined over time Cross-sectional: reverse causation

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Jain and Ducatman, 2019b	Location: US NHANES Years: 2005-14	Cross-sectional	Who: adults Ages: ≥20 N: 3,629	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: geometric mean = 2.9 ng/ml	Serum, fasting	Blood, fasting	TC: 0.50% (p=0.03) in obese men, no clear association in non-obese men or in women LDL: 0.79% (p=0.01) in obese men, no clear association in non-obese men or in women HDL: no association TG: no association Other: NA	Percent increase in lipid level for each 10% increase in PFOA	Adjusted for race/ethnicity, smoking, age, income, fasting time, lipid lowering medication, activity, survey year, and diet Stratified by gender	Large magnitude: no Statistical significance: yes for TC and LDL Dose-response: unclear Temporal association: no Subgroup only: yes, obese men Adjustments: not given	Overlap with other NHANES studies Stratified by obesity and gender Potential weaknesses: Cross-sectional: reverse causation
Liu et al., 2018b	Location: US NHANES Years: 2013-4	Cross-sectional	Who: adults Ages: ≥18 N: 1,871	Selection: randomized cluster sample Participation: unclear Equal groups: mostly, some differences in PFOA by age, race, income, and other factors Blinded: yes Levels: geometric mean = 1.86 ng/ml	Serum, likely fasting	Blood, likely fasting	TC: β = 5.58 (p <0.05) LDL: β = 4.47 (p >0.05) HDL: β = 1.93 (p <0.01) TG: β = -0.08 (p >0.05) Other: apolipoprotein B no association	Change in lipid level (in mg/dl) for each log increase in PFOA	Adjusted for age, gender, ethnicity, smoking, alcohol, income, waist circumference and relevant medications	Large magnitude: no Statistical significance: yes for TC and HDL Dose-response: log-linear Temporal association: no Subgroup only: no Adjustments: some decrease for HDL, small change for TC	Overlap with other NHANES studies Potential weaknesses: Cross-sectional: reverse causation
Chen et al., 2019	Location: Hvar, Croatia Years: 2007-8	Cross-sectional	Who: adults Ages: 44-56 N: 122	Selection: unclear Participation: unclear Equal groups: PFOA higher with lower education, otherwise similar Blinded: unclear Levels: geometric mean = 2.87 ng/ml	Plasma, fasting	Plasma, fasting	TC: no association in regression analyses; OR = 1.60 (0.64-4.00) for high TC (or lipid lowering medication use) in logistic regression LDL: no association HDL: no association TG: no association Other: NA	Change in lipid levels per one unit increase in ln PFOA; logistic regression	Adjusted for age, sex, SES, smoking, dietary pattern, and activity	Large magnitude: yes for TC Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Low statistical power Cross-sectional: reverse causation
Christen-sen et al., 2016	Location: Wisconsin Years: 2012-3	Cross-sectional	Who: male fishermen Ages: ≥50 N: 154	Selection: "interested in future studies" per online survey; flyers Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 2.50 ng/ml	Serum, fasting unknown	"have you ever been told by a doctor you have…high cholesterol"	TC: OR = 1.12 (0.85-1.50) for "high cholesterol" LDL: NA HDL: NA TG: NA Other: NA	Answered yes vs. no	Adjusted for age, BMI, work status, and alcohol consumption Males only	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -20º C Potential weaknesses: Self-reported non-specific outcome

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Donat-Vargas et al., 2019	Location: Sweden Years: 1990-2013	Cohort F/U: approxi-mately 10 years	Who: adults Ages: 40-60 at baseline N: 187	Selection: controls in a diabetes case-control study Participation: 56% Equal groups: unclear Blinded: unclear Levels: median = 2.9 ng/ml	Blood, fasting	Plasma, fasting	TC: no clear association in prospective analysis, possibly some decrease in repeated measures analysis LDL: NA HDL: NA TG: β = -0.10 mmol/L (-0.22-0.02) decrease for each one SD increase in PFOA Other: NA	Change in lipid level for tertiles of PFOA or per each SD increase in PFOA	Adjusted for gender, age, education, sample year, BMI, alcohol, exercise, energy intake, cotinine, and diet	Large magnitude: no, about a 7-8% decrease for TG Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	PFOA and PFOS levels decreased over time PFOA and PFOS highly correlated Potential weaknesses: Some inconsistency between repeated measures and prospective analysis
Graber et al., 2019	Location: New Jersey Years: 2016-17	Cross-sectional	Who: residents of an area contaminated with PFAS, likely from a nearby manufacturing facility Ages: ≥19 N: 105	Selection: claimants in a class action lawsuit, flers to all local residents Participation: unclear but likely low Equal groups: unclear Blinded: unclear Levels: geometric mean (95% CI) = 3.03 (2.70-3.40) ng/ml	Serum, fasting unknown	Self-reported physician diagnosed "high cholesterol"	TC: NA LDL: NA HDL: NA TG: NA Other: self-reported "high cholesterol" OR = 1.12 (0.94, 1.35)	Unclear	Adjusted for age and BMI	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: OR = 1.03 (0.94, 1.14) after adjustment for other PFAS	PFNA levels in water and serum were high Serum samples were collected and measured as part of a lawsuit, not collected or measured by the researchers Potential weaknesses: Methods are unclear Outcome not verified
Kishi et al., 2015	Location: Hokkaido, Japan Years: 2002-5	Cross-sectional	Who: pregnant women Ages: unknown N: 306	Selection: pregnant women, preterm care and delivery at participating hospital Participation: 28.6% Equal groups: higher PFOA associated with lower parity, lower caffeine intake, and male offspring Blinded: unclear Levels: median = 1.40 ng/ml	Serum, 23-35 weeks gestation, non-fasting	Blood, non-fasting	TC: No association Others not assessed	Linear regression with log10 PFOA and log10 TC	Adjusted for age, smoking, alcohol, income, parity, and gestation week	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Sample stored at -80º C Potential weaknesses:

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Lin et al., 2019	Location: 27 US medical centers Years: 1996-2014	Cohort and cross-sectional	Who: adults Ages: ≥25 N: 888	Selection: pre-diabetic adults Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 4.9 ng/ml	Plasma at baseline 1996-99, fasting unclear	Blood at baseline and annually, fasting	**Cross-sectional:** TC: β = 6.09 (3.14-9.04) LDL: β = 2.93 (0.22-5.63) HDL: β = -0.49 (-1.38-0.40) TG: β = 17.75 (9.77-25.74) Other: VLDL β = 3.66 (2.18-5.15) No effect modification by treatment group **Prospective:** Hypercholesterolemia: OR = 1.29 (1.05-1.57) per doubling of PFOA Greater effect in the placebo group Hypertriglyceridemia: OR = 1.48 (1.21-1.81) per doubling of PFOA Greater effect in the placebo group	Change in lipid level (mg/dl) per doubling of plasma PFOA; ORs	Adjusted for age, sex, race/ethnicity, marital status, education, smoking, alcohol, fat intake, fiber intake, activity, and waist circumference Cross-sectional: excluded people taking lipid lowering medications	Large magnitude: yes for multiple outcomes Statistical significance: yes for multiple outcomes Dose-response: linear Temporal association: yes Subgroup only: those not on special intervention Adjustments: not given	Randomized to lifestyle intervention or placebo Hypercholesterolemia defined by high TC, high LDL, or use of lipid lowering medication F/U: 15 years Potential weaknesses: Intervention may have altered results
Lin et al., 2020	Location: Taiwan Years: 2009-11	Cross-sectional	Who: adults Ages: 22-63 N: 597	Selection: controls in a coronary heart disease case-control study, recruited from a bulletin board announcement Participation: unclear Equal groups: PFOA higher in men, higher BMI, higher alcohol consumption, otherwise similar Blinded: unclear Levels: geometric mean = 3.77 ng/ml	Blood, fasting unknown	Blood, fasting unknown	TC: NA LDL: β = 6.12 (p=0.017) HDL: no association TG: β = 0.13 (p=0.004) Other: NA	Change in lipid level for each one unit ln increase in PFOA	Adjusted for age, gender, smoking alcohol, education, hypertension, BMI, and diabetes	Large magnitude: unclear Statistical significance: yes for LDL and TG Dose-response: possibly log-linear Temporal association: no Subgroup only: no Adjustments: little change	PFOA higher with higher BMI Potential weaknesses: Fasting status unknown Cross-sectional: reverse causation
Liu et al., 2018a	Location: Boston, MA Years: 2004-7	Cross-sectional	Who: participants in a weight loss program at baseline Ages: 30-70 N: 621	Selection: convenience sample Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 4.1 and 5.2 ng/ml in men and women, respectively	Plasma, fasting	Plasma, fasting	TC: R = 0.02 (p >0.05) LDL: R = 0.06 (p >0.05) HDL: R = -0.10 (p <0.05) TG: R = 0.08 (p <0.05) Other: NA	Partial Spearman correlation coefficients (R)	Adjusted for age, sex, race, education, smoking, alcohol, activity, menopause, hormone replacement, and dietary intervention group	Large magnitude: yes for HDL Statistical significance: yes for HDL and TG Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Change in lipid levels during weight loss also given but difficult to interpret Potential weaknesses: Cross-sectional: reverse causation

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Matilla-Santander et al., 2017	Location: Spain Years: 2003-8	Cross-sectional	Who: pregnant women Ages: first trimester N: 1,194	Selection: "population-based" Participation: <55% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: median (IQR) = 2.35 (1.63-3.30) ng/ml	First trimester plasma, non-fasting	First trimester serum, non-fasting	TC: β = 1.26 (0.01-2.54) 3.15 mg/dl difference between quartile 1 and 4 LDL: NA HDL: NA TG: β = -2.78 (-6.15-1.42) with inverted U-shapted dose-response Other: NA	Change in lipid level (mg/dl) per log10 increase in PFOA; PFOA quartiles	Adjusted for sub-cohort, place of birth, BMI, breast-feeding, parity, collection time, activity, and diet	Large magnitude: no Statistical significance: yes for TC Dose-response: log-linear (continuous), flattened (categorical) Temporal association: no Subgroup only: no Adjustments: mixed, effect size decreases in categorical analysis and increases in linear regression	Samples stored at -80º C Potential weaknesses: Inconsistent impact with adjustments Cross-sectional: reverse causation Little increase in effect after quartile 2 Non-fasting
Seo et al., 2018	Location: Korea Years: 2006-15	Cross-sectional	Who: adults Ages: 40-60 N: 786	Selection: residents of Seoul Participation: unclear Equal groups: PFOA and PFOS generally increased with age, and higher in males, lower in obese Blinded: unclear Levels: median = 4.06 ng/ml	Serum, appears non-fasting	Not discussed	*Most findings given in figure form* TC: increase from quartile 1 to 2 then flat (p-trend = 0.29) LDL: inverted U-shaped dose-response (p=0.27) HDL: no association TG: small increase across quartiles (p=0.12) Other: NA	Quartiles of PFOA	Unadjusted	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unadjusted only	Samples frozen at -20º C and analyzed within 30 days Potential weaknesses: Limited covariates Cross-sectional: reverse causation Fasting unknown
Starling et al., 2017	Location: Colorado Years: 2010-14	Cross-sectional	Who: pregnant women Ages: ≥16 N: 628	Selection: convenience sample from pregnancies at the University of Colorado Hospital Participation: <50% Equal groups: higher PFOA in non-Hispanic whites, higher education, higher income, and fewer prior pregnancies but otherwise similar Blinded: yes Levels: median = 1.1 ng/ml	Serum, fasting	Plasma, fasting	TC: 209.5; 213.4; 215.2 LDL: NA HDL: 62.2; 64.1; 65.3 TG: 159.6; 167.4; 157.8 Other: NA	Mean lipid levels (mg/dl) from 1st to 3rd tertile of PFOA	Unadjusted	Large magnitude: no Statistical significance: no Dose-response: possible for TC and HDL Temporal association: no Subgroup only: no Adjustments: not given	Samples collected at the 24th-30th week of gestation Samples stored at -80º C PFAS highly correlated No lipid data for PFOS Potential weaknesses: Adjusted results not found in article or supplement

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Yang et al., 2018	Location: China Years: 2015	Cross-sectional	Who: males, cases and controls in a study of metabolic syndrome Ages: 19-60 N: 145	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 1.90 ng/ml	Serum, fasting unknown	Blood, fasting unknown	**With metabolic syndrome:** TC: R = -0.052 LDL: R = -0.094 HDL: R = -0.082 TG: R = 0.133 Other: NA **Without metabolic syndrome:** TC: R = -0.272* LDL: R = -0.223 HDL: R = -0.058 TG: R = 0.001 Other: NA * p <0.05, all others >0.05	Spearman correlation coefficients (R)	Unadjusted	Large magnitude: yes for TC in those without metabolic syndrome Statistical significance: yes see above Dose-response: unclear Temporal association: no Subgroup only: without metabolic syndrome Adjustments: not given	Samples stored at -80º C Potential weaknesses: Limited co-variates Fasting unknown
Dong et al., 2019	Location: US NHANES Years: 2003-14	Cross-sectional	Who: adolescents Ages: 12-19 N: 2,947	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 2.9 ng/ml	Serum, fasting	Blood, fasting	TC: no association LDL: no association HDL: no association TG: NA Other: NA See other table entry for results in adults	Change in lipid level (mg/dl) per ng/ml increase in PFOA (most data in figure form only)	Adjusted for age, gender, race, BMI, income, waist circumference, activity, diabetes, smoking, and alcohol	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies PFOA and PFOS levels decreased over time PFOA and PFOS correlation = 0.69 Reference doses and benchmark doses also calculated Potential weaknesses: Cross-sectional: reverse causation Unclear if adequately adjusted for survey year
Jain and Ducatman (2018a)	Location: US NHANES Years: 2013-4	Cross-sectional	Who: children Ages: 6-11 N: 458	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: geometric mean = 1.78 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: no association LDL: NA HDL: no association TG: NA Other: non-HDL (TC – HDL), no association	Linear regression with log-lipid	Adjusted for gender, race, age, income, BMI, fasting time, and second hand smoke Little change when adjusted for other PFAS	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Low lipid levels in children
Domazet et al., 2016	Location: Denmark Years: 1997-2009	Cohort	Who: children and young adults Ages: 9-21 N: 444	Selection: unclear Participation: 48% Equal groups: unclear Blinded: unclear Levels: medians = 2.7-9.7 ng/ml	Plasma, fasting	Plasma, fasting	TC: NA LDL: NA HDL: NA TG: no association Other: NA	Change in outcome for each 10 ng/ml increase in PFOA	Adjusted for age, sex, outcome level at baseline, and ethnicity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	F/U: 12 years PFOA measured at ages 9 and 15; TG measured at ages 15 and 21 Samples stored at -80º C Tracking coefficients over time: 0.07-0.50 for PFOA Potential weaknesses: Low lipid levels in children

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Fassler et al., 2019	Location: Cincin-nati, OH Years: 2004-6	Cross-sectional	Who: girls Ages: 8 N: 353	Selection: recruited from schools and the breast cancer registry (family members) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 7.7 ng/ml	Serum, fasting	Plasma, fasting	TC: no association LDL: NA HDL: β = 0.0442 (p=0.0046) No association with structural equation modeling TG: no association Other: NA	Regression coefficient for log PFOA (ng/ml) and log lipid levels (units not given)	Initial models adjusted for age and race; structural equation modeling adjusted for multiple other factors	Large magnitude: no Statistical significance: possible for HDL Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -80º C Potential weaknesses: Structural equation modeling difficult to understand Inconsistent results between structural equation modeling and linear regressions Cross-sectional: reverse causation Low lipid levels in children
Kang et al., 2018	Location: Korea Years: 2012-4	Cross-sectional	Who: children Ages: 3-18 N: 150	Selection: subgroup from a nationwide survey Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 1.88 ng/ml	Serum, fasting	Serum, fasting	TC: no association LDL: no association HDL: NA TG: no association Other: NA	Linear regression	Adjusted for age, sex, BMI, second hand smoke, and income	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Stored at -70º C PFOA highly correlated with other PFAS Potential weaknesses: Low lipid levels in children
Khalil et al., 2018	Location: Dayton, OH Years: 2016	Cross-sectional	Who: obese children Ages: 8-12 N: 48	Selection: Lipid Clinic Dayton Children's Hospital Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 0.99 ng/ml	Blood, fasting	Blood, fasting	TC: β = 39.9 (8.00-65.7) LDL: β = 35.1 (8.57-61.6) HDL: no association TG: no association Other: NA	Linear regression, not log transformed	Adjusted for age, sex, and ethnicity	Large magnitude: unclear Statistical significance: yes for TC and LDL Dose-response: yes Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Limited co-variates Cross-sectional: reverse causation
Koshy et al., 2017	Location: New York Years: 2001-12	Cross-sectional	Who: children and adolescents Ages: 10-19 N: 402	Selection: World Trade Center exposure ("cases") and matched controls Participation: <48% Equal groups: unclear Blinded: unclear Levels: 1.39 and 1.81 ng/ml in controls and cases, respectively	Blood, 6 hour fast	Blood, 6 hour fast	TC: β = 0.09 (p <0.001) (9.2% increase) LDL: β = 0.11 (p=0.006) (11.5% increase) HDL: no association TG: β = 0.14 (p=0.03) (15% change) Other: NA	Change in lipid levels and percent changes for each log increase in PFOA	Adjusted for sex, race, calories, activity, cotinine, and BMI	Large magnitude: yes for LDL and TG Statistical significance: yes for TC, LDL, and TG Dose-response: linear Temporal association: no Subgroup only: no Adjustments: only small changes with adjustments	Potential weaknesses: Cross-sectional: reverse causation Low lipid levels in children
Manzano-Salgado et al., 2017	Location: Spain Years: 2003-12	Cohort	Who: children Ages: 4 N: 627	Selection: pregnant women recruited at baseline, methods unclear Participation: <51% Equal groups: unclear Blinded: unclear Levels: geometric mean = 2.32 ng/ml	First trimester maternal plasma, likely non-fasting	Children's blood, non-fasting	TC: no association LDL: no association HDL: see notes TG: no association Other: NA	SDs of lipid level per doubling of PFOA	Adjusted for region, birth place, prior breast-feeding, age, sex, parity, and BMI	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	F/U: 4 years Samples stored at -80º C HDL results for boys are unusual, CI does not include the effect estimate Potential weaknesses: In utero exposure, relevance unknown Non-fasting Low lipid levels in children

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Mora et al., 2018	Location: Boston, MA Years: 1999-2010	Cohort and cross-sectional	Who: children Ages: 7 N: 682	Selection: prenatal visits at a medical group in Boston Participation: <38% Equal groups: unclear Blinded: unclear Levels: median = 5.4 ng/ml	First trimester maternal plasma, non-fasting	Children's fasting plasma	**Prospective (prenatal):** TC: no association LDL: no association HDL: no association TG: no association Other: NA **Cross-sectional:** TC: β = 2.6 (-0.5-5.7) LDL: no association HDL: β = 1.5 (0.1-2.9) TG: no association Other: NA No effect modification by sex	Change in lipid level for each interquartile increase in PFOA	Adjusted for education, smoking, gestational age, sex, race, and child's age Adjusting for income, albumin, marital status, breast-feeding, activity, fast food and soda consumption did not change results	Large magnitude: no Statistical significance: possible for HDL Dose-response: linear Temporal association: no Subgroup only: no Adjustments: not given	F/U: 7 years Potential weaknesses: In utero exposure, relevance unknown Low lipid levels in children
Spratlen et al., 2020	Location: New York Years: 2001-2	Cross-sectional	Who: neonates Ages: 0 N: 222	Selection: World Trade Center cohort Participation: unclear Equal groups: higher total PFAS in Asians, lower education Blinded: unclear Levels: median = 2.46	Cord blood, likely non-fasting	Cord blood, likely non-fasting	TC: no association LDL: NA HDL: NA TG: 0.256% (0.129-0.383) Other: NA	Percent increase in lipid levels per 1% increase in PFOA	Adjusted for maternal age, child sex, maternal education, race, parity, BMI, marital status, smoking, and gestational age	Large magnitude: no Statistical significance: yes for TG Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Pregnant women 18-39 years old Potential weaknesses: Non-fasting Cross-sectional: reverse causation Low lipid levels in children

Rows are sorted by adult vs. children studies, then by whether the study was in NHANES or not, then by first author
Numbers in parentheses are 95% confidence intervals unless otherwise noted
Ages are in years unless otherwise noted
Abbreviations: β, regression coefficient; BMI, body mass index; CI, confidence interval; F/U: follow-up period; HDL, high density lipoprotein; LDL, low density lipoprotein; N, number of participants; NA, not assessed; NHANES, US National Health and Nutrition Examination Survey; OR, odds ratio; PFAS, per-and polyfluorinated substances; perfluorononanoic acid (PFNA); R, correlation coefficient; SD, standard deviation; SES, socioeconomic status; TC, total cholesterol; TG, triglycerides; VLDL, very low density lipoprotein

Table A7.9. Recent epidemiologic studies of PFOS and lipid levels

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Christen-sen et al., 2019	Location: US NHANES Years: 2007-14	Cross-sectional	Who: adults Ages: ≥20 N: 2,975	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 8.04 ng/ml	Blood, fasting	Blood, fasting, and use of lipid lowering medication	TC: NA LDL: NA HDL: OR for low HDL = 1.33 (0.80-2.21) TG: OR for high TG = 0.64 (0.37-1.08) Other: NA	ORs for 4th vs. 1st quartile of PFOS	Adjusted for survey year, age, sex, race/ethnicity, income, alcohol, and smoking	Large magnitude: yes for HDL and TG Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Large reduction in PFOS levels from 2007-8 to 2013-4 Presents results adjusted and unadjusted for NHANES sampling weights "High triglycerides" if TG >150 mg/dl or medication use; "Low HDL" if HDL <30 mg/dl or medication use Potential weaknesses: Low statistical power Cross-sectional: reverse causation
Dong et al., 2019	Location: US NHANES Years: 2003-14	Cross-sectional	Who: adults Ages: 20-80 N: 8,948	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 10.9 ng/ml	Serum, fasting	Blood, fasting	TC: β = 0.4 (0.06-0.6); means for each quintile are given in their Figure S4 LDL: small increase (figure form only) HDL: no association TG: NA Other: NA See separate entry for results in adolescents	Change in lipid level (mg/dl) per ng/ml increase in PFOS (most data in figure form only)	Adjusted for age, gender, race, BMI, income, waist circumference, activity, diabetes, smoking, and alcohol	Large magnitude: no Statistical significance: yes for TC Dose-response: unclear Temporal association: no Subgroup only: adults (see separate entry for children) Adjustments: not given	Overlap with other NHANES studies PFOA and PFOS levels decreased over time PFOA and PFOS correlation = 0.69 Reference doses and benchmark doses also calculated Potential weaknesses: Cross-sectional: reverse causation Unclear if adequately adjusted for survey year
He et al., 2018	Location: US NHANES Years: 2003-12	Cross-sectional	Who: adults Ages: ≥20 N: 7,904	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: mean = 14.51-20.80 ng/ml in females and males, respectively	Serum, likely fasting	Serum, likely fasting	TC: 2.28% (0.68-3.90%) and 1.36% (-0.11-2.85%) increase in men and women, respectively LDL: no clear association HDL: 2.51% and 3.08% increase (p <0.05) in men and women, respectively TG: no association Other: NA	Percent change in lipid level for quartile 4 vs. 1 of PFOS	Adjusted for age, race, BMI, education, alcohol, income, and activity Stratified by gender	Large magnitude: no Statistical significance: yes for TC and HDL Dose-response: yes Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Potential weaknesses: Cross-sectional: reverse causation Not adjusted for survey year
Huang et al., 2018	Location: US NHANES Years: 1999-2014	Cross-sectional	Who: adults Ages: ≥20 N: 10,859	Selection: randomized cluster sample Participation: unclear Equal groups: data only for total PFAS Blinded: yes Levels: median = 12.40 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: R = 0.105 LDL: R = 0.091 HDL: R = -0.013 TG: R = 0.075 Other: NA All p-values <0.01 except HDL	Spearman correlation coefficients (R)	Results appear to be unadjusted	Large magnitude: yes for TC Statistical significance: yes for all except HDL Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies Correlation with PFOA = 0.69 NHANES survey weights used PFOA and PFOS levels declined over time Potential weaknesses: Relatively small effect sizes Limited covariates including survey year Some lipid levels have also declined over time Cross-sectional: reverse causation

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Jain and Ducatman, 2019b	Location: US NHANES Years: 2005-14	Cross-sectional	Who: adults Ages: ≥20 N: 3,629	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: geometric mean = 9.3 ng/ml	Blood, fasting	Blood, fasting	TC: no association LDL: 0.36% (p=0.04) in obese women, no clear association in non-obese women or in men HDL: no association TG: -0.87% (p <0.01) in obese women, no association in non-obese women or in men Other: NA	Percent change in lipid level for each 10% change in PFOS	Adjusted for race/ethnicity, smoking, age, income, fasting time, lipid lowering medication, activity, survey year, and diet Stratified by obesity and gender	Large magnitude: no Statistical significance: yes for LDL and TG Dose-response: unclear Temporal association: no Subgroup only: yes, obese women Adjustments: not given	Overlap with other NHANES studies Potential weaknesses: Cross-sectional: reverse causation
Liu et al., 2018b	Location: US NHANES Years: 2013-4	Cross-sectional	Who: adults Ages: ≥18 N: 1,871	Selection: randomized cluster sample Participation: unclear Equal groups: mostly, some differences in PFOS by age, race, income, and other factors Blinded: yes Levels: geometric mean = 5.28 ng/ml	Serum, likely fasting	Blood, likely fasting	TC: no association LDL: no association HDL: no association TG: no association Other: apolipoprotein B no association	Change in lipid level (in mg/dl) for each log increase in PFOS	Adjusted for age, gender, ethnicity, smoking, alcohol, income, waist circumference and relevant medications	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: relatively small changes only except decrease for HDL	Overlap with other NHANES studies Potential weaknesses: Cross-sectional: reverse causation
Chen et al., 2019	Location: Hvar, Croatia Years: 2007-8	Cross-sectional	Who: adults Ages: 44-56 N: 122	Selection: unclear Participation: unclear Equal groups: PFOS higher with lower education, otherwise similar Blinded: unclear Levels: geometric mean = 8.91 ng/ml	Plasma, fasting	Plasma, fasting	TC: no association LDL: no association HDL: no association TG: no association Other: NA	Change in lipid levels per one unit increase in ln PFOS, levels unclear	Adjusted for age, sex, SES, smoking, dietary pattern, and activity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Low statistical power
Christian-sen et al., 2016	Location: Wisconsin Years: 2012-3	Cross-sectional	Who: male fishermen Ages: ≥50 N: 154	Selection: "interested in future studies" per online survey; flyers Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 19.00 ng/ml	Serum, fasting unknown	Self-report, "have you ever been told by a doctor you have…high cholesterol"	TC: NA LDL: NA HDL: NA TG: NA Other: OR = 1.02 (1.00-1.04) for ever told by a doctor the participant had high cholesterol	Answered yes vs. no	Adjusted for age, BMI, work status, and alcohol consumption	Large magnitude: no Statistical significance: unclear Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -20º C Potential weaknesses: Self-reported non-specific outcome Unusually narrow CI

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Donat-Vargas et al., 2019	Location: Sweden Years: 1990-2013	Cohort	Who: adults Ages: 40-60 at baseline N: 187	Selection: controls in a diabetes case-control study Participation: 56% Equal groups: unclear Blinded: unclear Levels: median = 20 ng/ml	Blood, fasting	Plasma, fasting	TC: no clear association in prospective analysis, possibly some decrease in repeated measures analysis but strong U-shaped dose-response curve LDL: NA HDL: NA TG: β = -0.14 mmol/L (-0.27 to -0.02) decrease for each one SD increase in PFOA Other: NA	Change in lipid level by tertile or per SD increase in PFOS	Adjusted for gender, age, education, sample year, BMI, smoking, alcohol, exercise, and diet	Large magnitude: about a 10% decrease for TG Statistical significance: yes for TG Dose-response: U-shaped Temporal association: yes Subgroup only: no Adjustments: not given	F/U: approximately 10 years PFOA and PFOS levels decreased over time PFOA and PFOS highly correlated Potential weaknesses: Some inconsistency between repeated measures and prospective analysis
Graber et al., 2019	Location: New Jersey Years: 2016-17	Cross-sectional	Who: residents of an area contaminated with PFAS, likely from a nearby manufacturing facility Ages: ≥19 N: 105	Selection: claimants in a class action lawsuit, flers to all local residents Participation: unclear but likely low Equal groups: unclear Blinded: unclear Levels: geometric mean (95% CI) = 5.37 (4.75-6.06) ng/ml	Serum, fasting unknown	Self-reported physician diagnosed "high cholesterol"	TC: NA LDL: NA HDL: NA TG: NA Other: self-reported "high cholesterol" OR = 1.08 (0.98, 1.21)	Unclear	Adjusted for age and BMI	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: OR = 1.10 (0.95, 1.27) after adjustment for other PFAS	PFNA levels in water and serum were high Serum samples were collected and measured as part of a lawsuit, not collected or measured by the researchers Potential weaknesses: Methods are unclear Outcome not verified
Kishi et al., 2015	Location: Hokkaido, Japan Years: 2002-5	Cross-sectional	Who: pregnant women Ages: unknown N: 306	Selection: pregnant women, preterm care and delivery at participating hospital Participation: 28.6% Equal groups: higher PFOS associated with younger age, lower parity, non-smoking, and earlier sampling week Blinded: unclear Levels: median = 5.60 ng/ml	Serum, 23-35 weeks gestation, fasting	Blood, non-fasting	TC: β = -0.130 (-0.253—0.011) Others not assessed	Linear regression with log10 PFOS and log10 TC	Adjusted for age, smoking, alcohol, income, parity, and gestation week	Large magnitude: unclear Statistical significance: yes (decrease) Dose-response: somewhat linear Temporal association: no Subgroup only: no Adjustments: small decrease in magnitude	Sample stored at -80º C Potential weaknesses:

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Lin et al., 2019	Location: 27 US medical centers Years: 1996-2014	Cohort and cross-sectional	Who: adults Ages: ≥25 N: 888	Selection: pre-diabetic adults Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 27.2 ng/ml	Plasma at baseline 1996-99, fasting unknown	Blood at baseline and annually, fasting	**Cross-sectional:** TC: β = 2.53 (-0.10–5.16) LDL: β = 1.38 (-1.02-3.77) HDL: β = -0.40 (-1.19-0.39) TG: β = 7.75 (0.63-14.88) Other: VLDL β = 1.57 (0.24-2.89) No effect modification by treatment group **Prospective:** Hypercholesterolemia: No association Hypertriglyceridemia: OR = 1.23 (1.03-1.46) per doubling of PFOS Greater effect in the placebo group	Change in lipid level (mg/dl) per doubling of PFOS; ORs	Adjusted for age, sex, race/ethnicity, marital status, education, smoking, alcohol, fat intake, fiber intake, activity, and waist circumference Cross-sectional: excluded people taking lipid lowering medications	Large magnitude: yes for TG Statistical significance: yes for TG and VLDL Dose-response: linear Temporal association: yes Subgroup only: those not on special intervention Adjustments: not given	F/U: 15 years Randomized to lifestyle intervention or placebo Hypercholesterolemia defined by high TC, high LDL, or use of lipid lowering medication Potential weaknesses:
Lin et al., 2020	Location: Taiwan Years: 2009-11	Cross-sectional	Who: adults Ages: 22-63 N: 597	Selection: controls in a coronary heart disease case-control study, recruited from a bulletin board announcement Participation: unclear Equal groups: PFOS higher in men, older age, less education, higher alcohol consumption, otherwise similar Blinded: unclear Levels: geometric mean = 3.77 ng/ml	Blood, fasting unknown	Blood, fasting unknown	TC: NA LDL: β = 5.90 (p=0.002) HDL: β = 1.31 (p=0.023) TG: no association Other: NA	Change in lipid level for each one unit ln increase in PFOS; levels unclear	Adjusted for age, gender, smoking alcohol, education, hypertension, BMI, and diabetes	Large magnitude: unclear Statistical significance: yes for LDL and HDL Dose-response: possibly log-linear Temporal association: no Subgroup only: no Adjustments: little change	Somewhat greater effect sizes in subjects with elevated levels of oxidative stress biomarkers Potential weaknesses: Fasting unknown Cross-sectional: reverse causation
Liu et al., 2018a	Location: Boston, MA Years: 2004-7	Cross-sectional	Who: participants in a weight loss program at baseline Ages: 30-70 N: 621	Selection: convenience sample Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 27.2 and 22.3 ng/ml in men and women, respectively	Plasma, fasting	Plasma, fasting	TC: R = 0.04 (p >0.05) LDL: R = 0.09 (p <0.05) HDL: R = 0.01 (p >0.05) TG: R = -0.02 (p >0.05) Other: NA	Partial Spearman correlation coefficients (R)	Adjusted for age, sex, race, education, smoking, alcohol, activity, menopause, hormone replacement, and dietary intervention group	Large magnitude: no Statistical significance: yes for LDL Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: not given	Change in lipid levels during weight loss also given but difficult to interpret Potential weaknesses: Cross-sectional: reverse causation
Matilla-Santander et al., 2017	Location: Spain Years: 2003-8	Cross-sectional	Who: pregnant women Ages: ≥16 N: 1,194	Selection: "population-based" Participation: <55% Equal groups: unclear Above detection: 100% Blinded: unclear Levels: geometric mean = 5.77 ng/ml	First trimester plasma, non-fasting	First trimester serum, non-fasting	TC: no association LDL: NA HDL: NA TG: β = -5.86 (-9.91 to -1.63) Other: NA	Change in lipid level (mg/dl) per log10 increase in PFOS; PFOS quartiles	Adjusted for sub-cohort, place of birth, BMI, breast-feeding, parity, collection time, activity, and diet	Large magnitude: no Statistical significance: yes for TG Dose-response: log-linear (continuous), flattened (categorical) Temporal association: no Subgroup only: no Adjustments: small decrease	Samples stored at -80º C Potential weaknesses: Cross-sectional: reverse causation Little increase in effect after quartile 2 Non-fasting

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Seo et al., 2018	Location: Korea Years: 2006-15	Cross-sectional	Who: adults Ages:40-60 N: 786	Selection: residents of Seoul Participation: unclear Equal groups: PFOA and PFOS generally increased with age, and higher in males, lower in obese Blinded: unclear Levels: median = 12.43 ng/ml	Serum, appears non-fasting	Blood, appears non-fasting	*Most findings given in figure form* TC: increase across quartiles (p-trend = 0.05) LDL: increase but mostly from quartile 1 to 2 (p-trend = 0.06) HDL: no association TG: increase across quartiles (p-trend = 0.08) Other: NA	PFOS quartiles	Unadjusted	Large magnitude: unclear Statistical significance: borderline for TC and LDL Dose-response: see results Temporal association: no Subgroup only: no Adjustments: not given	Samples frozen at -20º C and analyzed within 30 days Potential weaknesses: Limited covariates Cross-sectional: reverse causation Non-fasting
Yang et al., 2018	Location: China Years: 2015	Cross-sectional	Who: males, cases and controls in a study of metabolic syndrome Ages: 19-60 N: 144	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 3.00 ng/ml	Serum, fasting unknown	Blood, fasting unknown	**With metabolic syndrome:** TC: R = -0.004 LDL: R = -0.03 HDL: R = 0.02 TG: R = 0.140 Other: NA **Without metabolic syndrome:** TC: R = 0.097 LDL: R = 0.068 HDL: R = 0.142 TG: R = 0.030 Other: NA all p >0.05	Spearman correlation coefficients	Unadjusted	Large magnitude: yes for HDL and TG by subgroup Statistical significance: no Dose-response: unclear Temporal association: no Subgroup only: by metabolic syndrome Adjustments: not given	Samples stored at -80º C Potential weaknesses: Limited co-variates Subgroup inconsistencies Fasting unknown
Dong et al., 2019	Location: US NHANES Years: 2003-14	Cross-sectional	Who: adolescents Ages: 12-19 N: 2,947	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: median = 9.4 ng/ml	Serum, fasting	Blood, fasting	TC: no association LDL: no association HDL: no association TG: NA Other: NA See separate entry for results in adults	Change in lipid level (mg/dl) per ng/ml increase in PFOS (most data in figure form)	Adjusted for age, gender, race, BMI, income, waist circumference, activity, diabetes, smoking, and alcohol	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Overlap with other NHANES studies PFOA and PFOS levels decreased over time PFOA and PFOS correlation = 0.69 Reference doses and benchmark doses also calculated Potential weaknesses: Cross-sectional: reverse causation Unclear if adequately adjusted for survey year

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Jain and Ducatman, 2018b	Location: US NHANES Years: 2013-14	Cross-sectional	Who: children Ages: 6-11 N: 458	Selection: randomized cluster sample Participation: unclear Equal groups: unclear Blinded: yes Levels: geometric mean = 2.67 ng/ml	Serum, likely fasting	Serum, likely fasting	TC: β = 0.02738 (p=0.03) β = 0.0439 (p=0.03) when adjusted for other PFAS LDL: NA HDL: no association TG: NA Other: non-HDL (TC – HDL), no association	Linear regression with log-lipid level	Gender, race, age, income, BMI, fasting time, and second hand smoke; other PFAS	Large magnitude: no Statistical significance: yes for TC Dose-response: yes Temporal association: no Subgroup only: no Adjustments: some change when adjusted for other PFAS	Overlap with other NHANES studies Potential weaknesses: Low lipid levels in children Small effect size: β = 0.02738 is a 0.03% change per 10% change in PFOS Concerns about collinearity in model adjusted for other PFAS Cross-sectional: reverse causation
Domazet et al., 2016	Location: Denmark Years: 1997-2009	Cohort	Who: children and young adults Ages: 9-21 N: 444	Selection: unclear Participation: 48% Equal groups: unclear Blinded: unclear Levels: median = 9.1-44.5 ng/ml (medians vary by sex and age)	Plasma, fasting	Plasma, fasting	TC: NA LDL: NA HDL: NA TG: no association Other: NA	Change in lipid level for each 10 ng/ml increase in PFOS	Adjusted for age, sex, outcome level at baseline, and ethnicity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	F/U: 12 years PFOS measured at ages 9 and 15 TGs measured at ages 15 and 21 Samples stored at -80º C Tracking coefficients over time: 0.43-0.69 Potential weaknesses: Low lipid levels in children
Fassler et al., 2019	Location: Cincin-nati, OH Years: 2004-06	Cross-sectional	Who: girls Ages: 8 N: 353	Selection: recruited from schools and the breast cancer registry (family members) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 13.6 ng/ml	Serum, fasting	Plasma, fasting	TC: no association LDL: NA HDL: no association TG: no association Other: NA Actual coefficients not given	Linear regression coefficients	Initial models adjusted for age and race. Structural equation modeling adjusted for multiple other factors	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -80º C Potential weaknesses: Structural equation modeling difficult to understand Cross-sectional: reverse causation Low lipid levels in children
Kang et al., 2018	Location: Korea Years: 2012-14	Cross-sectional	Who: children Ages: 3-18 N: 150	Selection: subgroup from a nationwide survey Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 5.68 ng/ml	Serum, fasting	Serum, fasting	TC: no association LDL: no association HDL: NA TG: no association Other: NA	Linear regression	Adjusted for age, sex, BMI, second hand smoke, and income	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Stored at -70º C PFOA highly correlated with other PFAS Potential weaknesses: Low lipid levels in children
Khalil et al., 2018	Location: Dayton, OH Years: 2016	Cross-sectional	Who: obese children Ages: 8-12 N: 48	Selection: Lipid Clinic Dayton Children's Hospital Participation: unclear Equal groups: unclear Blinded: unclear Levels: median = 2.79 ng/ml	Blood, fasting	Blood, fasting	TC: β = 5.63 (-0.11 to 11.4) LDL: β = 5.70 (0.458 to 10.9) HDL: no association TG: no association Other: NA	Linear regression, not log transformed	Adjusted for age, sex, and ethnicity	Large magnitude: unclear Statistical significance: yes for LDL Dose-response: yes Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: Other potential confounders Cross-sectional: reverse causation

Reference	Location Years	Design	Who Ages N	Factors related to bias	Exposure method	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Koshy et al., 2017	Location: New York Years: 2001-12	Cross-sectional	Who: children and adolescents Ages:10-19 N: 402	Selection: World Trade Center exposure ("cases") and matched controls Participation: <48% Equal groups: unclear Blinded: unclear Levels: 2.78 and 3.72 ng/ml in controls and cases, respectively	Blood, 6 hour fast	Blood, 6 hour fast	TC: β = 0.08 (p <0.001) (8.5% increase) LDL: β = 0.10 (p <0.001) (10.7% increase) HDL: β = 0.06 (p=0.04) (6.6% increase) TG: no association Other: NA	PFOS and lipids were log transformed; percent change in lipid level for each log increase in PFOS	Adjusted for sex, race, calories, activity, cotinine, and BMI	Large magnitude: yes for LDL Statistical significance: yes for TC, LDL, and HDL Dose-response: linear Temporal association: no Subgroup only: no Adjustments: only small changes with adjustments	Potential weaknesses: Cross-sectional: reverse causation Low lipid levels in children
Manzano-Salgado et al., 2017	Location: Spain Years: 2003-12	Cohort	Who: children Ages: 4 N: 627	Selection: pregnant women recruited at baseline, methods unclear Participation: <51% Equal groups: unclear Blinded: unclear Levels: geometric mean = 5.80 ng/ml	First trimester maternal plasma, likely non-fasting	Children's blood, non-fasting	TC: no association LDL: no association HDL: no association TG: no association Other: NA	SD of lipid level per doubling of PFOS	Adjusted for region, birth place, prior breast-feeding, age, sex, parity, and BMI	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -80º C Potential weaknesses: In utero exposure, relevance unknown Non-fasting Low lipid levels in children
Mora et al., 2018	Location: Boston, MA Years: 1999-2010	Cohort and cross-sectional	Who: children Ages: 7 N: 682	Selection: prenatal visits at a medical group in Boston Participation: <38% Equal groups: unclear Blinded: unclear Levels: median = 5.4 ng/ml	First trimester maternal plasma, non-fasting	Children's fasting plasma	**Prospective (prenatal):** TC: no association LDL: no association HDL: no association TG: no association Other: NA **Cross-sectional:** TC: β = 1.8 (-0.2-3.7) LDL: no association HDL: β = 1.5 (0.4-2.5) TG: β = -2.5 (-4.3 to – 0.6) Other: NA No effect modification by sex	Change in lipid level for each interquartile increase in PFOS	Adjusted for education, smoking, gestational age, sex, race, and child's age Adjusting for income, albumin, marital status, breast-feeding, activity, fast food and soda consumption did not change results	Large magnitude: no Statistical significance: yes for HDL and TG in cross-sectional analysis Dose-response: linear Temporal association: no Subgroup only: no Adjustments: not given	Potential weaknesses: In utero exposure, relevance unknown Low lipid levels in children Some differences in cross-sectional and prospective results
Spratlen et al., 2020	Location: New York Years: 2001-2	Cross-sectional	Who: neonates Ages: 0 N: 222	Selection: World Trade Center cohort Participation: unclear Equal groups: higher total PFAS in Asians, lower education Blinded: unclear Levels: median = 2.46	Cord blood, likely non-fasting	Cord blood, likely non-fasting	TC: no association LDL: NA HDL: NA TG: no association Other: NA	Percent increase in lipid level per 1% increase in PFOS	Adjusted for maternal age, child sex, maternal education, race, parity, BMI, marital status, smoking, and gestational age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Pregnant women 18-39 years old Potential weaknesses: Non-fasting Low lipid levels in children

Numbers in parentheses are 95% confidence intervals unless otherwise noted
Ages are in years unless otherwise noted
Abbreviations: β, regression coefficient; BMI, body mass index; CI, confidence interval; F/U: follow-up period; HDL, high density lipoprotein; LDL, low density lipoprotein; N, number of participants; NA, not assessed; NHANES, US National Health and Nutrition Examination Survey; OR, odds ratio; PFAS, per-and polyfluorinated substances; perfluorononanoic acid (PFNA); R, correlation coefficient; SD, standard deviation; SES, socioeconomic status; TC, total cholesterol; TG, triglycerides; VLDL, very low density lipoprotein

As shown in Tables A7.8 and A7.9, there is a relatively large number of studies in children, as well as a relatively large number of studies that used data from NHANES. Overall, the results of these studies appeared to be somewhat heterogeneous. In order to help explore and identify the possible sources of this heterogeneity, these studies were divided into several categories (i.e., children vs. adults, NHANES vs. non-NHANES studies), and study quality summary tables (Tables A7.11 and A7.12) were created, with key study characteristics, results, and factors potentially related to study quality for each of these categories. The characteristics and study quality factors, as well as the codes used in these summary tables are shown in Table A7.10.

Table A7.10. Characteristics and codes for the study quality summary tables of recent epidemiologic studies of PFOA or PFOS and lipids

Category	Label	Definition	Codes
General	Age	Ages of the study participants	A for adults, otherwise age in years
	N	Number of participants in the study	
Results	TC	Result for total cholesterol	+: Statistically significant positive association[1] (+): Positive association but not statistically[2] significant -: Statistically significant negative association[3] (-): Negative association but not statistically significant[2] 0: No association U: unclear
	LDL	Result for low density lipoprotein	Same as above
	HDL	Result for high density lipoprotein	Same as above
	TG	Result for triglycerides	Same as above
Quality scoring factors	Design	Study design	XS: Cross-sectional CO: Prospective cohort study
	Selection	Participant selection	1: Population based selection 0: Convenience sample or not clear
	Demo	Was demographic information compared and mostly similar across PFOA/PFOS levels?	1: Yes or differences were adjusted for 0: No
	Blind	Were the study personnel assessing the exposures or the outcomes blinded?	1: Yes, reported 0: No or not reported
	Range	Range of PFOA or PFOS levels	1: Wide range of PFOA or PFOS exposure levels (this includes occupational studies or communities with known industrial contamination) 0: Population based studies with no known high exposure source
	Exposure	How was PFOA or PFOS exposure assessed?	SB: A single blood measurement MB: Multiple blood measurements U: Unknown or not mentioned 0: Other metrics including modeling of environmental exposure or ecologic assessment
	Outcome	How was the outcome (lipid levels) assessed?	SB: A single blood measurement MB: Multiple blood measurements M: Use of a lipid lowering medication or physician's diagnosis of abnormal lipid levels U: Unknown or not mentioned 0: Other

Category	Label	Definition	Codes
	Fasting	If serum, were samples collected after fasting?	1: Yes, fasting blood lipid level, or exposure assessed by a method not impacted by fasting (e.g., modeled PFOA serum levels) 0: No or not mentioned
	Age/Sex	Were the results adjusted for or otherwise controlled for age and sex?	1: Yes 0: No
	Diet/BMI	Were the results adjusted for some aspect of diet or BMI?	1: Yes (e.g., BMI, waist circumference, calorie intake, diet patterns, fat intake) 0: No
	SES	Were the results adjusted for some aspect of socioeconomic status?	1: Yes, including poverty indices, income, or education 0: No
	Year	If a cross-sectional study >3 years, were the results adjusted for possible changes in PFAS and lipid levels over time?	1: Yes, cross-sectional and not >3 years, or prospective design 0: No Note: this primarily applies to multi-year NHANES studies
	Clear Res	Were the results presented clearly and were they consistent across different analyses?	1: Yes 0: No
	Overall	Overall quality score (range 0-13)	Sum of the individual quality scoring factors listed above with prospective cohort studies receiving a score of 1 and cross-sectional studies receiving a score of 0; and exposure and outcome ratings of MB or SB receiving a quality score of 1 and all other metrics a quality score of 0
Overlap		Did participants of the study overlap with those of another study?	Y: Yes N: No

1. A statistically significant association between increasing PFOA or PFOS exposures and increasing lipid levels
2. Generally includes results that are not statistically significant but where effect sizes are >10 percent
3. A statistically significant association between increasing PFOA or PFOS exposures and decreasing lipid levels

Table A7.11. Study quality summary of recent epidemiologic studies of PFOA and lipid levels

General			Results				Quality scoring														
Reference	Age	N	TC	LDL	HDL	TG	Design	Selection	Demo	Blind	Range	Exposure	Outcome	Fasting	Age/Sex	Diet/BMI	SES	Year	Clear res	Overall	Overlap	Notes
ADULTS NHANES:																						
Christensen et al., 2019	A	2,975			(-)	(+)	XS	1	0	1	L	SB	SB, M	1	1	0	1	1	1	9	Y	NHANES 2007-14
Dong et al., 2019	A	8,948	+	(+)	0		XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-14
He et al., 2018	A	7,904	+	(+)	0	(+)	XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-12
Huang et al., 2018	A	10,859	+	+	-	+	XS	1	0	1	L	SB	SB	1	0	0	0	0	1	6	Y	NHANES 1999-2014; correlations
Jain & Ducatman, 2019b	A	3,629	+	+	0	0	XS	1	0	1	L	SB	SB	1	1	1	1	1	1	10	Y	NHANES 2005-14; in obese women
Liu et al., 2018b	A	1,871	+	(+)	+	0	XS	1	1	1	L	SB	SB	1	1	1	1	1	1	11	Y	NHANES 2013-4
ADULTS OTHER:																						
Chen et al., 2019	A	122	U	0	0	0	XS	0	1	0	L	SB	SB	1	1	1	1	1	0	8	N	Differences in ORs vs. regressions
Christiansen et al., 2016	A	154	U	U	U	U	XS	0	0	0	L	SB	M	0	1	1	0	1	0	4	N	Self-reported high cholesterol: no association
Donat-Vargas et al., 2019	A	187	U			(-)	CO	0	0	0	L	SB	SB	1	1	1	1	1	0	8	N	Differences prospective vs. repeated measures
Graber et al., 2019	A	105	U				XS	0	0	0	L	SB	M	0	0	0	0	1	0	3	N	Self-reported high cholesterol: no association
Kishi et al., 2015	A	306	0				XS	0	1	0	L	SB	SB	0	1	0	1	1	1	5	N	
Lin et al., 2019	A	888	+	+	(-)	+	CO	0	0	0	L	SB	SB, M	1	1	1	1	1	1	9	N	Similar CO and XS results
Lin et al., 2020	A	597		+	0	+	XS	0	1	0	L	SB	SB	0	1	1	1	1	1	8	N	
Liu et al., 2018a	A	621	0	0	-	+	XS	0	0	0	L	SB	SB	1	1	U	1	1	1	7	N	Correlations
Matilla-Santander et al., 2017	A	1,194	+			U	XS	1	0	0	L	SB	SB	0	0	1	0	1	0	5	N	U-shaped dose-response for TGs
Seo et al., 2018	A	786	U	U	0	(+)	XS	0	0	0	L	SB	U	0	0	0	0	0	0	1	N	Unusual dose-response
Starling et al., 2017	A	628	(+)		0	0	XS	0	0	1	L	SB	SB	1	0	0	0	1	0	5	N	Pregnant women
Yang et al., 2018	A	145	-	(-)	0	(+)	XS	0	0	0	L	SB	SB	0	0	0	0	1	0	3	N	In subgroups only
CHILDREN NHANES:																						
Dong et al., 2019	12-19	2,947	0	0	0		XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-14
Jain & Ducatman, 2018b	6-11	458	0		0		XS	1	0	1	L	SB	SB	1	1	1	1	1	1	10	Y	NHANES 2013-14
CHILDREN OTHER:																						
Domazet et al., 2016	9-21	444				0	CO	0	0	0	L	SB	SB	1	1	0	0	1	1	7	N	
Fassler et al., 2019	8	353	0		U	0	XS	0	0	0	L	SB	SB	1	1	1	1	1	0	7	N	Differences with structural equation models
Kang et al., 2018	3-18	150	0	0		0	XS	1	0	0	L	SB	SB	1	1	1	1	1	1	9	N	
Khalil et al., 2018	8-12	48	+	+	0	0	XS	0	0	0	L	SB	SB	1	1	0	0	1	1	6	N	
Koshy et al., 2017	10-19	402	+	+	0	+	XS	0	0	0	L	SB	SB	1	0	1	0	1	1	6	N	
Manzano-Salgado et al., 2017	4	627	0	0	U	0	CO	0	0	0	L	SB	SB	0	1	1	0	1	1	7	N	
Mora et al., 2018	7	682	U	0	U	0	CO	0	0	0	L	SB	SB	1	1	1	1	1	0	8	N	XS results: positive for TC and HDL
Spratlen et al., 2020	0	222	0			+	XS	0	1	0	L	SB	SB	0	1	1	1	1	1	8	N	

See Table A7.10 for abbreviations
Empty cells (darkened) indicate that no data was available for this variable

Table A7.12. Study quality summary of recent epidemiologic studies of PFOS and lipid levels

General			Results				Quality scoring															
Reference	Age	N	TC	LDL	HDL	TG	Design	Selection	Demo	Blind	Range	Exposure	Outcome	Fast	Age/Sex	Diet/BMI	SES	Year	Clear res	Overall	Overlap	Notes
ADULTS NHANES:																						
Christensen et al., 2019	A	2,975			(+)	(-)	XS	1	0	1	L	SB	SB, M	1	1	0	1	1	1	9	Y	NHANES 2007-14
Dong et al., 2019	A	8,948	+	(+)	0		XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-14
He et al., 2018	A	7,904	+	0	+	0	XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-12
Huang et al., 2018	A	10,859	+	+	0	+	XS	1	0	1	L	SB	SB	1	0	0	0	0	1	6	Y	NHANES 1999-2014; correlations
Jain & Ducatman, 2019b	A	3,629	0	+	0	-	XS	1	0	1	L	SB	SB	1	1	1	1	1	1	10	Y	NHANES 2005-14; in obese women
Liu et al., 2018b	A	1,871	0	0	0	0	XS	1	1	1	L	SB	SB	1	1	1	0	1	1	10	Y	NHANES 2013-4
ADULTS OTHER:																						
Chen et al., 2019	A	122	0	0	0	0	XS	0	1	0	L	SB	SB	1	1	1	1	1	0	8	N	Differences in ORs vs. regressions
Christiansen et al., 2016	A	154	U	U	U	U	XS	0	0	0	L	SB	M	0	1	1	0	1	0	4	N	Self-reported high cholesterol: unusual results
Donat-Vargas et al., 2019	A	187	U			-	CO	0	0	0	L	SB	SB	1	1	1	1	1	0	8	N	Differences prospective vs. repeated measures
Graber et al., 2019	A	105	U				XS	0	0	0	L	SB	M	0	0	0	0	1	0	3	N	Self-reported high cholesterol
Kishi et al., 2015	A	306	-				XS	0	1	0	L	SB	SB	0	1	0	1	1	1	5	N	
Lin et al., 2019	A	888	(+)	(+)	(-)	+	CO	0	0	0	L	SB	SB, M	1	1	1	1	1	1	9	N	Some differences between CO and XS results
Lin et al., 2020	A	597		+	+	0	XS	0	1	0	L	SB	SB	0	1	1	1	1	1	8	N	
Liu et al., 2018a	A	621	0	+	0	0	XS	0	0	0	L	SB	SB	1	1	U	1	1	1	7	N	Correlations
Matilla-Santander et al., 2017	A	1,194	0			-	XS	1	0	0	L	SB	SB	0	0	1	0	1	0	5	N	Unusual dose-response
Seo et al., 2018	A	786	+	(+)	0	(+)	XS	0	0	0	L	SB	U	0	0	0	0	0	0	1	N	
Yang et al., 2018	A	145	0	0	(+)	(+)	XS	0	0	0	L	SB	SB	0	0	0	0	1	0	3	N	In subgroups only
CHILDREN NHANES:																						
Dong et al., 2019	12-19	2,947	0	0	0		XS	1	0	1	L	SB	SB	1	1	1	1	0	1	9	Y	NHANES 2003-14
Jain and Ducatman, 2018b	6-11	458	+		0		XS	1	0	1	L	SB	SB	1	1	1	1	1	1	10	Y	NHANES 2013-14
CHILDREN OTHER:																						
Domazet et al., 2016	9-21	444				0	CO	0	0	0	L	SB	SB	1	1	0	0	1	1	7	N	
Fassler et al., 2019	8	353	0		0	0	XS	0	0	0	L	SB	SB	1	1	1	1	1	0	7	N	Differences with structural equation models
Kang et al., 2018	3-18	150	0	0		0	XS	1	0	0	L	SB	SB	1	1	1	1	1	1	9	N	
Khalil et al., 2018	8-12	48	(+)	+	0	0	XS	0	0	0	L	SB	SB	1	1	0	0	1	1	6	N	
Koshy et al., 2017	10-19	402	+	+	+	0	XS	0	0	0	L	SB	SB	1	0	1	0	1	1	6	N	
Manzano-Salgado et al., 2017	4	627	0	0	0	0	CO	0	0	0	L	SB	SB	0	1	1	0	1	1	7	N	
Mora et al., 2018	7	682	0	0	U	U	CO	0	0	0	L	SB	SB	1	1	1	1	1	0	8	N	XS results: positive for HDL, negative for TG
Spratlen et al., 2020	0	222	0			0	XS	0	1	0	L	SB	SB	0	1	1	1	1	1	8	N	

See Table A7.10 for abbreviations
Empty cells (darkened) indicate that no data was available for this variable

Developmental and Reproductive Toxicity

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018a), and others, OEHHA searched for all new human epidemiologic studies on PFOA or PFOS and developmental and reproductive toxicity (DART) published since the 2016 US EPA reviews. The search for literature on epidemiologic studies of DART effects of PFOA and PFOS exposure was performed sequentially in four bibliographic databases: PubMed, Embase, Scopus and SciFinder. The search in each database was limited to 1/1/2016 to 12/31/19. In PubMed the DART[sb] search (shown below) strategy was used to restrict to DART studies. The DART[sb] strategy was translated to the appropriate syntax for use in the other databases.

PubMed Search Strategy

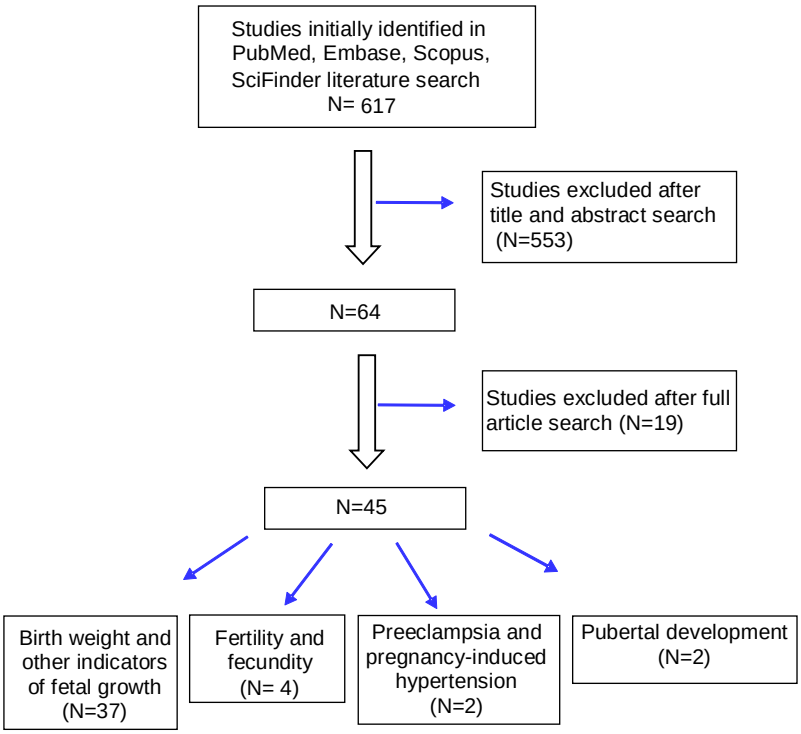
1	(“perfluorooctane sulfonic acid”[nm] OR PFOS [Tiab] OR “perfluoroalkyl sulphonate”[tiab] OR “perfluoro-n-octanesulfonic” [Tiab] OR “perfluoroctane sulfonic” [Tiab] OR “perfluorooctane sulfonic” [Tiab] OR perfluoroctanesulfonic [Tiab] OR perfluorooctanesulfonic [Tiab] OR “perfluorooctane sulphonic” [Tiab] OR “perfluoroctane sulphonic” [Tiab] OR perfluoroctanesulphonic [Tiab] OR perfluorooctanesulphonic [Tiab] OR “perfluorooctane sulfonate” [Tiab] OR “perfluoroctane sulfonate” [Tiab] OR perfluorooctanesulfonate [Tiab] OR perfluoroctanesulfonate [Tiab] OR “perfluorooctane sulphonate” [Tiab] OR “perfluoroctane sulphonate” [Tiab] OR perfluoroctanesulphonate [Tiab] OR perfluorooctanesulphonate [Tiab] OR “perfluorooctanyl sulfonate” [Tiab] OR “perfluorooctanyl sulphonate” [Tiab] OR perfluoroctylsulfonic [Tiab] OR “heptadecafluoro-1-octanesulfonic” [Tiab] OR “heptadecafluoro-1-octane sulfonic” [Tiab] OR “heptadecafluorooctane sulfonic” [Tiab] OR “heptadecafluoroctane sulfonic” [Tiab] OR “heptadecafluorooctane sulfonic” [Tiab] OR heptadecafluorooctanesulfonic [Tiab] OR “heptadecafluorooctane-1-sulphonic” [Tiab] OR “heptadecafluorooctane sulphonic” [Tiab] OR “1-octanesulfonic acid” [Tiab] OR “1-octanesulphonic acid” [Tiab] OR “1-perfluoroctanesulfonic” [Tiab] OR “1-perfluorooctanesulfonic [Tiab]” OR “octanesulfonic acid” [Tiab] OR “octanesulphonic acid” [Tiab] OR "ammonium perfluorosulfonate"[tiab] OR 1763-23-1 [rn] OR 2795-39-3 [rn] OR 29081-56-9 [rn] OR 29457-72-5 [rn] OR 4021-47-0 [rn] OR 70225-14-8 [rn] OR 307-35-7 [rn] OR 56773-42-3[rn] OR 251099-16-8[rn]) OR (“perfluorooctanoic acid”[nm] OR PFOA [Tiab] OR PFAA* [Tiab] OR APFO [Tiab] OR “fluorinated surfactants” [Tiab] OR fluorosurfactant* [Tiab] OR “fluorinated polymer*” [Tiab] OR (fluorinated [Tiab] AND (polymer [Tiab] OR polymers [Tiab])) OR (fluorocarbon [Tiab] AND (polymer [Tiab] OR polymers [Tiab])) OR fluoropolymer* [Tiab] OR (fluorinated [Tiab] AND telomer* [Tiab]) OR fluorotelomer* [Tiab] OR fluoro-telomer* [Tiab] OR fluorotelomer alcohol*[tiab] OR “telomer alcohol*” [Tiab] OR “polyfluoroalkyl* ” [Tiab] OR “N-ethyl perfluorooctanesulfonamido ethanol” [Tiab] OR “N-ethyl perfluorooctanesulfonamidoethanol” [Tiab] OR “N-EtFOSE” [Tiab] OR perfluoroalkyl* [Tiab] OR perfluorocarbon* [Tiab] OR perfluorocarboxyl* [Tiab] OR perfluorochemical* [Tiab] OR “perfluorinated*” [Tiab] OR (perfluorinated [Tiab] AND (C8 [Tiab] OR carboxylic [Tiab] OR chemical* [Tiab] OR compound* [Tiab] OR octanoic [Tiab])) OR (PFO [Tiab] AND (perfluoroalk* [Tiab] OR perfluorocarb* [Tiab] OR perfluorinat* [Tiab] OR perfluorooc* [Tiab])) OR (C8 [Tiab] AND (perfluoroalk* [Tiab] OR perfluorocarb* [Tiab] OR perfluorinat* [Tiab] OR perfluorooc* [Tiab])) OR perfluoroctanoic [Tiab] OR perfluorooctanoic [Tiab] OR “perfluoro octanoic” [Tiab] OR “perfluoro-n-octanoic” [Tiab] OR “perfluorinated octanoic acid” [Tiab] OR perfluorooctanoate [Tiab] OR perfluoroctanoate [Tiab] OR “perfluoro octanoate” [Tiab] OR perfluoroheptanecarboxylic [Tiab] OR “perfluoro-1-heptanecarboxylic” [Tiab] OR perfluorocaprylic [Tiab] OR perfluorocaprilate [Tiab] OR perfluorocaprylate [Tiab] OR	PFOS & PFOA terms

	perfluoroacrylate [Tiab] OR "perfluorooctanoyl chloride" [Tiab] OR "pentadecafluoro-1-octanoic" [Tiab] OR "pentadecafluoro-n-octanoic" [Tiab] OR pentadecafluorooctanoate* [Tiab] OR pentadecafluoroctanoate* [Tiab] OR pentadecafluoroctanoic [Tiab] OR pentadecafluorooctanoic [Tiab] OR fluorad [Tiab] OR "FC 143" [Tiab] OR FC143 [Tiab] OR 335-67-1 [rn] OR 3825-26-1 [rn] OR 335-95-5 [rn] OR 2395-00-8 [rn] OR 90480-56-1[rn] OR 335-93-3[rn])	
2	dart[sb]	DART terms
3	#1 AND #2	Combine Chemicals & DART
4	limit to 2016 to present	Add Date Limit

Results

A general description of OEHHA's literature search is shown in Figure A7.5. A list of studies excluded based on OEHHA's abstract or full article review is provided in Table A7.28.

Figure A7.5. Literature search: recent epidemiologic studies of PFOA or PFOS and developmental and reproductive toxicity*



*This figure is provided to document OEHHA's PubMed, Embase, Scopus, SciFinder. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

OEHHA identified 45 studies of PFOA or PFOS and developmental and reproductive toxicity published since January 2016. Two studies reported information on pubertal development, two on preecamplsia and pregnancy-induced hypertension, four on fertility and fecundity, and 37 studies reported information on birth weight and other indicators of fetal growth. Further details of the methods and results of these studies are shown in Tables A7.13-20.

Table A7.13. Recent epidemiologic studies on PFOA and preeclampsia and gestational hypertension

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wikstrom et al., 2019	Sweden 2007-2010	Prospective cohort	N=1,773 pregnant women from Varmland County (SELMA Pregnancy Cohort) N=64 cases (3.6%) N=42 cases among nulliparous	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR): 1.61 (1.12-2.31) ng/ml	Serum PFAS in early pregnancy (median 10 weeks gestation)	Preeclamp-sia	National birth registry	OR=2.40 (0.95-6.06) for highest quartile of serum PFOA compared to lowest All women OR=1.31 (0.93-1.87) Per unit increase in PFOA (log base 2)* Nulliparous women OR=1.38 (0.90-2.21) for serum PFOA (log base 2)* *one unit increase in log base 2 concentrations is a doubling of exposure	OR for highest vs. lowest exposure quartile OR per doubling in PFOA exposure	Adjusted for parity (except when stratified by parity), age, weight, smoking (serum cotinine) Twin pregnancies, education level, and month of sampling were not confounders	Magnitude: yes Statistical significance: no Dose-response: risk increased to a similar degree in 2nd-4th quartiles Temporal association: yes Subgroup only: no Adjustments: little change in nulliparous women. Unadjusted OR for preeclampsia in all women was 1.53 (1.13- 2.07).	Analyses were for individual PFAS exposures. Correlation between PFOA (log base 2) and PFOS (log base 2): r=0.60; correlations between PFOA and PFNA and PFDA were 0.66 and 0.72, respectively. PFNA was associated with preeclampsia: All women OR=1.38 (1.01-1.89) Nulliparous women OR=1.50 (1.04-2.16)
Huang et al., 2019	Shanghai, China 2011-2012	Cross-sectional	N=674 Women with singleton pregnancies who came to one of two hospitals to deliver	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR): 6.98 (4.95-9.54) ng/ml Sample selection was not described	Cord blood samples at delivery	Gestational hyper-tension Preeclamp-sia	Medical records	<u>Preeclampsia ORs</u> Tertile 2: 2.23 (0.67-7.44) Tertile 3: 1.41 (0.38-5.14) Continuous*: 1.12 (0.68-1.84) <u>Gestational hypertension ORs</u> Tertile 2: 0.33 (0.10-1.11) Tertile 3: 0.77 (0.30-2.01) Continuous*: 0.95 (0.61-1.48) PFOA was not selected in elastic net regression to select exposures related to preeclampsia or gestational hypertension *Continuous exposure: ln-transformed, centered, and standardized with 1 SD	ORs for 2nd and 3rd tertile of exposure compared to 1st tertile ORs per unit increase in ln-transformed, centered, and standardized exposure	Selected covariates based on a directed acyclic graph: age, education level, parity, pre-pregnancy BMI	<u>Preeclampsia</u> Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear <u>Gestational hypertension</u> Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Prevalence of smoking was 1.5% before and 0.4% during pregnancy, so was not included as a confounder (authors did not state whether based on self-report) Overall risk of preeclampsia was 2.8% and gestational hypertension 3.3% Correlation between PFOA and PFOS was 0.33, and with other PFAS ranged from 0.12 to 0.61

Abbreviations and units: β, regression coefficient; BMI, body mass index (kilogram/meter^2); IQR, interquartile range; ln, natural logarithm; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFAS, per-and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFNA, perfluorononanoic acid; r, correlation coefficient; SD, standard deviation; SES, socioeconomic status

Table A7.14. Recent epidemiologic studies on PFOS and preeclampsia and gestational hypertension

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wikstrom et al., 2019	Sweden 2007-2010	Prospective cohort	N=1,773 pregnant women from Varmland County (SELMA Pregnancy Cohort) N=64 cases (3.6%) N=42 cases among nulliparous	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Median (IQR) 5.39 (3.95-7.61) ng/ml	Serum PFAS in early pregnancy (median 10 weeks gestation)	Preeclamp-sia	National birth registry	OR=2.68 (1.17-6.12) for highest quartile of serum PFOS compared to lowest All women OR=1.53 (1.07-2.20) for serum PFOS (log base 2*) Nulliparous women OR=2.02 (1.26-3.29) for serum PFOS (log base 2*) *one unit increase in log base 2 concentrations is a doubling of exposure	OR for highest vs. lowest exposure quartile OR per doubling in PFOA exposure	Adjusted for parity (except when stratified by parity), age, weight, smoking (serum cotinine) Twin pregnancies, education level, and month of sampling were not confounders	Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: yes Subgroup only: no Adjustments: little change in nulliparous women. Unadjusted OR for preeclampsia in all women was 1.74 (1.23-2.46).	Analyses were for individual PFAS exposures. Correlation between PFOA (log base 2) and PFOS (log base 2): r=0.60; Correlations between PFOS and PFNA and PFDA were 0.55 and 0.57, respectively. PFNA was associated with preeclampsia: All women OR=1.38 (1.01–1.89) Nulliparous women 1.50 (1.04–2.16)
Huang et al., 2019	Shanghai, China 2011-2012	Cross-sectional	Women with singleton pregnancies who came to one of two hospitals to deliver N=674	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Median (IQR) 2.38 (1.81-3.23) ng/ml	Cord blood samples at delivery	Preeclamp-sia Gestational hypertensi on	Medical records	<u>Preeclampsia ORs</u> Tertile 2: 0.59 (0.19-1.87) Tertile 3: 0.70 (0.23-2.08) Continuous*: 0.83 (0.52-1.32) <u>Gestational hypertension ORs</u> Tertile 2: 0.54 (0.17-1.66) Tertile 3: 0.95 (0.36-2.49) Continuous*: 0.87 (0.57-1.34) *Continuous exposure: ln-transformed, centered, and standardized with 1 SD PFOS was not selected in elastic net regression to select exposures related to preeclampsia or gestational hypertension	ORs for 2nd and 3rd tertile of exposure compared to 1st tertile ORs per unit increase in ln-transformed, centered, and standardized exposure	Selected covariates based on a directed acyclic graph: age, education level, parity, pre-pregnancy BMI	<u>Preeclampsia</u> Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear <u>Gestational hypertension</u> Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Prevalence of smoking was 1.5% before and 0.4% during pregnancy, so was not included as a confounder. (authors did not state whether based on self-report) Overall risk of pre-eclampsia was 2.8% and gestational hypertension 3.3% Correlation between PFOA and PFOS was 0.33, and with other PFAS ranged from 0.01 to 0.87.

Abbreviations and units: β, regression coefficient; BMI, body mass index (kilogram/meter^2); IQR, interquartile range; ln, natural logarithm; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFAS, per-and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFNA, perfluorononanoic acid; r, correlation coefficient; SD, standard deviation; SES, socioeconomic status

Table A7.15. Recent epidemiologic studies on PFOA and indicators of fetal growth (see footnote for abbreviations and units)

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Alkhalawi et al., 2016	Duisberg, Germany 2000-2002	Retrospective cohort	N=148 mother-child pairs from the Duisberg Cohort	Selection: of 196 mothers who met inclusion criteria, sufficient blood/plasma for analysis (including imputation for missing data) was available for 148 Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (range) Cord plasma 1.75 (<0.4-6.54) Maternal plasma 2.44 (<0.4-9.2)	Maternal plasma at 32 weeks Cord blood or plasma collected at delivery See notes	Birth weight PI BL	"Child examination booklets"	BW β = –0.025 (–0.090 - 0.040) PI β = –0.412 (–0.788 - –0.037) kg/m^3 BL β = 0.152 (–0.199 - 0.503)	Per quartile increase in maternal plasma PFOA Quartiles (ng/ml): 1: <0.4–1.97 2: 1.99–2.73 3: 2.75–3.48 4: 3.52–9.20	Adjusted for pregnancy duration, pre-pregnancy BMI, maternal height, lead in maternal blood, gender, mode of delivery, mother born in Germany, and smoking during pregnancy	Magnitude: no Statistical significance: yes (PI) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	Missing values for PFOA, PFOS, and PFHxS in maternal and cord plasma were replaced by calculating conversion factors from geometric mean ratios of available paired cord plasma and cord whole blood samples, and paired cord plasma and maternal plasma samples. A factor of 2 was calculated to convert cord plasma to cord whole blood for all three PFAS; calculated conversion factors of 0.35 for PFOS and 0.76 for PFOA were used for cord plasma to maternal plasma. No mention of correlations among three PFAS studied. Associations between PFHxS and birth size were in the same direction as PFOA and PFOS.
Ashley-Martin 2017	10 cities in Canada 2008-2011	Prospective cohort	N=1,705 women ≥18 years old, with no history of pregnancy complications or known fetal or chromo-somal anomaly in the current pregnancy, and their live born singletons	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR), (range) 1.7, (1.2-2.4), (LOD-16)	First trimester maternal plasma	BW	Medical charts	BW *z*-score β = −0.10 (95% credible interval [CrI], −0.34 - 0.13)	Per log-unit increase in PFOA	Adjusted for: PFOS, PFHxS, maternal age, pre-pregnancy BMI, parity, income, and maternal smoking	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Pearson correlation coefficients for correlations between log-transformed PFAS ranged from r = 0.5 (PFOA and PFHxS) to r = 0.6 (PFOA and PFOS).
Bach et al., 2016	Aarhus, Denmark 2008-2013	Prospective cohort	N=1,507 women and their infants from the Aarhus Birth Cohort	Selection: random sample of eligible women (nulliparous, donated blood sample between 9-20 weeks gestation and gave birth to live singleton) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 2.0 (1.5-2.6)	Maternal serum at 12 weeks (96% within 13 weeks gestation)	BW BW *z*-scores, calculated by standardi-zation of BW for GA according to recent Scandi-navian reference BL HC	Collected by clinical staff immediately after delivery; after January 2013, registry records were used	BW Overall: β = 21 (–1 - 44) Adjusting for GA or restricting to term births attenuated this association Boys: β = 31 (4 - 59) Girls: β = 4 (–34 - 42) No associations with BW *z*-scores, BL, or HC	Per IQR of PFOA exposure	Adjusted for maternal age, pre-pregnancy BMI, education Some BW analyses adjusted for GA, were restricted to term infants, or were stratified by sex	Magnitude: no Statistical significance: yes (BW – boys) Dose-response: no Temporal association: no Subgroup only: BW- boys Adjustments: little change	Of 16 PFAS measured, 7 that were quantifiable in >50% of the study sample were presented. Spearman correlations among PFAS ranged from 0.14 to 0.85. The correlation for PFOS and PFOA was moderate, r = 0.40. The highest correlation for PFOA was with PFNA, r = 0.82.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Bell et al., 2018	New York State (excluding New York City) 2008-2010	Cross-sectional	N=6,171 infants born to 5,034 women in the upstate KIDS cohort study	Selection: Infants were sampled based on infertility treatment field on birth certificates, frequency matched 1:3 with infants conceived without infertility treatment by region; all mothers of twins were recruited Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) All 1.07 (0.67-1.60) Singletons 1.10 (0.69-1.63) Twins 1.01 (0.63-1.53)	Newborn dried blood spots from heel sticks, captured onto filter paper cards	BW BL HC PI	BW: birth certificate data BL, HC: maternal question-naire	No significant associations between PFOA and birth size (BW, BL, HC, or PI) when parity was included in the models.	Per increase in log-transformed and scaled PFOA concentration	Adjusted for maternal age, BMI, education, infertility treatment, parity	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Correlation between PFOS and PFOA r = 0.32. No other PFAS were considered. The addition of PFOS and BPA to the models did not alter the results.
Buck Louis et al., 2018	12 clinical sites in the US 2009-2013	Prospective cohort (National Institute for Child Health and Human Development Fetal Growth Studies)	N=2,106 healthy women with low-risk pregnancies who delivered live singleton infants and had plasma samples available.	Selection: unclear Participation: unclear Equal groups: non-Hispanic white women had higher PFOA concentrations (median 2.9); mean age and BMI of mothers varied by race Blinded: unclear Levels: median (IQR) 1.985 (1.297-3.001)	Maternal plasma at 10-13 weeks gestation	BW BL Upper arm length Upper thigh length Head circumference Umbilical circumference	Trained nurses completed the neonatal anthropo-metric assessment	<u>BL</u> Overall β = −0.23 (−0.35 - −0.10) Black women β = –0.47 (–0.73 - –0.21) <u>Upper thigh length</u> Overall β = −0.19 (−0.26-−0.12) No consistent, statistically significant associations with other measures of fetal growth	Per log (PFOA+1), scaled by SD	Adjusted for maternal age, race, education, gender, serum cotinine, delivery mode (in head circumference models), interaction of chemical and race/ethnicity	Magnitude: no Statistical significance: yes (BL, upper thigh length) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	Cohort was racially/ethnically diverse, mostly married, and had a high education level
Callan et al., 2016	Western Australia 2008-2011	Cross-sectional	N=98 pregnant women >18 years	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Mean ± SD, median (range) 1.00 ± 0.60, 0.86 (0.21-3.1)	Maternal whole blood collected ~2 weeks before due date	BW Proportion of optimal BW (POBW; see notes) BL Proportion of optimal BL (POBL; see notes) HC Proportion of optimal HC (POHC; see notes) PI (100*BW g /BL cm^3)	Participant question-naire	<u>BW</u> β = −48 (−203 - 108) <u>HC</u> β = −0.40 (−0.96 - 0.16) PFOA was not associated with BL, PI, POBW, POBL, POHC A possible trend for lower BW and POBW with increasing PFOA was visible among girls, but no statistics were reported.	Per ln-unit increase in PFOA (equivalent to a ~2.7-fold increase)	BW, BL, HC models adjusted for GA, maternal height, pre-pregnancy BMI, weight gain during pregnancy, and sex of infant. POBW, POBL, POHC: adjusted for weight gain during pregnancy, maternal age (except POHC), and annual household income.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Pearson's correlations among PFAS ranged from 0.20 to 0.84. For PFOS and PFOA, r = 0.75. PFOA was also highly correlated with PFNA, r = 0.84 and PFDA, r = 0.79. POBW, POBL, POHC calculations incorporate adjustments for GA, maternal height, parity, sex of infant, maternal age (for POHC only) based on a large cohort of Caucasian single births in Western Australia. Authors state that multiplication of the concentrations by 2 to account for the dilution of whole blood has been reported as an acceptable means of estimating serum concentrations.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Cao et al., 2018	Zhoukou City, China 2013-2015	Cross-sectional	N=337 mother-infant pairs	Selection: native Chinese mothers who had lived in the local residence for >1 year and their live singleton infant Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.25 (0.87-1.82)	Cord serum	BL BW PI	Hospital birth records	BW, PI: no associations BL, by tertile 2: β = –0.21 (–0.56 - 0.14) 3: β = –0.45 (–0.79 - –0.10) *p trend* = 0.01 Girls 2: β = –0.16 (–0.68 - 0.37) 3: β = –0.58 (–1.12 - –0.04) *p trend* = 0.04 Boys 2: β = –0.22 (–0.68 - 0.23) 3: β = –0.36 (–0.80 - 0.09) *p trend* = 0.11	Compared to 1st tertile of exposure Exposure tertiles (ng/ml): 1: <0.99 2: 0.99 – 1.59 3: >1.59	Adjusted for gender, maternal age, household income, paternal drinking, parity. Birth weight and PI analyses also included maternal education and paternal smoking.	Magnitude: no Statistical significance: yes (BL) Dose-response: yes, trend of shorter BL with higher PFOA exposure Temporal association: no Subgroup only: no Adjustments: unclear	The sum of all 11 measured PFAS was not associated with birth outcomes. PFNA, PFDA, and PFUnDA appeared to be positively associated with fetal growth. Correlations among PFAS were not reported.
Chen et al., 2017	Taipei and New Taipei, Taiwan 2004-2005	Cross-sectional	N=429 mother-infant pairs from the Taiwan Birth Panel Study. Mothers were non-smokers.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.9 (2.7)	Cord plasma	Age-specific *z*-scores for BW, BL, BMI	Medical records	BW *z*-score β = –0.07 (–0.18 - 0.03) BL *z*-score β = −0.04 (−0.16 - 0.08) BMI *z*-score β = −0.09 (−0.20 - 0.02)	Per ln-unit increase in PFOA	Adjusted for maternal age, pre-pregnancy BMI, education, infant sex, PTB, history of breastfeeding, ln-cotinine	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Spearman correlation coefficient for PFOA and PFOS r = 0.025 (p = 0.61)
Costa et al., 2019	Gipuzkoa, Sabadell, and Valencia, Spain 2004-2008	Prospective cohort	N=1,230 mothers with exposure, ultrasound, and delivery data from the INMA (Infancia y Medio Ambiente) Project	Selection: unclear (see note) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 2.35 (1.63-3.30)	Maternal plasma at end of 1st trimester (mean ± SD 13.5 ± 1.7 weeks)	Abdominal circumfer-ence (AC) Biparietal diameter (BPD) Femur length (FL) Estimated fetal weight (EFW)	Ultrasound scans performed by specialized obstetricians at 12, 20, and 34 weeks gestation.	No evidence of association between log_2 PFOA concentrations and fetal growth, except among smokers. Among smokers (N=382): FL at week 20: –6.8% (–12.4 - –1.0) EFW at week 20: –5.7% (–11.4 - –0.1) AC, BPD: no associations No associations at 12 or 34 weeks.	% change in growth per log_2-unit increase in PFOA	Adjusted for cohort (city), parity, maternal age and country of birth, smoking at week 12. Estimated glomerular filtration rate, plasma albumin, other PFAS, and other variables were evaluated but found to not be confounders.	Magnitude: no Statistical significance: yes (FL, EFW) Dose-response: no Temporal association: yes Subgroup only: yes, smokers Adjustments: little change	Women who were excluded based on missing data were somewhat less likely to have been born in Spain, more likely to smoke, and be of lower SES. Excluded women's children had small, non-significant increases in growth parameters, while children of included women had small, mostly non-significant decreases in growth parameters. PFAS compounds were correlated, with highest correlations between PFOA and PFNA (Pearson's partial correlation r = 0.74) and PFOS and PFHxS (r = 0.58) Results for PFNA were similar to those for PFOA

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de Cock et al. 2016	Netherlands 2011-2013	Cross-sectional	N=62 mother-child pairs with PFAS data Women were recruited at first antenatal visit. Children were singletons without major congenital anomalies.	Selection: unclear Participation: only 62 of 148 participants are included in unadjusted models (fewer in adjusted models), with explanation for only 57 of the 86 missing subjects Equal groups: unclear Blinded: unclear Levels: mean, median (range) 0.93, 0.87 (0.20-2.70)	Umbilical cord plasma	BW	Midwife registries	No associations with BW in boys or girls.	Compared to lowest exposure quartile Quartiles (ng/ml): 1: <0.591 2: 0.591 - 0.870 3: 0.871 - 1.150 4: ≥1.511	"Partially adjusted" models included: GA, maternal BMI, height, age, gestational weight gain, parity. "Fully adjusted" models additionally included smoking, alcohol intake, paternal BMI and height, fish and folic acid intake	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	No mention of correlations between PFOA and PFOS or with other chemicals examined (DDE, PCB-153, and three phthalate metabolites). 68% of women had bachelor's degree or higher education, compared with 28% in the general Dutch population. Male/female ratio of offspring was 1.7.
Govarts et al., 2016	Flanders, Belgium 2008-2009	Cross-sectional	N=213 mother-infant pairs recruited from general population Uncompli-cated live-born singletons	Selection: multi-stage sampling Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (IQR) 1.52 (1.10-2.10)	Cord plasma	BW	Medical records	PFOA was not significantly associated with BW in adjusted single exposure models. PFOA appeared to contribute to reduced BW of female infants in multi-exposure models, but measures of association for PFOA in these models were not reported.	Per unit (1 ng/ml) increase in PFOA	Adjusted for GA, sex, smoking during pregnancy, parity, pre-pregnancy BMI. Considered but not included: maternal age stress/pres-sure during pregnancy, education, smoking before pregnancy, alcohol use before/during pregnancy, maternal height, income, infections/ complications, folic acid use, cesarean section. Sex and smoking status were evaluated for effect modification.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Focus was on using cord blood vs. maternal blood biomarkers, and identifying effects of multiple simultaneous exposures PFOA and PFOS correlation: r = 0.50 PFOA was one of four chemicals that "enhanced the association" between arsenic and lower birth weight when both sexes were included.

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Govarts et al., 2018	Europe (Belgium, Norway, Slovakia, Netherlands) 2002-2012	Pooled analysis of cross-sectional data from 4 cohorts with PFOA exposure data	N=693 women and their live-born singletons	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: cord serum combined median (IQR): 0.550 (0.299-1.200) (medians for 4 cohorts ranged from 0.312-1.500)	Cord serum, observed and estimated based on breast milk concentrations Conversion factor: cord serum level = 13 x breast milk level	SGA, defined as BW <10th percentile for each week of pregnancy and each country and sex-specific reference weight curve	Medical records (BW) and question-naires or ultrasound (GA)	OR = 1.64 (0.97 - 2.76) Mothers who smoked during pregnancy: OR = 2.18 (1.02 - 4.64) Nonsmokers: OR = 1.51 (0.87 - 2.63) p-interaction = 0.33 Sex was not an effect modifier.	Per IQR increase in cord serum PFOA	Adjusted for sex, maternal height, pre-pregnancy BMI, education, age, parity, smoking during pregnancy. Effect varied by cohort (p-interaction <0.05), but the direction of the estimates was the same.	Magnitude: yes Statistical significance: yes (smokers) Dose-response: unclear Temporal association: no Subgroup only: stronger association in smokers Adjustments: unclear	For women in the Norwegian (N=196) and Slovakian (N=207) cohorts, cord serum PFOA levels were estimated based on breast milk samples. For women in the Dutch cohort (N=80), levels were a combination of observed and estimated PFOA concentrations. Correlation between PFOA and PFOS: r = 0.47, and between PFOA and *p,p'*-DDE: r = 0.59. The Belgian cohort (N=208) is included in the Govarts et al., 2016 study of BW.
Gyllen-hammar et al., 2018	Uppsala County, Sweden 1996-2011	Cross-sectional	N=381 first-time, Swedish-born mothers of singletons within the Persistent Organic Pollutants in Uppsala Primiparas (POPUP) study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR): 2.3 (1.6-3.0) ng/g	Maternal serum, sampled 3 weeks after delivery	Standard deviation scores (SDS) for BW, BL, HC (SDSs corrected for GA)	Swedish Medical Birth Register	PFOA exposure was not associated with BW, BL, or HC SDS. Adjustment for maternal estimated glomerular filtration rate (eGFR) did not markedly influence associations between PFAS and birth weight.	Per IQR increase in PFOA	Adjusted for sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, education, smoking during pregnancy, total fish consumption	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	eGFR was estimated using creatinine and cystatin C (GFR_{cc}) in serum at 3 weeks post-pregnancy. Both measures were associated with PFOA. GFR_{cc} was inversely associated with gestation length, but not birth weight SDS. Correlations between PFOA levels in 3rd trimester and 3 weeks after delivery in a sample of 20: r = 0.94, p <0.001

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Hjermit-slev et al., 2020	Greenland 2010-2011 and 2013-2015	Prospective cohort (authors called it cross-sectional but measure-ments were taken during pregnancy) 2 groups, recruited 2010-2011 and 2013-2015	N=482 pregnant Inuit women	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range), 1.06 (0.10-7.26)	Maternal serum collected at mean 26.2 (range 7-40) weeks gestation for 1st group and before end of gestation week 13 for 2nd group	BW BL HC LBW	Assessed by midwives and data were obtained from the Greenland Doctors Office	Results are adjusted for GA except as noted <u>BW</u> All β = –119 (–202 - –36.6) Females β = –161 (–283 - –40.1) Males β = –81.2 (–194 - 31.2) All (no adjustment for GA) β = –27.3 (–127 - 72.0) Restricting analysis to term births did not change GA-adjusted association with BW. <u>BL</u> All β = –0.37 (–0.76 - 0.02) <u>HC</u> All β = –0.35 (–0.59 - –0.10) Females β = –0.51 (–0.88 - –0.15) Males β = –0.22 (–0.56 - 0.12) <u>LBW</u>: no associations	Per unit increase in PFOA	"Core" adjustments: maternal age, pre-pregnancy BMI, parity, smoking status, alcohol use during pregnancy; additional adjustment: GA Stratification by gender	Magnitude: yes (BW) Statistical significance: yes (BW, HC) Dose-response: unclear Temporal association: yes Subgroup only: effects were larger for females Adjustments: core adjustments diminished the association and statistical significance for BW. Adjustment for GA increased the magnitude and statistical significance of the inverse associations between PFOA and BW, HC, and BL.	Generalizability may be limited due to unique population of Inuit women with high smoking rates and possibly high exposure to persistent organic pollutants through traditional diet. PFOA was statistically significantly associated with older GA at birth for both boys (0.42 weeks) and girls (0.48 weeks).
Kwon et al., 2016	Seoul, South Korea 2006-2010	Cross-sectional	N=268 pregnant women enrolled in the Ewha Birth & Growth Retrospective Cohort (EBGRC) study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range) 0.91 (0.68-1.15)	Cord serum	BW Effect modification by glutathione S-transferase M1 (GSTM1) and cytochrome P4501A1 (CYP1A1) polymor-phisms	Medical records	<u>BW</u> β = −77.93 (−153.56-−2.30) <u>Effect modification</u> For mothers with *CYP1A1 Val* variant, β = −128.89 (−265.71-7.93) *CYP1A1 Ile/Ile* variant, β = −13.15 (−119.37, 93.07) Association between PFOA and BW was not statistically significantly modified by GSTM1 or CYP1A1.	Per log-unit increase in PFOA	Adjusted for maternal age, pre-pregnancy BMI, history of alcohol consumption, GA, gender, and parity. Results were similar with adjustment for history of smoking.	Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: adjustment resulted in slightly larger coefficient. Unadjusted β = −47.04 (−121.22, 27.14).	Spearman's rank correlations were high for PFOA and PFNA (r = 0.78) and PFOS and PFNA (r = 0.60). Correlations for PFOA and PFOS were not reported.

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Lauritzen et al., 2017	Norway and Sweden 1986-1988	Case-cohort: the study sample comprised a 10% random sample and SGA cases from a high risk group* from a population-based cohort study. *defined as any of the following: prior SGA or LBW child, cigarette smoker, pre-pregnancy weight <50kg, previous perinatal death, chronic disease	N=424 pairs of parous women and children 143 SGA cases; 281 randomly sampled non-SGA controls Norway N=265 Sweden N=159	Selection: based on availability of maternal serum samples Participation: unclear Equal groups: see notes Blinded: unclear Levels: median, arithmetic mean (SD), Sweden: 2.33, 2.42 (1.00) Norway: 1.62, 1.83 (1.00)	Second trimester maternal serum	SGA (BW <10th percentile adjusted for GA, parity, and sex) BW BL HC	Measured and recorded at birth	In pooled analyses with data from both Norway and Sweden, and from Norway alone, there were no significant associations between PFOA and indicators of fetal growth after adjustment for important covariates. In the Swedish cohort: SGA Boys OR = 6.55 (1.14 - 37.5) Girls OR = 4.73 (0.79 - 28.3) All OR = 5.25 (1.68 - 16.4) BW Boys β = –526 (–828 - – 222) Girls β = –156 (–541 - 228) All β = –359 (–596 - – 122) BL Boys β = –1.6 (–2.9 - – 0.4) Girls β = –0.8 (–2.4 - 0.8) All β = –1.3 (–2.3 - –0.3) HC Boys β = –0.6 (–1.3 - 0.1) Girls β = –0.1 (–1.0 - 0.7) All β = –0.4 (–1.0 - 0.1)	Per ln-unit increase in PFOA	Adjusted for maternal age, height, pre-pregnancy BMI, education, smoking status, inter-pregnancy interval, parity (except SGA analyses). Adjustment for infant sex, hexachloro-benzene, PCB-153, alcohol, or weight gain did not change estimates.	Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: yes Subgroup only: Swedish women, stronger effects in boys Adjustments: unclear	Correlation between PFOA and PFOS, r = 0.56-0.73 Swedish women had slightly higher pre-pregnancy BMI, were less likely to smoke, and had higher PFAS and organochlorine levels than Norwegian women. Swedish offspring were also longer at birth.
Lee et al., 2016	Seoul, South Korea 2008	Cross-sectional	N=85 newborns delivered by cesarean section	Selection: unclear Participation: unclear Equal groups: participants treated by one gynecologist had greater mean GA and BW (3.33 kg, vs. 2.72 kg). A variable representing treatment by this clinician was included in models. Blinded: unclear Levels: mean ± SD (IQR) 1.11 ± 0.48 (0.83-1.29)	Cord serum	BW	Medical records	β = –0.03 (–0.25 - 0.18)	Per ln-unit increase in PFOA	Adjusted for GA, maternal age, infant gender, and clinician	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Concentrations of the 6 studied PFAS were correlated with each other. Spearman's rank correlations: PFOS and PFOA r = 0.5290 PFOS and PFNA r = 0.7181 PFOA and PFNA r = 0.6830

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Lenters et al., 2016	Greenland, Poland, Ukraine 2002-2004	Prospective cohort INUENDO Cohort	N=1,250 mother-child pairs. Women ≥18 years old and born in the country of study were enrolled at a prenatal visit. Infants were term singletons with complete exposure data.	Selection: unclear Participation: Ukraine 26%, Greenland 90%, Poland 68% Equal groups: unclear Blinded: unclear Levels: 5th, 50th, 95th percentiles Greenland 0.78, 1.84, 3.55 Poland 1.34, 2.51, 4.36 Ukraine 0.45, 0.96, 2.10	Maternal serum during pregnancy (timing of serum samples varied widely by location and overall)	BW	Hospital records	PFOA was selected by elastic net regression modeling as a predictor of BW in term infants. β = –42.77 (–108.19 - 22.65) Smokers: β = –80.35 (–211.94 - 51.23) Non-smokers: β = –36.93 (–109.75 - 35.88) p-interaction = 0.03 "Further adjusted" model: β = –63.77 (–122.83 - –4.71) g.	Per 2-SD increase in ln-transformed PFOA (1.18 ng/ml)	"Minimal sufficient adjustment set": study population (location), maternal age, pre-pregnancy BMI, parity. "Further adjusted models" also included: GA, sex, maternal height, alcohol consumption near conception, maternal serum cotinine, maternal serum vitamin D, ln-mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), ln-mono(4-methyl-7-oxo-octyl) phthalate (MOiNP), and ln-*p,p'*-DDE.	Magnitude: no Statistical significance: yes Dose-response: unclear Temporal association: yes Subgroup only: no Adjustments: further adjusted model suggested a stronger relationship between PFOA and birth weight	Elastic net regression modeling was used to select covariates and consistently selected PFOA. Spearman correlation between PFOA and PFOS r = 0.61; all other correlations with PFOA were lower. Authors note that there is no consensus on adjustment for GA, and that "further adjusted models" might over-adjust or adjust unnecessarily.
Li et al., 2017	Guangzhou, China 2013	Cross-sectional	N=317 mother-infant pairs Guangzhou Birth Cohort Study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Total PFOA 1.2 (0.9-1.8) n-PFOA (linear PFOA) 1.0 (0.7-1.6)	Cord serum	BW LBW	Medical records	BW Total PFOA β = –112.7 (–171.9 - –53.5) n-PFOA β = –85.0 (–133.6 - –36.5) LBW results not reported	Per ln-unit increase in PFOA isomers	Adjusted for GA (BW analyses), BW (GA analyses), delivery, education, parity, infant sex, maternal age, PIH, GDM, anemia.	Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: adjustment for maternal education and maternal age changed some coefficients substantially	Electrochemical fluorination, used in the manufacture of the majority of PFOS, PFHxS, and PFOA, and phased out in 2001, results in 20–30% branched isomers. The telomerization method for PFOA manufacturing produces isomers that are almost completely linear. Spearman correlations PFOA and PFOS r = 0.65
Lind et al., 2017	Odense, Denmark 2010-2012	Prospective cohort	N=638 pregnant women and singleton children Odense Child Cohort	Selection: all pregnant women in Odense were invited. Women were recruited at ultrasound information meeting or first antenatal visit. Sample for present study included singleton children of 200 women randomly selected in 2010, and 449 women selected from 2011-2012 with adequate data. Participation: 70.5% for the cohort. Equal groups: unclear Blinded: yes Levels: median (IQR) 1.7 (1.1-2.3)	Maternal serum collected at recruitment (median 10 weeks gestation, range 5-12 weeks)	BW HC Abdominal circum-ference	Birth records	BW Boys β = –5 (–92 - 82) Girls β = 6 (–90 - 102) Effects of PFAS on BW were modified by sex, though no β coefficients for PFOA (continuous or quartiles) and BW were statistically significant. PFOA was not associated with HC or abdominal circumference.	Per ln-unit increase in PFOA	Adjusted for GA, parity, maternal smoking during pregnancy, pre-pregnancy BMI. Maternal ethnicity and education were considered but were not associated with outcomes.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Correlations among PFAS not reported.

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Manzano-Salgado et al., 2017	Gipuzkoa, Sabadell, and Valencia, Spain 2003-2008	Prospective cohort	N=1,202 mother-child pairs from the INMA cohort Mothers were >16 years old, without communica-tion barriers and reproductive assistance, delivering in a reference hospital.	Selection: unclear Participation: participants were more educated, less likely to come from Gipuzkoa, and had slightly longer infants than excluded women Equal groups: yes Blinded: unclear Levels: median ± SD 2.35 ± 1.25	Maternal plasma collected at mean ± SD 12.3 ± 5.6 weeks gestation	BW, BL, HC (size measure-ments were standard-ized to week 40 of gestation) LBW SGA (below 10th percentile for GA and sex according to national references)	Infant size was measured by midwives and nurses GA was calculated based on last menstrual period	<u>BW</u> All: β = –9.33 (–38.81 - 20.16) Boys: β = –24.75 (–66.71 - 17.22) Girls: β = 13.81 (-26.67 - 54.30) [Interaction term for sex, p = 0.25] <u>SGA</u> All: OR = 0.92 (0.72 - 1.19) Boys: OR = 1.18 (0.82 - 1.69) Girls: OR = 0.72 (0.50 - 1.04) [Interaction term for sex, p = 0.08] LBW associations were similar to those for SGA. No statistically significant associations with BL and HC	Per doubling of PFOA	Adjusted for maternal age, parity, pre-pregnancy BMI, fish intake, type of delivery (HC only). Region was included as a random effect. Adjusting for smoking during pregnancy did not substantially change results.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Maternal GFR was calculated from plasma-creatinine measurements in the 1st trimester. GFR did not confound associations. Increasing PFAS concentrations were associated with lower BW in Sabadell and Valencia, and higher birth weight in Gipuzkoa. Region-specific PFOA concentrations were not reported. Spearman correlation coefficients for PFOS, PFOA, PFHxS, and PFNA ranged between 0.43 and 0.68. When all PFAS were included in a model, betas for the continuous outcomes were close to the null.
Marks et al., 2019	Former Avon Region in South West England 1991-1992	Prospective cohort	N=457 mother-son pairs enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC).	Selection: unclear; women were selected for the present analysis to maximize data on puberty and dual energy X-ray absorptiometry (DXA) scans. Participation: unclear Equal groups: "no…strong associations between PFAS and maternal characteristics. There was some evidence that mothers who reported taking folic acid had higher PFAS concentrations" Blinded: unclear Levels: median (IQR) 3.0 (2.3-3.8)	Maternal serum collected at median 30 weeks gestation	BW Crown to heel length (BL) HC	Medical records for BW BL and HC measured by study staff	PFOA was not associated with birth size outcomes in boys.	Per unit increase in PFOA	Adjusted for pregnancy weight gain, pre-pregnancy BMI, education level, vitamin use, folic acid use, smoking during pregnancy, parity, GA at sample collection. BW analyses also adjusted for maternal age and alcohol use during pregnancy.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Authors previously published a study of PFAS and birth size in girls. In a sensitivity analysis including only 1st trimester samples (N=115), associations were consistent with entire study sample. This sample of the ALSPAC cohort was disproportionately white, educated, older, and nonsmoking compared to the overall ALSPAC cohort. PFOS, PFHxS, and PFNA were also analyzed. PFAS concentrations were strongly correlated; PFOA and PFOS (r = 0.63) and PFOS and PFNA (r = 0.60) were most strongly correlated. BMI, parity, and education did not interact with PFAS' effects on birth size.

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Meng et al., 2018	Denmark 1996-2002	Prospective cohort	N=3,507 mother and singleton infant pairs from the Danish National Birth Cohort Three samples from sub-studies that had measured PFAS within the same source population were included.	Selection: unclear Participation: about 60% of invited women accepted Equal groups: unclear Blinded: yes Levels: median (IQR) 4.6 (3.3-6.0)	Maternal plasma in 1st (92%) and 2nd trimester Sample 1 plasma was measured at 3M for only PFOS and PFOA in 2007. Samples 2 and 3 were analyzed at Aarhus University in 2011 and 2014, respectively.	BW LBW	Hospital discharge register	BW, by quartile 2: β = –20.4 (–70.0 - 29.2) 3: β = –25.9 (–77.7 - 25.9) 4: β = –117.0 (–172.3 - –61.6) Continuous β = –35.6 (–66.3 - –5.0) LBW, by quartile 2: OR = 1.5 (0.8 - 3.1) 3: OR = 1.2 (0.5 - 2.5) 4: OR = 1.5 (0.7 - 3.3) Continuous OR = 1.0 (0.7 - 1.5)	Compared to 1st quartile of exposure, or per doubling of PFOA exposure Quartiles (ng/ml): 1: ≤3.3 2: >3.3 and ≤4.6 3: >4.6 and ≤6.0 4: >6.0	Adjusted for infant sex, birth year, maternal age, parity, socio-occupational status, pre-pregnancy BMI, smoking during pregnancy, alcohol use during pregnancy, gestational week of blood draw.	Magnitude: no Statistical significance: yes (BW) Dose-response: yes (BW) Temporal association: yes Subgroup only: no Adjustments: for BW, unadjusted continuous β = –90.1 (–117.9 - –62.2)	6 PFAS were analyzed in two different labs, with results differing by lab, though correlations of PFOS and PFOA concentrations measured in both labs were high (r = 0.94 for PFOS and r = 0.95 for PFOA) Correlations among PFAS: PFOS and PFOA r = 0.66 PFOS and PFHpS r = 0.89 PFOA and PFHpS r = 0.67 3 of the other 4 PFAS evaluated were associated with BW. Adjustment for other PFAS changed the direction of the association between PFOA and BW, though the associations were not statistically significant.
Minatoya et al., 2017	Hokkaido, Japan 2002-2005	Prospective cohort	N=168 pairs of mothers and their term singletons Sapporo Cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.4 (0.9-2.2)	Maternal serum, collected between 23 weeks gestation and delivery	BW PI	Birth records	BW β = –197 (–391 - –3) PI β = –1.32 (–2.66 - 0.02) kg/m^3 No interaction with sex for BW and PI.	Per log-unit increase in PFOA	Adjusted for BMI, maternal smoking during pregnancy, parity, gestation weeks at blood sampling, infant sex, GA	Magnitude: yes (BW) Statistical significance: yes (BW) Dose-response: yes (BW, p-trend <0.05) Temporal association: yes Subgroup only: no Adjustments: unclear	PFOA and PFOS were modestly correlated, Spearman r = 0.287 Adjustment for PFOS did not change coefficients
Sagiv et al., 2018	Boston, MA 1999-2002	Prospective cohort	N=1,645 women with singleton live births	Selection: unclear Participation: 78% Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.8 (3.8)	Maternal plasma at median 9 weeks gestation	Birth weight-for-gestational-age (BW-for-GA) *z*-score Term BW	Medical records (US national reference for BW-for-GA and BW-for-sex *z*-score)	BW-for-GA *z*-score β = –0.02 (–0.08 - 0.03) The effect was entirely among girls, though not statistically significant (data presented graphically). Additional adjustments for eGFR and albumin did not substantially change the estimate. Term BW β = −18.5 (−45.4 - 8.3)	Per IQR increase in PFOA	Adjusted for mother's age, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, pre-pregnancy BMI, GA at blood draw, sex, paternal education, household income. Plasma albumin concentration and estimated glomerular filtration rate (eGFR) were also included in some models.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Highly educated, high-income population. Authors state PFAS were "moderately correlated"; PFOS and PFOA Spearman r = 0.72 An objective of this study was to evaluate whether adjusting for pregnancy hemodynamics affected associations between PFAS and birth outcomes.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Shi et al., 2017	Beijing, China 2012	Cross-sectional	N=170 women who gave birth to singletons without congenital anomalies were recruited from one hospital	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: mean ± SD, median (IQR) 1.285 ± 0.721, 1.097 (0.817-1.443)	Cord serum	BW BL PI	Medical records	BW β = 163.28 (–127.66 - 454.23) BL β = 0.38 (–0.41 - 1.17) PI β = 0.06 (–0.10 - 0.22)	Per log-unit increase in PFOA	Adjusted for maternal age, pre-pregnancy BMI, parity, GA, gender, and height (birth length only). Participants were non-smokers and did not drink alcohol during pregnancy.	Magnitude: yes (BW) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: large difference between crude and adjusted coefficients for BW	The sample included 1 LBW infant
Shoaff et al., 2018	Cincinnati, OH 2003-2006	Prospective cohort	N=345 mother-child pairs Eligibility criteria: ≥18 years old, recruited at 16 ± 3 weeks gestation, living in Cincinnati area in a home built before 1978, no history of HIV, no medication for seizure or thyroid disorders, singleton pregnancy	Selection: unclear Participation: unclear Equal groups: unclear Blinded: yes Levels: median (IQR) 5.5 (3.8-7.7)	Maternal serum at ~16 weeks gestation if available (86%), at 26 weeks gestation (9%), or within 48 hours of delivery (5%) if neither earlier sample was available.	BW *z*-scores, standard-ized for gestational age using US reference data	Hospital records	Continuous β = –0.03 (–0.17 - 0.10) By tertiles: 2: β = 0.18 (–0.06 - 0.42) 3: β = –0.15 (–0.40 - 0.10) Sex did not modify associations between PFAS and BW *z*-scores.	Change in BW *z*-score per doubling of serum PFOA, or compared to 1st tertile of PFOA concentration Tertiles (ng/ml): 1: 0.5-4.3 2: 4.4-6.7 3: 6.8-26.4	Adjusted for maternal age, race, marital status, insurance status, income, education, parity, serum cotinine, depressive symptoms, mid-pregnancy BMI, food security, fruit/vegetable consumption during pregnancy, fish consumption during pregnancy, prenatal vitamin use.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS were moderately correlated, the highest correlation was for PFOS and PFOA, r = 0.60. Including all measured PFAS (PFOS, PFNA, PFHxS) in the model attenuated the association toward the null.
Starling et al., 2017	Colorado 2010-2014	Prospective cohort	N=628 participants selected based on availability of maternal serum and cord blood, from women ≥16 years old with singleton pregnancies	Selection: unclear Participation: 11 of 1,410 enrolled dropped out and the 628 included participants did not meaningfully differ from the larger cohort of 1,410 Equal groups: unclear Blinded: yes Levels: median (IQR) 1.1 (0.7-1.6)	Maternal serum from fasting blood collected at median 27 weeks gestation (range 20-34 weeks)	BW	Weighed by clinical personnel	Continuous β = –51.4 (–97.2 - –5.7) By tertiles: 2: β = −15.9 (−84.9 - 53.2) 3: β = −92.4 (−166.2 - – 18.5) In sensitivity analyses to evaluate possible confounding by other PFAS, PFOA remained inversely associated with lower BW, but the coefficients were smaller and not statistically significant. No significant interactions between PFOA and sex.	Per ln-unit increase in PFOA, or compared to 1st tertile Tertiles (ng/ml): 1: 0.1-0.8 2: 0.9-1.4* 3: 1.4-17.0 * Study reported 1.4 in both 2nd and 3rd tertiles.	Adjusted for maternal age, pre-pregnancy BMI, race/ethnicity, education, parity, smoking during pregnancy, gestational weight gain, sex, GA, gestational age at maternal blood draw	Magnitude: yes Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: yes, unadjusted continuous β = –9.9 g	PFAS concentrations showed moderate to high correlations; the highest Spearman's rank correlation was for PFOA and PFOS, r = 0.76. PFOA level was lower than among NHANES females (NHANES 2011-2012 geometric mean = 1.84) Authors estimated that maternal glucose mediated 3.0% of the effect of PFOA on BW.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Valvi et al., 2017	Faroe Islands 1997-2000	Prospective cohort	N=604 mother-child pairs recruited at 34 weeks' gestation with singleton pregnancies	Selection: unclear Participation: 92% of 656 recruited mother-child pairs with complete data on key variables were included Equal groups: unclear Blinded: yes Levels: median (IQR) 3.31 (2.54-3.99)	Maternal serum at gestation week 34	BW BL HC	Unclear for BW, HC BL was measured by midwife at 14 days postpartum	<u>BW</u> β = –11 (–88 - 67) Although not statistically significant, PFOA was slightly positively associated with BW in girls, and inversely associated for boys (data presented graphically only). <u>BL</u> β = –0.28 (–0.60 - 0.03) <u>HC</u> β = 0.00 (–0.22 - 0.23) Sex was not a modifier of associations with length.	Per doubling of PFOA	Adjusted for maternal age, education, parity, pre-pregnancy BMI, smoking during pregnancy, and sex Evaluated, but not included: gestational weight gain, family history of diabetes, vitamin D concentration, docosahexa-enoic acid concentration, and GA	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Authors were interested in gestational diabetes as a possible mediator of the association between environmental pollutants and offspring size, but did not find that it modified or mediated the associations with birth size.
Wang et al., 2016	Taiwan 2000-2001	Prospective cohort	N=223 mothers and term infants	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Female 2.34 (1.57-3.43) Male 2.37 (1.35-3.47)	Maternal serum in 3rd trimester	BW BL HC SGA	Measure-ments were taken by clinic nurses	<u>BW</u> Girls β = –0.08 (–0.18 - 0.01) Boys β = 0.04 (–0.05 - 0.12) PFOA was not associated with BL, HC, or SGA.	Per ln-unit increase in PFOA	Adjusted for maternal age, education, previous live births, pre-pregnancy BMI, and income. No adjustment for smoking and alcohol consumption due to low prevalence (2% and 1%, respectively). GA and maternal weight gain were not confounders.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFOA was not highly correlated with other perfluorocarboxylic acids (r≤0.34).

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wang et al., 2019	Hebei Province, China 2013	Cross-sectional	N=424 mother-infant pairs selected from healthy 20-40-year-old women recruited in first 12 weeks of pregnancy	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Mean ± SD, median (IQR) 2.64 ± 2.41, 1.99 (1.22-3.11)	Cord serum	BW BL HC PI	Measured at birth by obstetric nurses	No associations with BW or BL. HC (mm) β = –3.87 (–6.96 - –0.77) [possible error: the study's text shows β = –3.87 but Table 2 shows β = –3.70 (–7.00 - –0.40)] PI β = –0.05 (–0.10 - 0.01)	Per ln-unit increase in PFOA	Adjusted for: age, family income, maternal education, maternal career, husband's smoking, daily energy intake, daily physical activity, GA, parity, pre-pregnancy BMI, GDM, sex, delivery mode, gestational weight gain. No women smoked during pregnancy.	Magnitude: no Statistical significance: yes (HC) Dose-response: no Temporal association: no Subgroup only: no Adjustments: no large changes evident	Authors were also interested in effects of PFAS on estrogen homeostasis and role of estrogen as a mediator between PFAS and birth size.
Wikstrom et al., 2019	Sweden 2007-2010	Prospective cohort	N=1,533 mother and singleton infant pairs with complete data from the SELMA (Swedish Environ-mental, Longitudinal, Mother and child, Asthma and allergy) pregnancy cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.61 (1.11-2.30)	Maternal serum (measured at median 10 weeks gestation)	BW Sex- and GA-specific birth weight standard deviation score (BW-SDS) SGA	Swedish Medical Birth Register	BW All children β = –68 (–112 - –24) Girls β = –86 (–145 - –26) Boys β = –49 (–113 - 15) SGA All children OR = 1.43 (1.03 - 1.99) Girls OR = 1.96 (1.18 - 3.28) Boys OR = 1.16 (0.75 - 1.78) BW-SDS All children β = –0.152 (–0.251 - –0.052) Girls β = –0.191 (–0.325 - –0.057) Boys β = –0.111 (–0.258 - 0.036) Restricting analyses to 1st trimester serum samples did not change regression coefficients. GA did not mediate associations between PFAS and BW.	Per ln-unit increase in PFOA (correspond-ing to an increase from 25th to 75th percentile of exposure)	Adjusted for parity, maternal weight, cotinine concentration, sex (unless stratified by sex), GA (for BW only). Maternal age, education level, and week of sampling were not confounders.	BW Magnitude: yes overall and for girls Statistical significance: yes overall and for girls Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear SGA Magnitude: yes overall and for girls Statistical significance: yes overall and for girls Dose-response: yes, larger OR with higher exposure quartile Temporal association: yes Subgroup only: stronger association for girls Adjustments: unclear BW-SDS Magnitude: no Statistical significance: yes overall and for girls Dose-response: no Temporal association: yes Subgroup only: stronger association for girls Adjustments: unclear	No reporting of correlations among different PFAS. BW was inversely associated with PFOS, PFNA, PFDA, and PFUnDA. The finding of significance in girls, not boys, was also consistent with the same PFAS.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Woods et al., 2017	9 counties in Cincinnati, OH area 2003-2006	Prospective cohort	N=272 pregnant women enrolled in the Health Outcomes and Measures of Environment (HOME) Study	Selection: Women >18 years of age were recruited from prenatal clinics at 13 to <19 weeks pregnancy. Women who were free of specified health conditions and living in residences built before 1978 to select for lead exposure were included. Black women were oversampled. Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.4 (3.8-8.1)	Maternal serum at ~16 and ~26 weeks gestation	BW	Birth records	PFOA was associated with a small (~15 g) decrease in BW but the confidence interval was wide and included 0 (data presented graphically; statistics were not reported).	Per log-unit increase in PFOA (correspond-ing to a 10-fold increase)	Adjusted for maternal age, race, marital status, employment status, income, insurance, education level, tobacco use, gender, BMI, 56 other chemicals from 5 classes of potential endocrine disrupting chemicals, organo-chlorine pesticides, and heavy metals	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	All PFAS were associated with small, non-statistically significant decreases in BW. .
Workman et al., 2019	Winnipeg, Manitoba, Canada 2010-2011	Prospective cohort	N=414 mother-infant pairs with prenatal samples from the Canadian Healthy Infant Longitudinal Development Study Cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range) 0.89 (0.16-7.1)	Maternal prenatal plasma collected at 16 and 26 weeks	BW BL HC PI	Not reported	No significant associations between PFOA and size parameters (statistics not reported). Result for PI was not reported.	Regression coefficients were not reported	Adjusted for maternal age, smoking during pregnancy, hypertension during pregnancy, diabetes during pregnancy, parity, sex, GA, method of delivery (for HC)	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	
Xu et al., 2019	Hangzhou, China 2016-2017	Cross-sectional	N=98 mother-infant pairs Women with serious illnesses and clinical symptoms, multiple births, infants with congenital diseases were excluded.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.05 (0.779-1.33)	Umbilical cord serum	BW LBW SGA HC PI	Hospital birth records	<u>BW</u> β = 315.5 (–159.7 - 790.7) <u>SGA</u> OR = 0.790 (0.319 - 1.959) No associations with HC or PI. Because there were only 3 LBW infants, authors did not analyze LBW.	Per log-unit increase in PFOA	Adjusted for maternal age, GA (except in SGA analyses), abortion history, parity, pre-pregnancy BMI, pregnancy weight gain, education, job type, gender, tap vs. filtered drinking water	Magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: large changes with adjustment for BW: Unadjusted β = 165.6 (–285.8 - 617.1)	Correlation between PFOS and PFOA, r=0.35. Correlations between PFOA and other PFAS, |r| ≤ 0.35 Low exposure contrast (small range of exposure)

Studies are in alphabetical order by first author and year

For BW, a change of >50 g was considered "large magnitude"

Numbers in parentheses are 95% confidence intervals unless otherwise noted

PFOA concentrations are in ng/ml unless otherwise noted

Abbreviations and units: β, regression coefficient; BL, birth (body) length (cm unless otherwise noted); BMI, body mass index (kg/m^2); BW, birth weight (g unless otherwise notes); BW-SDS, sex- and gestational age-specific birth weight standard deviation score; CI, confidence interval; cm, centimeter; CrI, credible interval; *p,p'*-DDE, dichlorodiphenyldichloroethylene; g, gram; GA, gestational age; GDM, gestational diabetes mellitus; GFR, glomerular filtration rate; GSTM 1, glutathione S-transferase M1; HC, head circumference (cm unless otherwise noted); IQR, interquartile range; kg, kilogram; LBW, low birth weight (birth weight <2,500 g); ln, natural logarithm; LOD, limit of detection; m, meter; N, number of participants; ng/ml, nanograms/milliliter; NHANES, US National Health and Nutrition Examination Survey; OR, odds ratio; PCB-153, polychlorinated biphenyls; PFAS, per-and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFNA,

perfluorononanoic acid; PFUnDA, perfluoroundecanoic acid PI, ponderal index (calculated as [1000*BW/BL] g/cm^3 or [100*BW/BL] g/cm^3 unless otherwise noted); PIH, pregnancy-induced hypertension; PTB, preterm birth; r, correlation coefficient; SD, standard deviation; SES, socioeconomic status; SGA, small for gestational age (<10th percentile for age and sex unless otherwise noted)

Table A7.16. Recent epidemiologic studies on PFOS and indicators of fetal growth (see footnote for abbreviations and units)

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Alkhalawi et al., 2016	Duisberg, Germany 2000-2002	Retrospective cohort	N=148 mother–child pairs from the Duisberg Cohort	Selection: of 196 mothers who met inclusion criteria, sufficient blood/plasma for analysis (including imputation for missing data) was available for 148 Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (range) Cord plasma 2.83 (0.53-11.7) Maternal plasma 9.04 (1.70-21.93 [possible error: 29.2 in text]	Maternal plasma at 32 weeks gestation Cord blood or plasma collected at delivery See notes	BW PI BL	"Child examination booklets"	BW β = –0.030 (–0.091 - 0.030) PI β = –0.355 (–0.702 - –0.008) kg/m^3 BL β = 0.103 (–0.221-0.428)	Per quartile increase in maternal plasma PFOS Quartiles (ng/ml): 1: 1.70-6.98 2: 7.02-9.31 3: 9.33-11.80 4: 11.86-21.93	Adjusted for pregnancy duration, pre-pregnancy BMI, maternal height, lead in maternal blood, sex, mode of delivery, mother born in/outside Germany, and smoking during pregnancy	Magnitude: no Statistical significance: yes (PI) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	Missing values for PFOA, PFOS, and PFHxS in maternal and cord plasma were replaced by calculating conversion factors from geometric mean ratios of available paired cord plasma and cord whole blood samples, and paired cord plasma and maternal plasma samples. A factor of 2 was calculated to convert cord plasma to cord whole blood for all three PFAS and a factor of 0.35 for PFOS for cord plasma to maternal plasma. Using these calculations, 81 maternal and 83 cord plasma samples were increased to 186 ng/ml each. There was an unexplained discrepancy in sample size between the publication text and Table 2 No mention of correlations among three PFAS studied. Associations between PFHxS and birth size were in the same direction as PFOA and PFOS.
Ashley-Martin 2016	10 cities in Canada 2008-2011	Prospective cohort	N=1,705 women ≥18 years old, with no history of pregnancy complications or known fetal or chromo-somal anomaly in the current pregnancy, and their live born singletons	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR), (range) 4.6 (3.2-6.8), (LOD-36)	First trimester maternal plasma	BW	Medical charts	BW z-score β = 0.05 (95% credible interval [CrI], −0.18 - 0.29)	Per log-unit increase in PFOS	Adjusted for: PFOA, PFHxS, maternal age, pre-pregnancy BMI, parity, income, and maternal smoking. Gestational weight gain was not a confounder or effect modifier.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Pearson correlation coefficients for correlations between log-transformed PFAS ranged from r = 0.5 (PFOA and PFHxS) to r = 0.6 (PFOA and PFOS)

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Bach et al., 2016	Aarhus, Denmark 2008-2013	Prospective cohort	N=1,507 women and their infants from the Aarhus Birth Cohort	Selection: random sample of eligible women (nulliparous, donated blood sample between 9-20 weeks gestation and gave birth to live singleton) Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 8.30 (6.03-10.80)	Maternal serum at 12 weeks (96% within 13 weeks gestation)	BW BW *z*-scores, calculated by standard-ization of BW for GA according to recent Scandi-navian reference BL HC GA PTB	Collected by clinical staff immediate-ly after delivery; in 2013, registry records were used	BW All births By PFOS exposure quartile, compared to lowest quartile: 2: β = –86 (–159 - –13) 3: β = –21 (–91 - 48) 4: β = –50 (–123 - 23) Per IQR (4.8 ng/ml): All: β = –2 (–30 - 26) Boys: β = 26 (–13 - 65) Girls: β = –32 (–71 - 7) Term births By exposure quartile: 2: β = –93 (–157 - –29) 3: β = –50 (–113 - 13) 4: β = –62 (–126 - 3) Per IQR: β = –14 (–40 - 11) PFOS was not associated with BW *z*-scores, BL, HC, GA, or PTB.	Compared to lowest exposure quartile, or per IQR of PFOS exposure Quartiles (ng/ml): 1: <6.02 2: 6.03-8.29 3: 8.30-10.80 4: 10.81-36.0	Adjusted for maternal age, pre-pregnancy BMI, education. Some BW analyses adjusted for GA, were restricted to term infants, or were stratified by sex.	Magnitude: yes Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: no Adjustments: little change	Of 16 PFAS measured, 7 that were quantifiable in >50% of the study sample were presented. Spearman correlations among PFAS ranged from r = 0.14 to r = 0.85. The correlation for PFOS and PFOA was moderate, r = 0.40. The highest correlation for PFOS was with PFNA, r = 0.46.
Bell et al., 2018	New York State (excluding New York City) 2008-2010	Cross-sectional	N=6,171 infants born to 5,034 women in the upstate KIDS cohort study. Infants were sampled based on infertility treatment field on birth certificates, frequency matched 1:3 with infants conceived without infertility treatment by region. All mothers of twins were recruited.	Selection: possible, based on infertility treatment; twins Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) All 1.69 (1.12-2.40) Singletons 1.72 (1.14-2.44) Twins 1.64 (1.09-2.33)	Newborn dried blood spots from heel sticks, captured onto filter paper cards	BW BL HC PI	BW: birth certificate data BL, HC: maternal question-naire	No significant associations with BW, BL, or HC when parity was included in the models. Models remained non-significant with the addition of PFOA and BPA.	Per increase in log-transformed and scaled PFOS concentration	Adjusted for maternal age, BMI, education, infertility treatment, parity	Magnitude: no Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: singletons Adjustments: little change	Correlation between PFOS and PFOA r = 0.32. No other PFAS were considered.

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Buck Louis et al., 2018	12 clinical sites in the US 2009-2013	Prospective cohort (National Institute for Child Health and Human Develop-ment Fetal Growth Studies)	N=2,106 healthy women with low-risk pregnancies who delivered live singleton infants and had plasma samples available	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.133 (3.39-7.981)	Maternal plasma at 10-13 weeks gestation	BW BL Upper arm length Upper thigh length HC Umbilical circum-ference	Trained nurses completed the neonatal anthropo-metric assess-ment	No consistent statistically significant associations with measures of fetal growth	Per log (PFOS+1), scaled by SD	Adjusted for maternal age, race, education, gender, serum cotinine, delivery mode (in HC models), interaction of chemical and race/ethnicity	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Cohort was racially/ethnically diverse, mostly married, and had a high education level.
Callan et al., 2016	Western Australia 2008-2011	Cross-sectional	N=98 pregnant women >18 years old	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range), 1.99 (0.45-8.1); mean ± SD, 2.32 ± 1.42	Maternal whole blood collected ~2 weeks before due date	BW Proportion of optimal BW (POBW; see notes) BL Proportion of optimal BL (POBL; see notes) HC Proportion of optimal HC (POHC; see notes) PI (g/cm^3 *100)	Participant question-naire	BW: −69 (−231 - 94) HC: −0.39 (−0.98 - 0.20) PFOS was not associated with BL, PI, POBW, POBL, POHC. A possible trend for lower BW and POBW with increasing PFOS was visible among girls, but no statistics were reported.	Per ln-unit increase in PFOS (equivalent to a ~2.7-fold increase)	BW, BL, HC models adjusted for GA, maternal height, pre-pregnancy BMI, weight gain during pregnancy and sex of infant. POBW, POBL, POHC models adjusted for weight gain during pregnancy, maternal age (except POHC), and income.	Magnitude: yes (BW) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Pearson correlations among PFAS ranged from r = 0.20 tor = 0.84. For PFOS and PFOA, r = 0.75. POBW, POBL, POHC calculations incorporate adjustments for GA, maternal height, parity, sex of infant, maternal age (for POHC only) based on a large cohort of Caucasian single births in Western Australia. Authors state that multiplication of the concentrations by 2 to account for the dilution of whole blood has been reported as an acceptable means of estimating serum concentrations.
Cao et al., 2018	Zhoukou City, China 2013-2015	Cross-sectional	N=337 mother-infant pairs	Selection: native Chinese mothers who had lived in the local residence for >1 year and their live singleton infant Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 1.01 (0.60-1.76)	Cord serum	BW BL PI	Hospital birth records	PFOS was not associated with BW, BL, or PI.	Compared to 1st tertile of exposure Tertiles (ng/ml): 1: <0.74 2: 0.74-1.52 3: >1.52	Adjusted for gender, maternal age, household income, paternal drinking, parity. BW and PI analyses also included maternal education and paternal smoking.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	The sum of all 11 measured PFAS was not associated with birth outcomes. PFNA, PFDA, and PFUndA appeared to be positively associated with fetal growth. Correlations among PFAS were not reported.

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Chen et al., 2017	Taipei and New Taipei, Taiwan 2004-2005	Cross-sectional	N=429 mother-infant pairs from the Taiwan Birth Panel Study. Mothers were non-smokers.	Selection: unclear. Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.7 (5.0)	Cord plasma	Age-specific *z*-scores for BW, BL, BMI	Medical records	BW *z*-score β = −0.14 (−0.26 - −0.01) BL *z*-score β = −0.16 (−0.31 - −0.02) BMI *z*-score β = −0.11 (−0.25 - 0.02)	Per ln-unit increase in PFOS	Adjusted for maternal age, pre-pregnancy BMI, education, infant sex, preterm birth, breastfeeding, ln-cotinine	Magnitude: no Statistical significance: yes (BW *z*-score, BL *z*-score) Dose-response: greater exposure appeared to be associated with strongest effects on BW, BL, BMI Temporal association: no Subgroup only: no Adjustments: little change	Spearman correlation coefficient for PFOA and PFOS r = 0.025 (p =0.61).
Costa et al., 2019	Spain (3 locations) 2004-2008	Prospective cohort	N=1,230 mothers with exposure, ultrasound, and delivery data from the INMA (Infancia y Medio Ambiente) Project	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 6.05 (4.52-7.82)	Maternal plasma at end of 1st trimester (mean ± SD 13.5 ± 1.7 weeks)	Fetal growth parameters: Abdominal circum-ference (AC) Biparietal diameter (BPD) Femur length (FL) Estimated fetal weight (EFW)	Ultrasound scans performed by specialized obstetri-cians at 12, 20, and 34 weeks gestation	No evidence of association between log_2 PFOS concentrations and fetal growth, except among smokers. Among smokers, PFOS was associated with 6.3 % (0.1 - 12.3) increase in BPD at week 34.	% change in growth per log_2 unit increase in PFOS	Adjusted for cohort (city), parity, maternal age and country of birth, smoking at week 12 (PFOS and BPD), estimated glomerular filtration rate (eGFR), plasma albumin; other variables, including other PFAS, were evaluated but found to not be confounders	Magnitude: no Statistical significance: yes (BPD at 34 weeks) Dose-response: no Temporal association: yes Subgroup only: smokers Adjustments: little change	PFAS were correlated, with highest correlations between PFOA and PFNA (Pearson's partial correlation r = 0.74) and PFOS and PFHxS (r = 0.58). The correlation between PFOA and PFOA was 0.5.
de Cock et al. 2016	Nether-lands 2011-2013	Cross-sectional	N=62 mother-child pairs with PFAS data Women were recruited at first antenatal visit. Children were singletons without major congenital anomalies.	Selection: unclear Participation: only 62 of 148 participants are included in unadjusted models (fewer in adjusted models), with explanation for only 57 of the 86 missing subjects Equal groups: unclear Blinded: unclear Levels: mean, median (range) 1.60, 1.60 (0.57-3.20)	Umbilical cord plasma	BW	Midwife registries	PFOS exposure was not associated with BW in boys. For girls in the highest exposure quartile: Unadjusted β = 65.6 (–431.46 - 562.71) "Partially adjusted" β = 224.3 (–193.07 - 641.68) "Fully adjusted" β = 595.9 (88.77 - 1103.04) No other PFOS exposure quartiles were significantly associated with BW in girls.	Compared to lowest exposure quartile. Quartiles (ng/ml): 1: <0.996 2: 0.996 - 1.600 3: 1.601 - 2.000 4: ≥2.001	"Partially adjusted" models included: GA, maternal BMI, height, age at birth, gestational weight gain, parity. "Fully adjusted" models additionally included smoking, alcohol intake, paternal BMI and height, fish and folic acid intake.	Magnitude: yes (girls, "fully adjusted" model) Statistical significance: yes for "fully adjusted" model Dose-response: unclear Temporal association: no Subgroup only: girls Adjustments: large changes with adjustment	No mention of correlations between PFOA and PFOS or with other chemicals examined (DDE, PCB-153, and three phthalate metabolites). 68% of women had bachelor's degree or higher education, compared with 28% in the general Dutch population. Male/female ratio of offspring was 1.7.

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Govarts et al., 2016	Flanders, Belgium 2008-2009	Cross-sectional	N=213 mother-infant pairs recruited from general population Uncompli-cated live-born singletons	Selection: multi-stage sampling Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (IQR) 2.63 (1.70-3.80)	Cord plasma	BW	Medical records	PFOS was not significantly associated with BW in adjusted single exposure models. Measures of association for PFOS in multi-exposure models were not reported. PFOS appeared to contribute to reduced BW of female infants in multi-exposure models.	Per unit (1 ng/ml) increase in PFOS	Adjusted for GA, sex, smoking during pregnancy, parity, pre-pregnancy BMI. Considered but not included: maternal age, stress during pregnancy, education, smoking before pregnancy, alcohol use before/during pregnancy, maternal height, income, infections/compli-cations, folic acid use, cesarean section. Sex and smoking status were evaluated for effect modification.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Focus was on using cord blood vs. maternal blood biomarkers, and identifying effects of multiple simultaneous exposures PFOA and PFOS correlation: r = 0.50
Govarts et al., 2018	Europe (Belgium, Norway, Slovakia, Nether-lands) 2002-2012	Pooled analysis of cross-sectional data from 4 cohorts with PFOS exposure data	N=688 women and their live-born singletons	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: cord serum PFOS combined median (IQR): 1.984 (1.200-3.008) (medians for 4 cohorts ranged from 0.960-2.700)	Cord serum, observed and estimated based on breast milk concentra-tions Conversion factor: cord serum level = 32 x breast milk level	SGA, defined as BW <10th percentile for each week of pregnancy and each country and sex-specific reference weight curve	Medical records (BW) and question-naires or ultrasound (GA)	Mothers who smoked during pregnancy: OR = 1.63 (1.02 - 2.59) Nonsmokers: OR = 0.66 (0.61 - 0.72) p-interaction = 0.0004 Sex was not an effect modifier.	Per IQR increase in cord serum PFOS	Adjusted for sex, maternal height, pre-pregnancy BMI, education, age, parity, smoking during pregnancy. Sex and smoking status were evaluated for effect modification.	Magnitude: yes, in different directions for smokers and nonsmokers Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: yes Adjustments: unclear	For women in the Norwegian (N=196) and Slovakian (N=204) cohorts, cord serum PFOS levels were estimated based on breast milk samples. For women in the Dutch cohort (N=80), levels were a combination of observed and estimated PFOS concentrations. Correlation between PFOA and PFOS: r = 0.47, and between PFOA and *p,p'*-DDE: r = 0.59. The Belgian cohort (N=208) is included in the Govarts et al., 2016 study of BW.
Gyllen-hammar et al., 2018	Uppsala County, Sweden 1996-2011	Cross-sectional	N=381 first-time, Swedish-born mothers of singletons within the Persistent Organic Pollutants in Uppsala Primiparas (POPUP) study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Median (IQR): 13 (7.4-19) ng/g	Maternal serum, sampled 3 weeks after delivery	Standard deviation scores (SDS) for BW, BL, and HC (SDSs corrected for GA)	Swedish Medical Birth Register	PFOS exposure was not associated with BW, BL, or HC SDS. Adjustment for maternal estimated glomerular filtration rate (eGFR) did not markedly influence association between PFOS and BW.	Per IQR increase in PFOS	Adjusted for sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, education, smoking during pregnancy, total fish consumption	Magnitude: no Statistical significance: no Dose response: no Temporal association: no Subgroup only: no Adjustments: no	eGFR was estimated using creatinine or cystatin C (GFR_{cc}) in serum at 3 weeks post-pregnancy. Neither was associated with maternal serum PFOS 3 weeks after delivery. GFR_{cc} was inversely associated with gestation length, but not BW SDS. Correlation between PFOS levels in 3rd trimester and 3 weeks after delivery in a sample of 20: r = 0.82, p <0.001 No mention of correlations among different PFAS.

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Hjermits-lev et al., 2020	Greenland 2010-2011 and 2013-2015	Prospective cohort (authors called it cross-sectional but measure-ments were taken during pregnancy) 2 groups, recruited 2010-2011 and 2013-2015	N=482 pregnant Inuit women	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Blinded: unclear Levels: median (range) 8.99 (1.50-61.3)	Maternal serum collected at mean 26.2 (range 7-40) weeks gestation for 1st group and before end of gestation week 13 for 2nd group.	BW BL HC LBW	Assessed by midwives and data were obtained from the Greenland Doctors Office	(Adjusted for GA) BW β = –5.47 (–12.6 - 1.67) BL β = –0.009 (–0.04 - 0.03) HC: β = –0.01 (–0.03 - 0.01) LBW: no associations; data not reported	Per unit increase in PFOS	"Core" adjustments: maternal age, pre-pregnancy BMI, parity, smoking status, alcohol use during pregnancy. Additional adjustment: GA. Stratification by gender.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Generalizability may be limited due to unique population of Inuit women with high smoking rates and possibly high exposure to persistent organic pollutants through traditional diet.
Kwon et al., 2016	Seoul, South Korea 2006-2010	Cross-sectional	N=268 pregnant women enrolled in the Ewha Birth & Growth Retrospec-tive Cohort study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range) 0.64 (0.29-1.09)	Cord serum	BW Effect modifica-tion by glutathione S-transferase M1 (GSTM1) and cytochrome P4501A1 (CYP1A1) polymorph-isms	Medical records	β = −49.41 (−95.57 - −3.25) No effect modification by GSTM1 or CYP1A1	Per log-unit increase in PFOs	Adjusted for maternal age, pre-pregnancy BMI, history of alcohol consumption, GA, gender, and parity. Results were similar with adjustment for history of smoking.	Magnitude: no Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: no Adjustments: Adjustment resulted in slightly larger coefficients. Unadjusted β = −22.26 (−68.76-24.24)	Spearman's rank correlations were high for PFOA and PFNA (r = 0.78) and PFOS and PFNA (r = 0.60). Correlations for PFOA and PFOS were not reported.
Lauritzen et al. 2017	Norway and Sweden 1986-1988	Case-cohort: study sample comprised a 10% random sample and SGA cases from a high risk group* from a population-based cohort study. *defined as any of the following: prior SGA or LBW child, cigarette smoker, pre-pregnancy weight <50kg, previous perinatal death, chronic disease	N=424 pairs of parous women and children 143 SGA cases; 281 randomly sampled non-SGA controls Norway N=265 Sweden N=159	Selection: based on availability of maternal serum samples Participation: unclear Equal groups: see notes Blinded: unclear Levels: median, arithmetic mean (SD) Sweden: 16.4, 17.3 (7.45) Norway: 9.74, 11.3 (7.02)	Second trimester maternal serum	SGA (BW below the 10th percentile adjusted for GA, parity, and sex) BW BL HC	Measured and recorded at birth	In pooled analyses with data from both Norway and Sweden, no significant associations between PFOS and indicators of fetal growth after adjustment for important covariates. PFOS was not associated with indicators of fetal growth in the Norwegian cohort. In the Swedish cohort: SGA OR = 2.51 (0.93 - 6.77) BW β = –292 (–500 - –84) BL β = –1.2 (–2.1 - –0.3) HC β = –0.4 (–0.9 - 0.04)	Per ln-unit increase in PFOS	Adjusted for maternal age, height, pre-pregnancy BMI, education, smoking status, inter-pregnancy interval, parity (except SGA analyses). Alcohol and weight gain did not change estimates in sensitivity analyses.	Magnitude: yes (SGA, BW) Statistical significance: yes (BW, BL) Dose-response: unclear Temporal association: yes Subgroup only: yes, Swedish women Adjustments: unclear	Correlations between PFOA and PFOS: Norway r = 0.56, Sweden r = 0.73 Swedish women had slightly higher pre-pregnancy BMI, were less likely to smoke, and had higher PFAS and organochlorine levels than Norwegian women. Swedish offspring were also longer at birth.

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Lee et al., 2016	Seoul, South Korea 2008	Cross-sectional	N=85 newborns delivered by cesarean section	Selection: unclear Participation: unclear Equal groups: participants treated by one gynecologist had greater mean GA and BW (3.33 kg vs. 2.72 kg). A variable representing treatment by this clinician was included in models. Blinded: unclear Levels: mean ± SD (IQR) 0.87 ± 0.46 (0.56-1.02)	Cord serum	BW	Medical records	β = –0.14 (–0.33 - 0.03)	Per ln-unit increase in PFOA	Adjusted for GA, maternal age, infant gender, and clinician	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Concentrations of the 6 studied PFAS were correlated with each other. Spearman's rank correlations: PFOS and PFOA r = 0.5290 PFOS and PFNA r = 0.7181 PFOA and PFNA r = 0.6830
Lenters et al., 2016	Greenland, Poland, Ukraine 2002-2004	Prospective cohort INUENDO Cohort	N=1,250 mother-child pairs with singleton, term infants with complete exposure data	Selection: unclear Participation: Ukraine 26%, Greenland 90%, Poland 68% Equal groups: unclear Blinded: unclear Levels: 5^{th}, 50^{th}, 95^{th} percentiles Greenland 10.23, 20.09, 49.47 Poland 4.38, 7.81, 12.40 Ukraine 2.27, 5.04, 9.48	Maternal serum during pregnancy (timing of serum samples varied widely by location and overall)	BW	Hospital records	PFOS was not selected in elastic net regression, and associations decreased markedly when modeled with other exposures, suggesting absence of an independent relationship with BW. The pooled relationship between PFOS and BW was non-linear, which appeared to be due to different exposure ranges and a slight positive slope for Poland. For the Greenland sample, which had higher PFOS concentrations, the slope was highly negative (i.e., PFOS was associated with lower BW).	Per 2-SD increase in ln-transformed PFOS (1.60 ng/ml)	"Minimal sufficient adjustment set:" study population (location), maternal age, pre-pregnancy BMI, parity. "Further adjusted models" add: GA, sex, maternal height, alcohol consumption near conception, maternal serum cotinine, maternal serum vitamin D. Multiple exposure models included ln-mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), ln-mono(4-methyl-7-oxo-octyl) phthalate (MOiNP), ln-dichlorodiphenyl-dichloroethylene (*p,p'*-DDE;).	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Elastic net regression modeling was used to select covariates and consistently selected PFOA, but not PFOS. Spearman correlation between PFOA and PFOS r = 0.61 Timing of serum samples varied widely by population/location and overall. Authors note that there is no consensus on adjustment for GA, and that "further adjusted models" might over-adjust or adjust unnecessarily.

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Li et al., 2017	Guang-zhou, China 2013	Cross-sectional	N=317 mother-infant pairs Guangzhou Birth Cohort Study	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Total PFOS 3.0 (1.7, 4.6) n-PFOS (linear PFOS) 2.1 (1.2, 3.6) Total branched (Br) PFOS 0.7 (0.4, 1.3) 1m-PFOS 0.1 (0.1-0.2) iso-PFOS 0.2 (0.1-0.3) 3 + 4 + 5m-PFOS 0.4 (0.2-0.7) See notes for isomer nomenclature details.	Cord serum	BW LBW	Medical records	BW Total PFOS Boys β = –150.6 (–225.4 - –75.7) Girls β = –26.6 (–125.1 - 71.8) n-PFOS Boys β = –100.2 (–160.5 - –39.9) Girls β = –10.0 (–83.0 - 63.0) Total Br-PFOS Boys β = –190.8 (–277.04 - 104.46) Girls β = –48.0 (–168.4 - 72.5) 1m-PFOS Boys β = –167.3 (–263.02 —71.64) Girls β = –15.0 (–139.1 - 109.2) Iso-PFOS Boys β = –156.1 (–231.91 - –80.37) Girls β = –66.9 (–168.5 - 34.8) 3 + 4 + 5m-PFOS Boys β = –184.7 (–269.9 - –99.5) Girls β = –38.7 (–156.9 - 79.5) PTB and LBW results not reported	Per ln-unit increase in PFOS isomers	Adjusted for GA, delivery, education, parity, infant sex, maternal age, PIH, GDM, anemia.	Magnitude: yes Statistical significance: yes (boys) Dose-response: unclear Temporal association: no Subgroup only: boys Adjustments: adjustment for maternal education and maternal age changed some coefficients substantially	Isomer nomenclature for branched PFOS: the abbreviations denote the structure of each isomer in relation to the position of the substituted perfluoromethyl, "m." The number preceding "m" indicates the carbon position of the branching point. E.g., sodium perfluoro-1-methylheptanesulfonate is 1m-PFOS, sodium perfluoro-3-methylheptanesulfonate is 3m-PFOS. Sodium perfluoro-6-methylheptanesulfonate is iso-PFOS. Spearman correlations PFOA and PFOS r = 0.65 PFOS and PFOS isomers: all r>0.97. Correlations with other PFAS were much lower.
Lind et al., 2017	Odense, Denmark 2010-2012	Prospective cohort	N=638 pregnant women and singleton children Odense Child Cohort	Selection: all pregnant women in Odense were invited. Women were recruited at ultrasound information meeting or first antenatal visit. Sample for present study included singleton children of 200 women randomly selected in 2010, and 449 women selected from 2011-2012 with adequate data. Participation: 70.5% for the cohort. Equal groups: unclear Blinded: yes Levels: median (IQR) 8.1 (6.0-11.0)	Maternal serum collected at recruitment (median 10 weeks gestation, range 5-12 weeks)	BW HC Abdominal circum-ference	Birth records	BW Girls β = 92 (–15 - 199) Boys β = –17 (–130 - 97) Effects of PFAS on BW were modified by sex, though no β coefficients for PFOS and BW were statistically significant. No associations with HC or abdominal circumference.	Per ln-unit increase in PFOS	Adjusted for GA, parity, maternal smoking during pregnancy, pre-pregnancy BMI. Maternal ethnicity and education were considered but were not associated with outcomes.	Magnitude: yes (girls) Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: tendency towards reduced BW in boys and greater BW in girls Adjustments: unclear	Correlations among PFAS not reported.

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Manzano-Salgado et al., 2017	Gipuz-koa, Sabadell, and Valencia, Spain 2003-2008	Prospective cohort	N=1,202 mother-child pairs from the INMA cohort Mothers were >16 years old, without communicati on barriers and reproductive assistance, delivering in a reference hospital.	Selection: unclear Participation: participants were more educated, less likely to come from Gipuzkoa, and had slightly longer infants than excluded women Equal groups: yes Blinded: unclear Levels: median ± SD 6.05 ± 2.74	Maternal plasma collected at mean ± SD 12.3 ± 5.6 weeks	BW, BL, HC (size measure-ments were standard-ized to week 40 of gestation) LBW SGA (below 10th percentile for GA and sex according to national references)	Infant size was measured by midwives and nurses GA was calculated based on last menstrual period	PFOS was not statistically significantly associated with BW, BL, HC, GA, SGA, and PTB. <u>LBW</u> Boys OR = 1.90 (0.98 - 3.68) Girls OR = 0.73 (0.46 - 1.19); [Interaction term for sex, p = 0.01]	Per doubling of PFOS	Adjusted for maternal age, parity, pre-pregnancy BMI, fish intake, type of delivery (HC only). Region was included as a random effect. Adjusting for smoking during pregnancy did not substantially change results.	Magnitude: yes (LBW) Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: little change	Maternal GFR was calculated from plasma-creatinine measurements in the 1st trimester. GFR did not confound associations. Increasing PFAS concentrations were associated with lower BW in Sabadell and Valencia, and higher BW in Gipuzkoa. Region-specific PFOS concentrations were not reported. Spearman correlation coefficients for PFOS, PFOA, PFHxS, and PFNA ranged between 0.43 and 0.68. When all PFAS were included in a model, betas for the continuous outcomes were close to the null.
Marks et al., 2019	Former Avon Region in South West England 1991-1992	Prospective cohort	N=457 mother-son pairs enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC).	Selection: unclear; women were selected for the present analysis to maximize data on puberty and dual energy X-ray absorptiometry (DXA) scans. Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 13.8 (11.0-7.7)	Maternal serum at median 30 weeks gestation (IQR 12-33 weeks)	BW Crown to heel length (BL) HC	Medical records for BW Length and HC measured by study staff	<u>BW</u> β = −8.50 (−15.93 - −1.07) <u>BL</u> β = −0.04 (−0.08 - −0.01) <u>HC</u> β = −0.02 (−0.04 - −0.002)	Per unit increase in PFOS	Adjusted for pregnancy weight gain, maternal age, pre-pregnancy BMI, education level, vitamin use, folic acid use, smoking during pregnancy, alcohol use during pregnancy, parity, GA at sample collection. BW analyses also adjusted for maternal age and alcohol use during pregnancy.	Magnitude: no Statistical significance: yes Dose-response: no Temporal association: yes Subgroup only: boys Adjustments: unclear	Authors previously published a study of PFAS and birth size in girls. In a sensitivity analysis including only 1st trimester samples (N=115), associations were consistent with entire study sample. This sample of the ALSPAC cohort was disproportionately white, educated, older, and nonsmoking compared to the overall ALSPAC cohort. PFOA, PFHxS, and PFNA were also analyzed. PFAS concentrations were strongly correlated; PFOA and PFOS (r = 0.63) and PFOS and PFNA (r = 0.60) were most strongly correlated. BMI, parity, and education did not interact with PFAS effects on birth size.

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Meng et al., 2018	Denmark 1996-2002	Prospective cohort	N=3,535 mother and singleton infant pairs from the Danish National Birth Cohort Three samples from sub-studies within the same source population were included.	Selection: unclear Participation: about 60% of invited women accepted Equal groups: unclear Blinded: yes Levels: median (IQR) 30.1 (22.9-39.0)	Maternal plasma in 1st (92%) and 2nd trimester Sample 1 plasma was measured at 3M for only PFOS and PFOA in 2007. Plasma from Samples 2 and 3 was analyzed at Aarhus University in 2011 and 2014, respectively, and included 16 PFAS.	BW LBW	Hospital discharge register	BW β = –45.2 (–76.8 - –13.6) LBW OR = 1.3 (0.9 - 2.0)	Per doubling of PFOS exposure	Adjusted for infant sex, birth year, maternal age, parity, socio-occupational status, pre-pregnancy BMI, smoking during pregnancy, alcohol use during pregnancy, gestational week of blood draw.	Magnitude: yes (LBW) Statistical significance: BW-yes, LBW-no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: little change	6 PFAS were analyzed in two different labs, with results differing by lab, though correlations of PFOS and PFOA concentrations measured in both labs were high (r = 0.94 for PFOS and r = 0.95 for PFOA). Adjustment for other PFAS augmented the association between PFOS and BW, though the associations were not statistically significant. Correlations among PFAS: PFOS and PFOA r = 0.66, PFOS and PFHpS r = 0.89, PFOA and PFHpS r = 0.67. 3 of the other 4 PFAS evaluated were associated with BW.
Minatoya et al., 2017	Hokkaido, Japan 2002-2005	Prospective cohort	N=168 pairs of mothers and their term singletons Sapporo Cohort .	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.1 (3.7-6.7)	Maternal serum, collected between 23 weeks gestation and delivery	BW PI	Birth records	BW All: β = –29 (–289 - 232) Girls: β = –251 (–645 - 143) Boys: β = 190 (–162 - 543) PI β = –2.25 kg/m^3 (– 4.01 - –0.50)	Per log-unit increase in PFOS	Adjusted for BMI, maternal smoking during pregnancy, parity, gestation weeks at blood sampling, infant sex, GA. Analyses were conducted for male and female infants combined and separately.	Magnitude: yes (BW - girls and boys) Statistical significance: yes (PI) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	PFOA and PFOS were modestly correlated, Spearman r = 0.287
Rokoff et al., 2018	Eastern Massa-chusetts 1999-2002	Prospective cohort (Project Viva)	N=1,597 pregnant women recruited at first prenatal visit. Exclusions: multiple gestation, inability to answer in English, gestation >22 weeks at recruitment, plans to move from the area.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: Mean ± SD 29.1± 16.5 ng/ml	Maternal plasma at first prenatal visit (median 9.9 weeks gestation)	Sex-specific BW for GA (BW/GA) *z*-score based on US national reference data	Hospital medical record	β = –0.03 (–0.08 - 0.02) from best-fitting model (multipollutant additive model with no interaction terms)	Per IQR increase in PFOS (16.1 ng/ml)	Adjusted for maternal age, education, race/ethnicity, parity, pre-pregnancy body mass index, and season of birth, date of birth. Smoking and black carbon exposure in 1st trimester were also added in some models. GFR, plasma albumin, and week of gestation did not significantly change estimates.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Authors were interested in interactions of early pregnancy exposures to multiple pollutants and associations with BW/GA. PFOA was not evaluated in this study because authors had found null associations with BW/GA in this cohort. Included women were more likely to be white, but were otherwise similar to excluded women.

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Sagiv et al., 2018	Boston, MA 1999-2002	Prospective cohort	N=1,645 women with singleton live births	Selection: unclear Participation: 78% Equal groups: unclear Blinded: unclear Levels: median (IQR) 25.7 (16.0)	Maternal plasma at median 9 weeks gestation	BW for GA (BW/GA) *z*- score Term BW	Medical records (US national reference for BW/GA and BW for sex *z*-score)	BW/GA *z*-score β = –0.04 (–0.08 - 0.01) Additional adjustments for eGFR and albumin did not substantially change the estimate. Term BW β = −17.9 (−40.9 - 5.1)	Per IQR increase in PFOS	Adjusted for mother's age, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, pre-pregnancy BMI, GA at blood draw, sex, paternal education, household income. Plasma albumin concentration and estimated glomerular filtration rate (eGFR) using plasma creatinine values were also included in some models.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: first adjustments (primarily parity) attenuated estimates, but addition of eGFR and plasma albumin concentration did not	Highly educated, high-income population. PFAS were "moderately correlated;" PFOS and PFOA Spearman correlation was 0.72. An objective of this study was to evaluate whether adjusting for pregnancy hemodynamics affected associations between PFAS and birth outcomes.
Shi et al., 2017	Beijing, China 2012	Cross-sectional	N=170 women who gave birth to singletons without congenital anomalies, recruited from one hospital	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean ± SD, median (IQR) 1.228 ± 0.899, 0.974 (0.626-1.584)	Cord serum	BW BL PI	Medical records	BW β = 160.45 (–11.85 - 332.75) BL β = 0.33 (–0.14 - 0.79) PI β = 0.07 (–0.03 - 0.16)	Per log-unit increase in PFOS	Models included maternal age, pre-pregnancy BMI, parity, GA, gender, and height (BL only). Participants were non-smokers and did not drink alcohol during pregnancy.	Magnitude: yes (BW) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	The sample included 1 LBW infant.
Shoaff et al., 2018	Cincin-nati, OH 2003-2006	Prospective cohort	N=345 mother-child pairs Eligibility criteria: ≥18 years old, recruited at 16 ± 3 weeks gestation, living in Cincinnati area in a home built before 1978, no history of HIV, no medication for seizure or thyroid disorders, singleton pregnancy.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: yes Levels: median (IQR) 14 (9.6-18)	Maternal serum at ~16 weeks gestation if available (86%), or 26 weeks gestation (9%), or within 48 hours of delivery (5%) if neither earlier sample was available.	BW *z*-scores, standard-ized for GA using US reference data	Hospital records	Continuous β = –0.06 (–0.16 - 0.04) By tertiles: 2: β = –0.05 (–0.29 - 0.19) 3: β = –0.12 (–0.36 - 0.13) p trend <0.36 Sex did not modify associations between PFAS and BW *z*-scores.	Change in BW *z*-score per doubling of serum PFOS, or compared to 1st tertile of PFOS concen-tration PFOS tertiles (ng/ml): 1: 0.4-10.8 2: 10.9-16.5 3: 16.6-57.2	Adjusted for maternal age, race, marital status, insurance status, income, education, parity, serum cotinine, depressive symptoms, mid-pregnancy BMI, food security, fruit/vegetable consumption during pregnancy, fish consumption during pregnancy, prenatal vitamin use	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	PFAS were moderately correlated, the highest correlation was for PFOS and PFOA, r = 0.60. Including all measured PFAS (PFOA, PFNA, PFHxS) in the model attenuated the association toward the null.

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Starling et al., 2017	Colorado 2010-2014	Prospective cohort	N=628 participants selected based on availability of maternal serum and cord blood, from women ≥16 years old with singleton pregnancies	Selection: unclear Participation: 11 of 1,410 enrolled dropped out and the 628 included participants did not meaningfully differ from the larger cohort of 1,410. Equal groups: unclear Blinded: yes Levels: median (Q1, Q3) 2.4 (1.5-3.7)	Maternal serum from fasting blood collected at median 27 weeks gestation (range 20-34 weeks)	BW	Weighed by clinical personnel	β = –13.8 (–53.8 - 26.3) No significant interactions between PFAS and sex.	Per ln-unit increase in PFOS	Adjusted for maternal age, pre-pregnancy BMI, race/ethnicity, education level, parity, smoking during pregnancy, gestational weight gain, sex, GA at birth, GA at maternal blood draw	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	PFAS concentrations showed moderate to high correlations; the highest Spearman rank correlation was for PFOA and PFOS, ρ=0.76. PFOS level was lower than among NHANES females (NHANES geometric mean=5.10).
Valvi et al., 2017	Faroe Islands 1997-2000	Prospective cohort	N=604 mother-child pairs recruited at 34 weeks gestation with singleton	Selection: unclear Participation: 92% of 656 recruited mother-child pairs with complete data on key variables were included Equal groups: n/a Blinded: yes Levels: median (IQR) 27.2 (23.1-33.1)	Maternal serum at week 34	BW BL HC	Unclear for BW and HC BL was measured by midwife at 14 days postpartum	<u>BW</u> All children: β = –81 (–173 - 11) Boys: β = ~–150 p <0.05 (presented graphically) <u>BL</u> β = 0.05 (–0.33 - 0.43) Sex was not a modifier of associations with length <u>HC</u> All children: β = 0.00 (–0.28 - 0.27) Girls: β = ~0.5 cm, p <0.05 Boys: β = ~–0.3 cm, p >0.05 (presented graphically)	Per doubling of PFOS	Adjusted for maternal age, education, parity, pre-pregnancy BMI, smoking during pregnancy, and sex. Evaluated, but not included: gestational weight gain, family history of diabetes, vitamin D concentration, docosahexaenoic acid concentration, and GA.	Magnitude: yes (BW) Statistical significance: yes (BW - boys, and HC - girls) Dose-response: no Temporal association: yes Subgroup only: yes (BW and boys, and HC and girls) Adjustments: no change	Authors were interested in GDM as a possible mediator of the association between environmental pollutants and offspring size, but did not find that it modified or mediated the associations with birth size. Among the PFAS, the highest correlation was between PFOS and PFNA, r=0.63. High PFOS levels. Among the 5 PFAS presented, PFOS appeared to have the strongest effects.
Wang et al., 2019	Hebei Province, China 2013	Cross-sectional	N=424 mother-infant pairs, from 924 healthy 20-40-year-old women recruited in first 12 weeks of pregnancy	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: mean ± SD, median (IQR) 1.10 ± 1.34, 0.65 (0.40-1.19)	Cord blood serum	BW BL HC PI	Measured at birth by obstetric nurses	<u>PI</u> β = –0.04 (–0.09 - 0.001) No associations with BW, BL, or HC	Per ln-unit increase in PFOS	Adjusted for maternal age, family income, maternal education, maternal career, husband's smoking, daily energy intake, daily physical activity, GA, parity, pre-pregnancy BMI, GDM, sex, delivery mode, gestational weight gain. Only 0.5% smoked before pregnancy and none smoked during pregnancy.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Authors were also interested in effects of PFAS on estrogen homeostasis and role of estrogen as a mediator between PFAS and birth size. Ln PFOS was positively associated with estrone and estriol. Estriol mediated the association between ln PFOS and reduced BW.

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Wikstrom et al., 2019	Sweden 2007-2010	Prospective cohort	N=1,533 mother and singleton infant pairs with complete data from the SELMA (Swedish Environ-mental, Longitudinal, Mother and child, Asthma and allergy) pregnancy cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 5.38 (3.97-7.60)	Maternal serum (measured at median 10 weeks gestation)	BW Sex- and GA-specific BW standard deviation score (BW-SDS) SGA	Swedish Medical Birth Register	<u>BW</u> All children β = –46 (–88 - –3) Girls β = –85 (–145 - –25) Boys β = –13 (–73 - 47) <u>SGA</u> All children OR = 1.19 (0.87 - 0.64) Girls OR = 1.40 (0.83 - 2.35) Boys OR = 1.08 (0.72 - 1.63) <u>BW-SDS</u> All children β = –0.100 (–0.197 - –0.004) Girls β = –0.167 (–0.301 - –0.034) Boys β = –0.027 (–0.166 - 0.112) p-interaction for sex and BW was not significant (p=0.06)	Per ln-unit increase in PFOS (correspond-ing to an increase from 25th to 75th percentile of exposure)	Adjusted for parity, maternal weight, cotinine concentration, sex (unless stratified by sex), GA (BW only). Maternal age, education level, and week of sampling were not confounders.	Magnitude: yes (BW - girls, SGA - girls) Statistical significance: yes (BW and BW-SDS overall, and for girls) Dose-response: yes (BW and BW-SDS for girls: larger change with higher exposure quartile) Temporal association: yes Subgroup only: yes - girls Adjustments: unclear	Restricting analyses to serum samples collected in the first trimester did not change regression coefficients. Correlations among PFAS not reported. BW was inversely associated with PFOS, PFNA, PFDA, and PFUnDA. The finding of significance for girls but not boys was also consistent with the same PFAS. GA did not mediate associations between PFOS and BW.
Woods et al., 2017	9 counties in Cincinnati, OH area. 2003-2006	Prospective cohort	N=272 pregnant women enrolled in the Health Outcomes and Measures of Environment (HOME) Study	Selection: women >18 years old were recruited from prenatal clinics at 13 to <19 weeks pregnancy. Women who were free of specified health conditions and living in residences built before 1978 to select for lead exposure were included. Black women were oversampled. Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) 14.4 (10-17.9)	Maternal serum at ~16 and ~26 weeks gestation (log_{10} transformed and averaged if multiple measure-ments were available)	BW	Birth records	PFOS was associated with a small (~10 g) decrease in BW but the confidence interval was wide and included 0 (data presented graphically; statistics were not reported).	Per log-unit increase in PFOS (10-fold increase)	Adjusted for maternal age, race, marital status, employment status, income, insurance, education level, tobacco use, gender, BMI, 56 other chemicals from 5 classes of potential endocrine disrupting chemicals, organochlorine pesticides, and heavy metals	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	All PFAS compounds were associated with small, non-statistically significant decreases in BW. PFOA and PFOS were dropped from the LASSO (least absolute shrinkage and selection operator) and elastic net regression models.
Workman et al., 2019	Winnipeg, Manitoba, Canada 2010-2011	Prospective cohort (subset of Canadian Healthy Infant longitudinal Develop-ment Study cohort)	N=414 mother-infant pairs with prenatal samples from the Canadian Healthy Infant Longitudinal Developmen t Study Cohort	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (range) in prenatal plasma 2.2 (0.18-21)	Maternal prenatal plasma collected at 16 and 26 weeks gestation	BW BL HC PI	Not reported	No significant associations between PFOS and size parameters (statistics not reported). Result for PI was not reported.	Regression coefficients were not reported	Adjusted for maternal age, smoking during pregnancy, hypertension during pregnancy, diabetes during pregnancy, parity, sex, GA, method of delivery (for HC)	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	

Author year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Xu et al., 2019	Hangzhou, China 2016-2017	Cross-sectional	N=98 mother-infant pairs Women with serious illnesses and clinical symptoms, multiple births, infants with congenital diseases were excluded.	Selection: unclear Participation: unclear Equal groups: n/a Blinded: unclear Levels: median (IQR), mean ± SD 4.07 (2.86-8.05), 5.69 ± 4.19	Umbilical cord serum	BW LBW SGA HC PI	Hospital birth records	<u>BW</u> β = –417.3 (–742.1 - –92.4) <u>PI</u> β = –0.005 (–0.008 - –0.002) <u>BL</u> β = 1.101 (–0.042 - 2.159) <u>SGA</u> OR = 4.138 (1.072 - 15.977) <u>HC</u> No association Because there were only 3 LBW infants, authors did not analyze LBW.	Per log-unit increase in PFOS	Adjusted for maternal age, GA (not in SGA analyses), abortion history, parity, pre-pregnancy BMI, pregnancy weight gain, education, job type, infant gender, tap vs. filtered drinking water.	Magnitude: yes (BW, SGA) Statistical significance: yes (BW, PI, SGA) Dose-response: no Temporal association: no Subgroup only: no Adjustments: large changes with adjustment for BW (unadjusted β = –334.1 (–636.5 - –31.6)) and SGA (unadjusted OR = 1.642 (0.699 - 3.856))	PFOS associated with tap (not filtered) water consumption, overweight (pre-pregnancy BMI>22.5). Correlation between PFOS and PFOA, r=0.35. Correlations between PFOS and other PFAS, |r| ≤ 0.60

Studies are in alphabetical order by first author and year
For BW, a change of >50 g was considered "large magnitude"
Numbers in parentheses are 95% confidence intervals unless otherwise noted
PFOS concentrations are in ng/ml unless otherwise noted
Abbreviations and units: β, regression coefficient; BL, birth (body) length (cm unless otherwise noted); BMI, body mass index (kg/m^2); BW, birth weight (grams unless otherwise notes); BW-SDS, sex- and gestational age-specific birth weight standard deviation score; CI, confidence interval; cm, centimeter; CrI, credible interval; *p,p'*-DDE, dichlorodiphenyldichloroethylene; g, gram; GA, gestational age; GDM, gestational diabetes mellitus; GFR, glomerular filtration rate; GSTM 1, glutathione S-transferase M1; HC, head circumference (cm unless otherwise noted); IQR, interquartile range; kg, kilogram; LBW, low birth weight (birth weight <2,500 g); ln, natural logarithm; LOD, limit of detection; m, meter; N, number of participants; ng/ml, nanograms/milliliter; NHANES, US National Health and Nutrition Examination Survey; OR, odds ratio; PCB-153, polychlorinated biphenyls; PFAS, per-and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFUnDA, perfluoroundecanoic acid PI, ponderal index (calculated as [1000*BW/BL] g/cm^3 or [100*BW/BL] g/cm^3 unless otherwise noted); PIH, pregnancy-induced hypertension; PTB, preterm birth; r, correlation coefficient; SD, standard deviation; SES, socioeconomic status; SGA, small for gestational age (<10th percentile for age and sex unless otherwise noted)

Table A7.17. Recent epidemiologic studies on PFOA and pubertal development

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Ernst et al., 2019	Denmark 2000-2003	Retrospective cohort	N=1,167 children from the Danish National Birth Cohort (DNBC) Sample 1: N=722 from the Puberty Cohort Sample 2: N=445 children from another study within the DNBC	Selection: unclear Participation: 66% of invited children from the Puberty Cohort (nested within the DNBC) responded. Children from the larger cohort who had responded to the 11-year questionnaire were also included, which increased the participation to 71% of the Puberty Cohort. Equal groups: unclear Blinded: yes Levels: Median (10^{th}, 90^{th} percentile) ng/ml Sample 1 Boys 5.1 (2.8, 8.3) Girls 4.8 (2.7, 8.2) Sample 2 Boys 4.3 (2.2, 6.7) Girls 4.1 (2.3, 6.4)	First trimester maternal plasma (median 9 gestational weeks, IQR 8-11 weeks).	Age at menarche, voice break, first ejaculation, and Tanner stages 2-5 for pubic hair, breast, genital develop-ment, and a combined puberty indicator	Self-assessment through web-based question-naires administered biannually from age 11 years	No consistent pattern of associations between PFOA concentrations and markers of male or female pubertal development, or the combined puberty indicator.	Change in onset of puberty indicators compared to lowest exposure tertile	Adjusted for: highest social class of parents, maternal age at menarche, maternal age at delivery,parity, pre-pregnancy BMI, and smoking during first trimester	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Sample 1 and Sample 2 were analyzed by different labs, with some overlap. Correlations between lab measurements were 0.95 for PFOA and 0.92 for PFOS.
Di Nisio et al., 2020	Veneto Region, Italy 2018-2019	Retrospective cohort with ecologic exposure	N=1,226 female high school students (mean age 18.1 years) who were lifelong residents of the area. 146 were considered "exposed" and 1,080 were "controls" based on residence.	Selection: unclear Participation: 88.5% in the "exposed" group and 90.0% in the "control" group Equal groups: yes Blinded: unclear Levels: median (IQR) ng/ml, based on a subset of 68 "exposed" and 56 "control" participants "exposed" 28.71 (12.36-46.21) "control" 2.59 (1.81-4.75)	Residence as a proxy for exposure	Age at menarche	Question-naire	Mean age at menarche was 164 days later in the exposed group (12.72 vs. 12.27 years), p <0.001	"Exposed" vs. "control" area of residence	Age at survey, BMI	Magnitude: yes Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Authors express interest in in utero exposure, but do not mention any effort to determine whether participants' mothers lived in the area while pregnant. Thus exposure may have represented postnatal exposure more than in utero exposure. Authors report that previous research showed concordance between geographic selection criteria and PFAS exposure patterns, and consistent exposure in all subjects from the area.

Numbers in parentheses are 95% confidence intervals unless otherwise noted
Abbreviations and units: β, regression coefficient; BMI, body mass index; CI, confidence interval; DNBC, Danish National Birth Cohort; IQR, interquartile range; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFAS, per-and polyfluoroalkyl substances

Table A7.18. Recent epidemiologic studies on PFOS and pubertal development

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Ernst et al., 2019	Denmark 2000-2003	Retrospective cohort	N=1,167 children from the Danish National Birth Cohort (DNBC) Sample 1: N=722 from the Puberty Cohort Sample 2: N=445 children from another study within the DNBC	Selection: unclear Participation: 66% of invited children from the Puberty Cohort (nested within the DNBC) responded. Children from the larger cohort who had responded to the 11-year questionnaire were also included, which increased the participation to 71% of the Puberty Cohort. Equal groups: unclear Blinded: yes Levels: Median (10^{th}, 90^{th} percentile) ng/ml Sample 1 Boys 31.9 (19.2, 51.2) Girls 32.3 (19.3, 50.8) Sample 2 Boys 27.2 (16.7, 45.2) Girls 27.9 (16.5, 42.2)	First trimester maternal plasma (median 9 gestational weeks, IQR 8-11 weeks).	Age at menarche, voice break, first ejaculation, and Tanner stages 2-5 for pubic hair, breast, genital develop-ment, and a combined puberty indicator	Self-assessment through web-based question-naires	In girls, the middle tertile of PFOS exposure was associated with earlier onset for all individual outcomes except acne; CIs excluded null for Tanner breast stages 2-4. The middle tertile was also associated with earlier puberty based on the combined puberty indicator, β=−3.73 (−6.59-−0.87) months. The highest tertile of PFOS exposure had weaker associations and CIs included the null for all Tanner breast stages, and were mostly null or nonsignificantly associated with later onset for other indicators. In boys, mean ages at onset of Tanner stages of genital development and voice break were earlier with similar differences across the middle and highest tertiles, but only Tanner genital stage 2 for the middle tertile was statistically significant. Tanner pubic hair, axillary hair, acne, and first ejaculation were similar across exposure tertiles.	Change in onset of puberty indicators compared to lowest exposure tertile Middle exposure tertiles, ng/ml: Sample 1: 28.1-38.4 Sample 2: 23.3-31.5	Adjusted for: highest social class of parents, maternal age at menarche, maternal age at delivery, parity, pre-pregnancy BMI, and smoking during first trimester	Girls Magnitude: no Statistical significance: yes (Tanner breast stages 2-4 only) Dose-response: non-monotonic Temporal association: yes Subgroup only: no Adjustments: unclear Boys Magnitude: no Statistical significance: no (except Tanner genital stage 2) Dose-response: no Temporal association: yes Subgroup only: no Adjustments: unclear	Sample 1 and Sample 2 were analyzed by different labs, with some overlap. Correlations between lab measurements were 0.95 for PFOA and 0.92 for PFOS.

Numbers in parentheses are 95% confidence intervals unless otherwise noted
PFOS concentrations are in ng/ml unless otherwise noted
Abbreviations and units: β, regression coefficient; BMI, body mass index; CI, confidence interval; DNBC, Danish National Birth Cohort; IQR, interquartile range; N, number of participants; ng/ml, nanograms/milliliter

Table A7.19. Recent epidemiologic studies on PFOA and fertility and fecundability

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Bach et al., 2018	Denmark 1996-2002	Cross-sectional	N=1,251 pregnant women 613 parous women, 638 nulliparous Sample of women from the Lifestyle During Pregnancy Study nested in the Danish National Birth Cohort Women had a planned pregnancy, a live birth and information on time to pregnancy (TTP)	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Nulliparous women 5.03 (4.02 - 6.13) Parous women 3.43 (2.60 - 4.53)	Plasma sampled in early pregnancy, median (IQR) 8 (7-10) weeks gestation	Fecunda-bility (probability of pregnancy per cycle), indicated by TTP	Woman's information on TTP	For nulliparous women, PFOA was not associated with fecundability (Model A*). For parous women, Model A fecundability ratio (FR) = 0.63 (0.47-0.86) Model B FR = 0.79 (0.75 - 0.83) Model C FR = 0.85 (0.62 - 1.16) * see Confounding column for adjustments in the different models	Fecundability in highest quartile of plasma PFOA compared to lowest quartile Exposure quartiles: Nulliparous women 1: 1.21-4.02 2: 4.03-5.03 3: 5.0-6.13 4: 6.14-13.80 Parous women 1: 0.61-2.60 2: 2.61-3.43 3: 3.44-4.53 4: 4.54-15.00	Model A adjustments: age, SES; pre-pregnancy BMI Parous women: Model B adjustments: Model A adjustments plus interpreg-nancy interval Model C: Model A adjustments using PFOA quartiles corrected to median interpreg-nancy interval (2.6 years)	Parous women: Magnitude: yes Statistical significance: yes Dose-response: no Temporal association: no Subgroup only: yes Adjustments: substantial changes; see Models B and C vs. Model A results	Most women who had a second pregnancy became pregnant again within 5 years with a peak at approximately 2.5 years. Spearman's correlation between PFOA levels and the interpregnancy interval, r=0.32 PFOA concentrations were higher in women with longer interpregnancy intervals. Used directed acyclic graphs and sensitivity analyses to evaluate unmeasured confounding in parous women. Censoring at 6 months instead of 12 months resulted in similar associations for nulliparous women and slightly attenuated associations for parous women. Authors concluded that associations observed "in parous women may be biased by confounders related to previous pregnancies and exposure measurement error."
Crawford et al., 2017	North Carolina 2008-2009	Prospective cohort	N=99 women attempting to conceive for three months or less, ages 30-44 years, and English-speaking. Women with history of infertility, polycystic ovarian disease, pelvic inflammatory disease, endometriosis, pelvic radiation, or with a partner with a history of infertility were excluded.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (95% CI) 2.79 (2.48 - 3.16)	Serum from initial study visit	Fecunda-bility using cycle-specific and day-specific models Anti-mullerian hormone (AMH) as an indicator of ovarian reserve	Blood samples and daily diaries Pregnancy was defined by a positive home pregnancy test	PFOA was not associated with fecundability or ovarian reserve. FR Cycle-specific FR = 1.15 (0.66 - 2.01) Day-specific FR = 0.96 (0.31 - 1.94) AMH β = –0.56 (p=0.75)	Highest quartile compared to three lower quartiles of exposure (quartile cut points were not reported)	Adjusted for maternal age (dichotomized at 35 years) and mean cycle length (cycle-specific models only)	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Women in the highest combined exposure group (sum of PFOA, PFOS, PFNA, and PFHxS levels) "were more likely to have longer mean cycle lengths (30.7 versus 28.7 days, p=0.02) and less likely to achieve pregnancy at study end (54% versus 75%, p=0.04) as compared to women with lower PFC exposures." Comparing highest quartile with lowest quartile may have yielded different results. Authors found no significant differences in fecundability by parity. Correlations: PFOA and PFOS, r = 0.37 PFOA and other PFAS, r ≤ 0.55 No correlation between PFOA and thyroid stimulating hormone or AMH. PFOA was positively correlated with triiodothyronine (r = 0.23, p = 003). Authors hypothesized that greater exposure to PFAS could be associated with thyroid dysfunction and decreased ovarian reserve, which would result in lower fecundability.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
McCoy et al. 2017	South Carolina 2013-2014	Prospective cohort	N=34 women undergoing in vitro fertilization (IVF) at a fertility clinic, from whom follicular fluid and stimulatory phase plasma samples were collected. No exclusions were applied.	Selection: unclear Participation: of 50 women enrolled, baseline and stimulatory phase and follicular fluid samples were collected from only 26 women. Baseline and stimulatory plasma samples were collected from 8 additional women. No explanation for the absence of information on the remaining women. Equal groups: unclear Blinded: unclear Levels: mean (± SEM) plasma 2.44 (± 0.30) ng/g; follicular fluid 1.94 (± 0.20) ng/g	Plasma at baseline (prior to gonadotropin stimulation phase) Follicular fluid collected at oocyte retrieval	Ovarian function Ovarian response to administration of human chorionic gonadotropin Fertilization	Correlation between plasma and follicular fluid and the following measures: estradiol, follicle count, change in peak 17β-estradiol, change in follicle number, number of oocytes retrieved, fertilization rate, blastocyst conversion (see Notes)	No statistically significant association between plasma or follicular fluid PFOA and any outcomes	Correlations and partial correlations	Corrected for age	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: unclear	Correlations among PFAS: <u>Follicular fluid</u> PFOA and PFOS r=0.524 All: range, r = 0.276 - 0.850 <u>Plasma</u> PFOA and PFOS, r=0.540 All: range, r = 0.173 - 0.814 PFOA levels in follicular fluid were similar to those in plasma and highly correlated (r = 0.96) Blastocyst conversion, an important measure of IVF cycle quality, was defined as the number of blastocysts generated by day 6 of development divided by the number of embryos cultured past day 3 of development.
Wang et al., 2017	China 2014-2015	Case-cohort	Cases N=157 women diagnosed with endometriosis-related infertility. Controls N=178 women with no reproductive endocrine disorders seeking infertility treatment because of male reproductive dysfunction	Selection: unclear Participation: unclear Equal groups: cases were more educated than controls; household income data were missing for 51.6% of cases and 75.3% of controls. Blinded: unclear Levels: median (IQR) cases 14.67 (7.32 - 23.73) controls 12.09 (7.33 - 22.59)	Plasma	Endometriosis-related infertility Infertility was defined as having unprotected intercourse for 12 months without conceiving spontaneously	Diagnosed at infertility clinic	PFOA was not associated with endometriosis-related infertility	Compared to lowest tertile Exposure tertiles: 1: 1.52-8.74 2: >8.74-19.6 3: >19.6-72.1	Adjusted for age, BMI, household income, education No adjustment for alcohol and tobacco use because the vast majority of women were non-smokers and non-drinkers.	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: 3rd tertile unadjusted OR = 1.36 (0.80–2.30), adjusted OR = 1.05 (0.58–1.91)	Imputed values were used for missing household income and education. Sensitivity analyses including only nulliparous women and only women without relevant gynecologic disorders as the reference group did not materially change results. Controls did not undergo laparoscopic examination, so some might have had asymptomatic endometriosis. PFBS was associated with endometriosis-related infertility. Correlations among PFAS: PFOA and PFOS, r = 0.31 PFOA and PFBS, r = 0.30 PFOA and other PFAS, range, r=0.21 - 0.50

Studies are in alphabetical order by first author and year
Numbers in parentheses are 95% confidence intervals unless otherwise noted
PFOA concentrations are in ng/ml unless otherwise noted
FRs greater than 1.2 or less than 0.83 were considered large magnitude and labeled "yes" for magnitude in the table.
Abbreviations and units: AMH, Anti-mullerian hormone; β, regression coefficient; BMI, body mass index (kg/m^2); CI, confidence interval; FR, fecundability ratio; IQR, interquartile range; IVF, in vitro fertilization; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFBS, perfluorobutanesulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; r, correlation coefficient; SEM, standard error of the mean; SES, socioeconomic status; TTP, time to pregnancy

Table A7.20. Recent epidemiologic studies on PFOS and fertility and fecundability

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Bach et al., 2018	Denmark 1996-2002	Cross-sectional	N=1,251 pregnant women 613 parous women, 638 nulliparous Sample of women from the Lifestyle During Pregnancy Study nested in the Danish National Birth Cohort. Women had a planned pregnancy and information on time to pregnancy (TTP).	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: median (IQR) Nulliparous women 30.2 (23.4-38.0) Parous women 26.0 (20.7-33.6)	Plasma sampled in early pregnancy, median (IQR) 8 (7-10) weeks gestation	Fecunda-bility, indicated by TTP	Woman's information on TTP	For nulliparous women, PFOS was not associated with fecundability (Model A). For parous women, Model A fecundability ratio (FR) = 0.60 (0.44 - 0.82) Model B FR = 0.69 (0.66 - 0.73) Model C FR= 0.84 (0.61 - 1.15) * see Confounding column for adjustments in the different models	Fecundability in highest quartile of plasma PFOS compared to lowest quartile Exposure quartiles: Nulliparous women 1: 6.7-23.4 2: 23.5-30.2 3: 30.3-38.0 4: 38.1-117.0 Parous women 1: 6.3-20.7 2: 20.8-26.0 3: 26.1-33.6 4: 33.7-127.0	Model A adjustments: age, SES; pre-pregnancy BMI Parous women: Model B adjustments: Model A adjustments plus interpreg-nancy interval Model C: Model A adjustments using PFOS quartiles corrected to the median interpregnancy interval (2.6 years)	Parous women: Magnitude: yes Statistical significance: yes Dose-response: unclear Temporal association: no Subgroup only: yes Adjustments: substantial change; see Model C vs. Model A results	Most women who had a second pregnancy became pregnant again within 5 years with a peak at approximately 2.5 years. Spearman's correlation between PFOS levels and the interpregnancy interval r=0.13. PFOS concentrations were higher in women with longer interpregnancy intervals. Used directed acyclic graphs (DAGs) and sensitivity analyses to evaluate unmeasured confounding in parous women. Authors concluded that associations observed "in parous women may be biased by confounders related to previous pregnancies and exposure measurement error." Censoring at 6 months instead of 12 months resulted in similar associations for nulliparous women and slightly attenuated associations for parous women.
Crawford et al., 2017	North Carolina 2008-2009	Prospective cohort	N=99 women attempting to conceive for three months or less, ages 30-44 years, and English-speaking. Women with history of infertility, polycystic ovarian disease, pelvic inflammatory disease, endometriosis, pelvic radiation, or with a partner with a history of infertility were excluded.	Selection: unclear Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean (95% CI) 9.29 (8.31 - 10.38)	Serum sampled at initial study visit	Fecund-ability using a cycle-specific and day-specific model Anti-mullerian hormone (AMH) as an indicator of ovarian reserve	Blood samples and daily diaries Pregnancy was defined by a positive home pregnancy test	PFOS was not associated with fecundability or ovarian reserve. FR Cycle-specific FR = 0.89 (0.49 - 1.60) Day-specific FR = 0.99 (0.28 - 2.32) AMH β = 0.07 (p = 0.73) .	Highest quartile compared to three lower quartiles of exposure (quartile cut points were not reported)	Adjusted for maternal age (dichotomized at 35 years), and mean cycle length (cycle-specific models only)	Magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: no	Women in the highest combined exposure group (sum of PFOA, PFOS, PFNA, and PFHxS levels) "were more likely to have longer mean cycle lengths (30.7 versus 28.7 days, p=0.02) and less likely to achieve pregnancy at study end (54% versus 75%, p=0.04) as compared to women with lower PFC exposures." Comparing highest quartile with lowest quartile may have yielded different results. Correlations: PFOA and PFOS, r = 0.37 PFOS and other PFAS, r ≤0.36 PFOS and TSH, r = 0.07 PFOS and AMH, r = -0.01 Authors found no significant differences in fecundability by parity.

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
McCoy et al. 2017	South Carolina 2013-2014	Prospective cohort	N=34 women undergoing IVF at a fertility clinic, from whom follicular fluid and stimulatory phase plasma samples were collected. No exclusions were applied.	Selection: unclear Participation: of 50 women enrolled, baseline and stimulatory phase and follicular fluid samples were collected from only 26 women. Baseline and stimulatory plasma samples were collected from 8 additional women. No explanation for the absence of information on the remaining women. Equal groups: unclear Blinded: unclear Levels: mean (± SEM) plasma 6.52 (± 0.50) ng/g; follicular fluid 5.33 (± 0.42) ng/g	Plasma at baseline (prior to gonadotropin stimulation phase) Follicular fluid collected at oocyte retrieval	Ovarian function Ovarian response to admini-stration of human chorionic gonado-tropin Fertilization	Correlation between plasma and follicular fluid and the following measures: estradiol, follicle count, change in peak 17β-estradiol, change in follicle number, number of oocytes retrieved, fertilization rate, blastocyst conversion (see Notes)	In plasma PFOS was negatively correlated with plasma estradiol, r = –0.47 pg/ml ($p < 0.05$) No other statistically significant associations with outcomes	Correlations and partial correlations	Corrected for age	Magnitude: yes (estradiol) Statistical significance: yes (estradiol) Dose response: unclear Temporal association: yes Subgroup only: no Adjustments: unclear	Correlations among PFAS: <u>Follicular fluid</u> PFOA and PFOS, r = 0.524 All: range, r = 0.276 - 0.850 <u>Plasma</u> PFOA and PFOS, r = 0.540 All: range, r = 0.173 - 0.814 PFOS levels in follicular fluid were similar to those in plasma and correlated (r = 0.81) Blastocyst conversion, an important measure of IVF cycle quality, was defined as the number of blastocysts generated by day 6 of development divided by the number of embryos cultured past day 3 of development.
Wang et al., 2017	China 2014-2015	Case-control	Cases N=157 women diagnosed with endometriosis-related infertility. Controls N=178 women without reproductive endocrine disorders seeking infertility treatment because of male reproductive dysfunction	Selection: unclear Participation: unclear Equal groups: cases were more educated than controls; household income data were missing for 51.6% of cases and 75.3% of controls. Blinded: unclear Levels: median (IQR) cases 6.40 (4.02 - 11.42) controls 6.60 (3.92 - 13.54)	Plasma	Endome-triosis-related infertility	Diagnosed at infertility clinic	ORs by exposure tertile: <u>Entire sample</u> 2: OR = 1.11 (0.61 - 1.99) 3: OR = 0.66 (0.36 - 1.21) <u>Sensitivity analyses</u> Women without pregnancy history: 2: OR = 1.00 (0.45 - 2.21) 3: OR = 0.62 (0.26 - 1.46) Women without other gynecologic pathology: 2: OR = 0.87 (0.44 - 1.73) 3: OR = 0.47 (0.22 - 0.99)	Compared to lowest tertile Exposure tertiles: 1: 0.54-4.70 2: >4.70-9.36 3: >9.36-138	Adjusted for age, BMI, household income, education	Magnitude: yes Statistical significance: no Dose-response: yes (women without other gynecologic pathology) Temporal association: no Subgroup only: no Adjustments: unadjusted entire sample, 3rd tertile OR = 0.88 (0.52 - 1.50)	Imputed values were used for missing household income and education. When analyses did not use imputed values for missing data, associations were attenuated. Controls did not undergo laparoscopic examination, so some might have had asymptomatic endometriosis. PFBS was associated with endome-triosis-related infertility Correlations among PFAS: PFOS and PFOA, r = 0.31 PFOS and PFBS, r = 0.13 PFOS and other PFAS range, r=0.17 - 0.81

Studies are in alphabetical order by first author and year
Numbers in parentheses are 95% confidence intervals unless otherwise noted
PFOS concentrations are in ng/ml unless otherwise noted
FRs greater than 1.2 or less than 0.83 were considered large magnitude and labeled "yes" for magnitude in the table.
Abbreviations and units: AMH, Anti-mullerian hormone; β, regression coefficient; BMI, body mass index (kg/m^2); CI, confidence interval; FR, fecundability ratio; IQR, interquartile range; IVF, in vitro fertilization; ln, natural logarithm; N, number of participants; ng/ml, nanograms/milliliter; OR, odds ratio; PFBS, perfluorobutanesulfonic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; pg/ml, picograms/ml; r, correlation coefficient; SEM, standard error of the mean; SES, socioeconomic status; TTP, time to pregnancy

Cancer

Literature search and methods

In addition to reviewing the results of previous reviews by the US EPA (US EPA, 2016b; 2016d), ATSDR (2018a), and others, OEHHA reviewed the epidemiologic literature on PFOA and PFOS and cancer, with a focus on cancer of the kidney, testis, bladder, breast, prostate, and pancreas. All cancer types were considered but these types were selected for greater focus because they were the cancers of concern noted in previous reviews and because at least some positive associations were seen for these types in OEHHA's preliminary review of the literature. This literature search included relevant review articles and documents (Chang et al., 2014; IARC, 2018a; ATSDR 2018a; US EPA 2016b and d), and the bibliographies of all study publications meeting OEHHA's inclusion and exclusion criteria. PubMed and Embase searches were also performed for articles published since these reviews (i.e., those published from December 2016 to September 20, 2020). The outcome portion of the search string used is shown below and was derived from the NTP's Draft Handbook for Preparing Report on Carcinogens Monographs (NTP 2016b).

("angiogenesis inducing agents"[mh] OR "myelodysplastic-myeloproliferative diseases"[mh] OR "neoplasms"[mh] OR "carcinogenicity tests"[mh] OR "carcinogens"[mh] OR (sentinel-lymph-node[tiab] NOT biopsy[tiab]) OR (ASCO[tiab] NOT fungi[tiab]) OR (WAGR[tiab] AND syndrome[tiab]) OR 5q-syndrome[tiab] OR leukostasis[tiab] OR acanthoma[tiab] OR acanthomas[tiab] OR acrochordon[tiab] OR acrochordons[tiab] OR acrospiroma[tiab] OR acrospiromas[tiab] OR adamantinoma[tiab] OR adamantinomas[tiab] OR adenoacanthoma[tiab] OR adenoacanthomas[tiab] OR adenoameloblastoma[tiab] OR adenoameloblastomas[tiab] OR adenocanthoma[tiab] OR adenocanthomas[tiab] OR adenocarcinoma[tiab] OR adenocarcinomas[tiab] OR adenofibroma[tiab] OR adenofibromas[tiab] OR adenolipoma[tiab] OR adenolipomas[tiab] OR adenolymphoma[tiab] OR adenolymphomas[tiab] OR adenoma[tiab] OR adenomas[tiab] OR adenomatosis[tiab] OR adenomatous[tiab] OR adenomyoepithelioma[tiab] OR adenomyoepitheliomas[tiab] OR adenomyoma[tiab] OR adenomyomas[tiab] OR adenosarcoma[tiab] OR adenosarcomas[tiab] OR adenosis[tiab] OR aesthesioneuroblastoma[tiab] OR aesthesioneuroblastomas[tiab] OR ameloblastoma[tiab] OR ameloblastomas[tiab] OR amyloidoses[tiab] OR amyloidosis[tiab] OR anaplasia[tiab] OR androblastoma[tiab] OR androblastomas[tiab] OR angioblastoma[tiab] OR angioblastomas[tiab] OR angioendothelioma[tiab] OR angioendotheliomas[tiab] OR angioendotheliomatosis[tiab] OR angiofibroma[tiab] OR angiofibromas[tiab] OR angiofibrosarcoma[tiab] OR angiokeratoma[tiab] OR angiokeratomas[tiab] OR angioleiomyoma[tiab] OR angioleiomyomas[tiab] OR angiolipoma[tiab] OR angiolipomas[tiab] OR angioma[tiab] OR angiomas[tiab] OR angiomatosis[tiab] OR angiomyolipoma[tiab] OR angiomyolipomas[tiab] OR angiomyoma[tiab] OR angiomyomas[tiab] OR angiomyxoma[tiab] OR angiomyxomas[tiab] OR angioreticuloma[tiab] OR angioreticulomas[tiab] OR angiosarcoma[tiab] OR angiosarcomas[tiab] OR apudoma[tiab] OR apudomas[tiab] OR argentaffinoma[tiab] OR argentaffinomas[tiab] OR arrhenoblastoma[tiab] OR arrhenoblastomas[tiab] OR astroblastoma[tiab] OR astroblastomas[tiab] OR astrocytoma[tiab] OR astrocytomas[tiab] OR astroglioma[tiab] OR astrogliomas[tiab] OR baltoma[tiab] OR basiloma[tiab] OR basilomas[tiab] OR blastoma[tiab] OR blastomas[tiab] OR Buschke-Lowenstein[tiab] OR cancer[tiab] OR cancerous[tiab] OR cancers[tiab] OR carcinogen[tiab] OR carcinogenesis[tiab] OR carcinogenic[tiab] OR carcinogenicity-test*[tiab] OR carcinogens[tiab] OR carcinoid[tiab] OR carcinoma[tiab] OR carcinomas[tiab] OR carcinomatosis[tiab] OR carcinosarcoma[tiab] OR carcinosarcomas[tiab] OR cavernoma[tiab] OR cavernomas[tiab] OR cementoma[tiab] OR cementomas[tiab] OR ceruminoma[tiab] OR ceruminomas[tiab] OR chemodectoma[tiab] OR chemodectomas[tiab] OR cherubism[tiab] OR chloroma[tiab] OR chloromas[tiab] OR cholangiocarcinoma[tiab] OR cholangiocarcinomas[tiab] OR cholangiohepatoma[tiab] OR cholangioma[tiab] OR cholangiomas[tiab] OR cholangiosarcoma[tiab] OR cholesteatoma[tiab] OR

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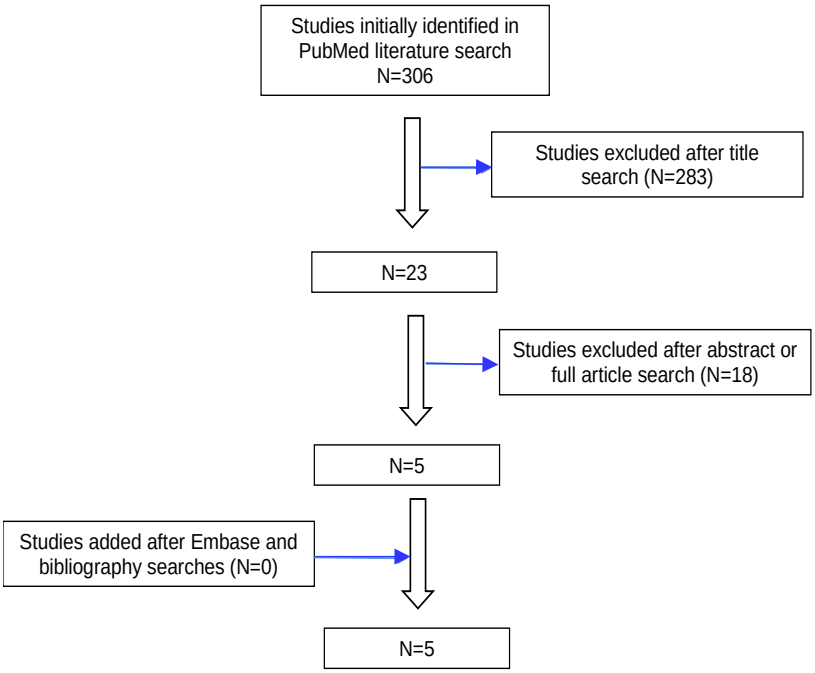
A number of studies of PFOA and PFOS and cancer were cross-sectional, that is, serum PFOA or PFOS levels were measured at or near the time of cancer diagnosis. If the latency period between exposure and cancer development and diagnosis is fairly short, PFOA or PFOS levels measured during these times could potentially provide an accurate metric for assessing the relevant exposure periods since the half-life of PFOA and PFOS in human serum is several years (Olsen et al., 2007). Currently however, the latency period for PFOA or PFOS associated cancer is unknown. Overall, although the results of some cross-sectional studies are discussed in this review, OEHHA excluded cross-sectional studies of cancer from its main analyses given the long latency usually associated with environmentally caused cancer, and the possibility that

cancer diagnosis or treatment could lead to medication use or a change in behaviors that could change PFOA or PFOS exposure levels. The study by Consonni et al. (2013) was reviewed but was excluded from OEHHA's main analyses because of the significant co-exposures to tetrafluoroethylene (TFE), which has been linked to kidney cancer, liver cancer, testicular cancer, and leukemia in rodents (IARC, 2018b). OEHHA included studies of cancer mortality and incidence, with the understanding that mortality studies may be less informative for cancer types with low mortality rates. Studies of cancer mechanisms or biomarkers (e.g., DNA methylation, prostate specific antigen) were not included in this section. In several instances there were multiple publications involving the same study population. When this occurred, a greater emphasis was usually placed on results from the most recent update, although all results were considered.

Results

A general description of the literature search is provided in Figure 7.6. A list of studies excluded after abstract and title search is provided in Table A7.28. Detailed reviews of the studies meeting OEHHA's inclusion criteria are shown in Tables A7.21-27.

Figure A7.6. Literature search: recent epidemiologic studies of PFOA or PFOS and cancer*



*This figure is provided to document OEHHA's PubMed, Embase, and bibliography literature searches. It does not include relevant publications identified from other sources such as previously published reviews from other agencies or other authors.

Table A7.21. Epidemiologic studies of PFOA and bladder cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012 Steen-land et al., 2015	Where: DuPont, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years (mortality)	Who: worked at least 1 day from 1948-2002 Ages: adults N: 5,791 (mortality), 3,713 (incidence)	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum 0.113 µg/ml (incidence) and 0.35 µg/ml (mortality)	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Bladder incidence and mortality	Incidence: self-reports Mortality: NDI, death certificates	Incidence: RR = 0.23 (0.05-0.93) N=29 cases Similar results with 10-year lag Mortality: SMR = 0.36 (0.10-2.01) for the 4th quartile (≥2,700 ppm-years) N=1 case in the highest category Note: SMR = 2.49 (0.97-5.78) in 2nd vs. 1st quartile N=6 cases, 904-<1,520 ppm-years)	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies Potential weaknesses: TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Bladder incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	Incidence: HRs of 1.00 (ref), 0.81, 0.78, 1.50, and 1.66 by exposure quartiles HR = 1.66 (0.86-3.18) in the highest quartile (>7.9 x 10^{-4} $µg/m^3$-years) N=12 cases p-trend not given Mortality: Similar results	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age and sex	Large magnitude: yes Statistical significance: no Dose-response: possible Temporal association: yes Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure Potential weaknesses: Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Bladder incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 1.00 (0.89-1.12) for each log increase Similar results for 10- and 20-year lag Similar results for community members and workers	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6 and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) Potential weaknesses: Self-reported cancer Modeled exposures

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Comparison	Confounding	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: serum 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Bladder incidence	West Virginia and Ohio cancer registries	OR = 0.60 (0.2-1.5) for the highest category (30.8-109 ng/ml) of modeled serum levels N=4 cases (69 exposed cases overall)	Other cancers except kidney, pancreas, testicular and liver	Adjusted for age, sex, diagnosis year, smoking, and insurance	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic Few highly exposed cases
Mastrantonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contaminated areas, 588,012 in the uncontaminated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of over 500 ng/L in 2013-2015 in water	Bladder mortality	Death records for 1980-2013	RR = 1.04 (0.91-1.19) N=282 exposed cases RRs of 1.12 and 1.15 in men and women	Communities in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contaminated vs. uncontaminated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: no Statistical significance: no Dose-response: not assessed Temporal association: no Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA
Eriksen et al., 2009	Where: Denmark Years: 1993-2006	Nested case-control F/U: 12-13 years	Who: general population Ages: 50-64 N: 332 cases, 772 controls	Selection: all people ages 50-64, born in Denmark, no diagnosis of cancer and living in the Copenhagen, Frederiksberg, Aarhus, Hinnerup or Hørning municipalities Participation: approximately 40% (see Tjonneland et al., 2007) Equal groups: likely Blinded: yes Levels: median serum = 6.9 ng/ml	Serum PFOA collected at recruitment	Bladder incidence	Danish Cancer Registry 1993-2006 ("virtually complete ascertainment")	IRRs by quartile all near or below 1.0 No effect modification by sex	Randomly sampled cohort members without cancer; quartiles	Adjusted for age, smoking, education, occupational exposures Matched on sex	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -150º C Information on confounders from detailed questionnaires at baseline (i.e.,the beginning of the follow-up period) PFOA and PFOS serum concentrations highly correlated (R = 0.70) <u>Potential weaknesses:</u> Single measurement

For all cancer tables:
Rows are sorted by exposure level, from high (top) to low (bottom)
Data in parentheses are 95% confidence intervals unless otherwise stated
Ages are in years unless otherwise stated
Abbreviations: F/U, follow-up; HR, hazard ratio; IH, industrial hygiene; IRR, incidence rate ratio; NA, not applicable; NDI, National Death Index; OR, odds ratio; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFOSA, perfluorooctanesulfonamide; R, correlation coefficient; RR, rate ratio; SMR, standardized mortality ratio

Table A7.22. Epidemiologic studies of PFOA and breast cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day in 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Breast mortality	NDI, death certificates	Only 3 cases, no SMRs reported	Other DuPont workers in the Appalachian region	NA	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: NA	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for breast cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies <u>Potential weaknesses:</u> TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Breast cancer subtypes not examined Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1×10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2,538 ng/ml (95% CI, 1626-3,961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Breast incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	<u>Mortality:</u> HR = 0.54 (0.15-1.94) for the 3rd and 4th quartile combined (>1.5×10^{-4} $\mu g/m^3$-years) quartile N not given <u>Incidence:</u> HR = 0.85 (0.29-2.46) for the 4th quartile (>7.9×10^{-4} $\mu g/m^3$-years) N=55 cases Note: HR = 1.47 (0.77-2.80) for the 3rd quartile N=14 cases	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age	Large magnitude: no (but see 3rd quartile) Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure <u>Potential weaknesses:</u> Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding Breast cancer subtypes not examined
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Breast incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 0.94 (0.89-1.00) for each log increase Similar results for 10- and 20-year lag Similar results for community members and workers	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) <u>Potential weaknesses:</u> Self-reported cancer Modeled exposures Breast cancer subtypes not examined

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed from 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Breast incidence	West Virginia and Ohio cancer registries	OR = 1.4 (0.9-2.3) for the highest category (30.8-109 ng/ml) of modeled serum levels N=29 cases ORs near 1.0 for all other categories	Other cancers except kidney, pancreas, testicular and liver	Adjusted for age, diagno-sis year, smoking, and insur-ance	Large magnitude: yes Statistical significance: no Dose-response: not linear Temporal association: yes Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic Breast cancer subtypes not examined
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontami-nated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Breast mortality	Death records for 1980-2013	RR = 1.11 (1.02-1.20) N=809 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contamin-ated vs. uncontamin-ated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: no Statistical significance: yes Dose-response: not assessed Temporal association: unclear Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Small increase in RR Breast cancer subtypes not examined Multiple PFAS, but it appears the highest exposures were for PFOA
Mancini et al., 2019	Where: France Years: 1990-2013	Nested case-control F/U: to 2013	Who: had national health insurance for workers Ages: 40-65 at enrollment N: 194 cases, 194 controls	Selection: unclear Participation: >97% follow-up Equal groups: yes Blinded: unclear Levels: serum median = 6.64 ng/ml	Blood collected in 1993-4	Breast incidence	Self-reports and NDI	No clear increase overall ORs of 3-7 for estrogen (ER) or progesterone receptor (PR) negative cases but p-trend = 0.59 and 0.90 respectively	Controls without breast cancer randomly selected from the cohort	Adjusted for lipids, BMI, smoking, exercise, educa-tion, family history, and many other known risk factors Matched by age, meno-pause status, BMI, and year of blood collection	Large magnitude: mixed Statistical significance: no Dose-response: no Temporal association: no Subgroup only: elevated risks with ER or PR negative cases Adjustments: little change	Information on confounders collected at baseline <u>Potential weaknesses:</u>

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Bonefeld-Jorgen-sen et al., 2014 Ghisari et al., 2017	Where: Denmark Years: 1996-2010	Nested case-control F/U: 10-15 years	Who: pregnant women Ages: adults N: 250 breast cancer cases and 233 controls	Selection: Danish National Birth Cohort recruitment in 1996-2002 Participation: ½ of all Danish pregnant women invited, about 60% agreed Equal groups: yes (see their Table 1) Blinded: unclear Levels: mean serum = 5.2 ng/ml	Blood at 1st and 2nd trimester of pregnancy	Breast incidence	Cancer registry	All RRs near 1.0 Highest category: >6.53 ng/ml Some RRs above 1.0 in women >40 years old, but unusual dose-response pattern	Other randomly selected members of the cohort	Adjusted for age, BMI, parity, oral contra-ceptive use, men-arche, smoking, alcohol, educa-tion, exercise Matched on age and parity	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: little change	Samples stored at -80º C F/U through 2010 Mostly premenopausal women Questionnaires administered during pregnancy and 6 months post-partum Correlation between PFOA and PFOS = 0.69 Positive association seen for PFOSA, negative association seen for PFHxS Possible interaction with CYP19 CC genotype but multiple comparisons (Ghisari et al., 2017) <u>Weaknesses:</u> Only 1 or 2 serum samples Breast cancer subtypes not examined
Cohn et al., 2019	Where: California Years: 1959-2013	Nested case-control F/U: 54 years, through 2013	Who: Kaiser members, in utero at time of recruitment (1959-67) Ages: adults N: 102 cases and 310 controls	Selection: >99% of eligible women enrolled Participation: <50% Equal groups: unclear Blinded: yes Levels: median serum = 0.4 ng/ml	Perinatal serum, mostly 1-3 days after delivery	Breast incidence	Vital status records, cancer registry (>99% complete), and self-reports	"no association"; actual results not given	Controls randomly selected from the cohort	Adjusted for choles-terol, DDT, age, race, BMI, parity, maternal history of breast cancer Matched on birth year and trimester of blood draw	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -20º C Includes mothers and their daughters Questionnaire information collected at baseline (i.e., at the start of the follow-up period) <u>Weaknesses:</u> Single measurement in mother Limited results Breast cancer subtypes not examined

Table A7.23. Epidemiologic studies of PFOA and kidney cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Girardi and Merler, 2019	Where: Trissino, Veneto, Italy Years: 1960-2018	Occupational Retrospective cohort F/U: average of 31.7 years	Who: worked at least 1 day from 1948-2002 Ages: adults N: 462 chemical or office workers, 1,383 railroad workers	Selection: all male workers, at least 6 months, from 1960-2008 Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean = 4.048 µg/ml	Job activities and tasks based on records and some interviews used to classify workers as ever, never exposed, or office workers. Jobs, years worked, and serum levels in a subsample (N = 120 workers) used to model cumulative exposure	Kidney mortality	Local and national death records	Two cases, SMRs not provided	Regional mortality rates (for SMRs), and local railroad workers (for RRs); tertiles of cumulative exposure	Adjusted for age Males only	Large magnitude: not given Statistical significance: not given Dose-response: unknown Temporal association: not given Subgroup only: no Adjustments: not given	F/U 1970 to 2018 Average length of employment = 17.1 years Other chemicals produced at the plant include fluoroaromatics, benzotrifluorides, and PFOS (mean serum PFOS = 0.148 µg/ml, correlation with PFOA = 0.59) Elevated SMRs for suicides <u>Potential weaknesses:</u> Small number of cases No measure of association provided
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Kidney mortality	NDI, death certificates	SMR = 2.68 (1.15-5.24) for the 4th quartile (≥2,700 ppm-years) N=8 cases p-trend = 0.02 SMR increases somewhat with 10- and 20-year lags	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: yes Statistical significance: yes Dose-response: linear trend Temporal association: yes Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for kidney cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies <u>Potential weaknesses:</u> TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Exposure duration could be short in some workers Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Kidney incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	<u>Incidence:</u> No increase N=16 cases Little change with 10-year lag Highest category: >7.9 x 10^{-4} µg/m^3-years <u>Mortality:</u> No increase	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); categorical	Adjusted for age and sex	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure <u>Potential weaknesses:</u> Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Kidney incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HRs = 1.00, 1.23, 1.48, and 1.58 by quartile of cumulative exposure HR = 1.58 (0.88-2.84) for the 4th quartile N=105 cases overall p-trend = 0.18 Similar results for 10- and 20-year lag Workers: HRs of 1.00, 0.84 (0.21-3.40), 4.20 (1.07-16.44), and 0.83 (0.20-3.55) by quartiles N=18 cases p-trend = 0.54	Subjects without the cancer of interest; quartiles	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: yes Statistical significance: not in highest quartile Dose-response: possible Temporal association: yes Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) <u>Potential weaknesses:</u> Self-reported cancer Modeled exposures
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Kidney incidence	West Virginia and Ohio cancer registries	OR = 0.8, 1.2, 2.0 (1.3-3.2), and 2.0 (1.0-3.9) by categories of exposure vs. unexposed (modeled serum levels) N=9 cases in the highest category (30.8-109 ng/ml) of modeled serum levels OR in women = 3.5 (1.4-8.3)	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, sex, diagno-sis year, smoking, and insur-ance	Large magnitude: yes Statistical significance: yes Dose-response: plateau Temporal association: yes Subgroup only: highest OR in women Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncon-taminated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Kidney mortality	Death records for 1980-2013	RR = 1.07 (0.90-1.28) in men N=225 exposed cases RR = 1.32 (1.06-1.65) in women N=103 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontami-nated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: yes Statistical significance: yes Dose-response: not assessed Temporal association: unclear Subgroup only: women Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Shearer et al., 2021	Where: US (10 sites) Years: 1993-2014	Nested case-control F/U: 8.9 years on average	Who: Adults Ages: 55-74 at baseline N: 324 cases; 324 controls	Selection: convenience sample Participation: unclear Equal groups: yes Blinded: yes Levels: median serum levels of 5.5 ng/ml	Serum	Renal cell carcinoma	Annual question-naires, physicians or relatives, the National Death Index, or local cancer registries	Adjusted ORs of 1.00 (ref), 1.47 (0.77-2.80), 1.24 (0.64-2.41), and 2.63 (1.33-5.20) by quartile if exposure in controls	Lowest quartile	Adjusted for age, sex, race/ ethnicity, study center, year of blood draw, eGFR, BMI, smoking, hyperten sion, freeze-thaw cycles of samples, and calendar year of blood draw	Large magnitude: yes Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: little change	<u>Potential weaknesses:</u> Single blood sample

Table A7.24. Epidemiologic studies of PFOA and liver cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Girardi and Merler, 2019	Where: Trissino, Veneto, Italy Years: 1960-2018	Occupational Retrospective cohort F/U: average of 31.7 years	Who: worked at least 1 day from 1948-2002 Ages: adults N: 462 chemical or office workers, 1,383 railroad workers	Selection: all male workers, at least 6 months, from 1960-2008 Participation: unclear Equal groups: unclear Blinded: unclear Levels: geometric mean = 4.048 µg/ml	Job activities and tasks based on records and some interviews used to classify workers as ever, never exposed, or office workers. Jobs, years worked, and serum levels in a subsample (N = 120 workers) used to model cumulative exposure	Liver mortality	Local and national death records	SMR = 4.71 (1.52-14.6) for those ever working at the PFAS facility N=3 cases (7 cases total) SMRs of 1.02 (0.12-7.21), 2.76 (0.69-11.0), and 3.07 (1.15-8.18) by tertile of cumulative exposure p-trend = 0.027 Higher mortality RRs when compared to railroad workers	Regional mortality rates (for SMRs), and local railroad workers (for RRs); tertiles of cumulative exposure	Adjusted for age Males only	Large magnitude: yes Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: not given	F/U 1970 to 2018 Average length of employment = 17.1 years Other chemicals produced at the plant include fluoroaromatics, benzotrifluorides, and PFOS (mean serum PFOS = 0.148 µg/ml, correlation with PFOA = 0.59) Elevated SMRs for suicides <u>Potential weaknesses:</u> Increased SMR in those never working in the PFAS area (SMR = 2.71; 95% CI, 1.02-7.22) (geometric mean serum levels = 977 ng/ml; range = 19–15,786 ng/ml) Limited information on confounding Small numbers of cases

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day from 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Liver mortality	NDI, death certificates	SMRs of 2.39 (0.65-6.13), 0 (0-1.81), 2.01 (0.65-4.68), and 0.32 (0.01-1.76) by quartile of exposure vs. the referent population N=1 case in the highest quartile and 10 cases overall	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: unclear Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for liver cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies <u>Potential weaknesses:</u> TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Small numbers of cases Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1×10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Liver incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	<u>Incidence:</u> Few cases, HRs not calculated <u>Mortality:</u> HRs of 1.00 (ref), 2.09 (0.69-6.31), and 0.67 (0.14-3.27) for quartile 1-2 combined, and quartile 3-4 combined, respectively N=8 cases total in exposed quartiles 1-4	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age and sex	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure <u>Potential weaknesses:</u> Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding Small numbers of cases
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Liver incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 0.73 (0.43-1.23) for each log increase N=9 cases overall Similar results for 10- and 20-year lag Only 1 occupationally exposed case	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) <u>Potential weaknesses:</u> Self-reported cancer Modeled exposures Small number of cases

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Liver incidence	West Virginia and Ohio cancer registries	OR = 1.0 (0.3-3.1) for the "high" category (30.8-109 ng/ml) of modeled serum levels N=3 cases (11 exposed cases overall)	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, sex, diagno-sis year, smoking, and insur-ance	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic Few highly exposed cases
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontam-inated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Liver mortality	Death records for 1980-2013	RR = 0.89 (0.78-1.03) N=242 exposed cases Similar RRs in men and women	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontami-nated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: no Statistical significance: no Dose-response: not assessed Temporal association: no Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA
Eriksen et al., 2009	Where: Denmark Years: 1993-2006	Nested case-control F/U: 12-13 years	Who: general population Ages: 50-64 N: 67 cases, 772 controls	Selection: all people ages 50-64, born in Denmark, no diagnosis of cancer and living in the Copenhagen, Frederiksberg, Aarhus, Hinnerup or Hørning municipalities Participation: approximately 40% (see Tjonneland et al., 2007) Equal groups: likely Blinded: yes Levels: median serum = 6.9 ng/ml	Serum PFOA collected at recruitment	Liver incidence	Danish Cancer Registry 1993-2006 ("virtually complete ascertain-ment")	IRRs by quartile all near or below 1.0 N=67 cases total No effect modification by sex	Randomly sampled cohort members without cancer; quartiles	Adjusted for age, smoking, educa-tion, occupa-tional expo-sures Matched on sex	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -150º C Information on confounders from detailed questionnaires at baseline (i.e.,the beginning of the follow-up period) PFOA and PFOS serum concentrations highly correlated (R = 0.70) <u>Potential weaknesses:</u> Single measurement

Table A7.25. Epidemiologic studies of PFOA and pancreatic cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Pancreas mortality	NDI, death certificates	SMR = 0.92 (0.30-2.16) for the 4th quartile (≥2,700 ppm-years) N=5 cases in the highest quartile	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for pancreatic cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies Potential weaknesses: TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1×10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Pancreas incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	Incidence: HR = 1.36 (0.59-3.11) for the highest two quartiles combined (>1.5×10^{-4} $µg/m^3$-years) N=9 cases in quartiles 3-4 p-trend not given Mortality: Similar results	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age and sex	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure Potential weaknesses: Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Pancreas incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 1.00 (0.89-1.12) for each log increase N=24 cases overall Similar results for 10- and 20-year lag Similar results for community members and workers	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) Potential weaknesses: Self-reported cancer Modeled exposures

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Pancreas incidence	West Virginia and Ohio cancer registries	OR = 0.6 (0.1-2.5) for the "very high" category (30.8-109 ng/ml) of modeled serum levels N=2 cases in this category	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, sex, diagno-sis year, smoking, and insur-ance	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontami-nated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Pancreas mortality	Death records for 1980-2013	RR = 1.11 (0.99-1.25) in men N=361 exposed cases RR = 0.99 (0.87-1.12) in women N=302 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontami-nated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA
Eriksen et al., 2009	Where: Denmark Years: 1993-2006	Nested case-control F/U: 12-13 years	Who: general population adults Ages: 50-64 N: 128 cases, 772 controls	Selection: all people ages 50-64, born in Denmark, no diagnosis of cancer and living in the Copenhagen, Frederiksberg, Aarhus, Hinnerup or Hørning municipalities Participation: approximately 40% (see Tjonneland et al., 2007) Equal groups: likely Blinded: yes Levels: median serum = 6.9 ng/ml	Serum PFOA collected at recruitment	Pancreas incidence	Danish Cancer Registry 1993-2006 ("virtually complete ascertain-ment")	IRR by quartile of 1.00, 0.88, 1.33, 1.55 (0.85-2.80) Quartile cut-off points not given N=32 cases in the highest quartile Trend per 1 ng/ml IRR = 1.03 (0.98-1.10)	Randomly sampled cohort members without cancer; quartiles	Adjusted for age, smoking, diet Matched on sex	Large magnitude: yes Statistical significance: no Dose-response: possible linear Temporal association: yes Subgroup only: no Adjustments: not given	Samples stored at -150º C Information on confounders from detailed questionnaires at baseline (i.e.,the beginning of the follow-up period) PFOA and PFOS serum concentrations highly correlated (R = 0.70) No effect modification by sex <u>Potential weaknesses:</u> Single measurement

Table A7.26. Epidemiologic studies of PFOA and prostate cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012 Steen-land et al., 2015	Where: DuPont plant, West Virginia Years: 1948-2008	Occupational Retrospective cohort F/U: mean 30 years	Who: worked at least 1 day 1948-2002 Ages: adults N: 5,791 (mortality), 3,713 (incidence)	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.113 µg/ml (incidence) and 0.35 µg/ml (mortality)	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Prostate incidence and mortality	Incidence: self-reports Mortality: NDI, death certificates	Incidence: RR = 1.00, 1.81, 2.45, and 1.88 by quartiles RR = 1.88 (0.72-4.88) for the highest quartile N=129 cases overall Mortality: SMR = 0.57 (0.16-1.46) for the 4th quartile (≥2,700 ppm-years) N=4 cases in the highest category	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age	Large magnitude: yes Statistical significance: no Dose-response: plateau Temporal association: yes Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies Potential weaknesses: TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupational Retrospective cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respectively)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1×10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Prostate incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	Mortality: HR = 1.32 (0.61-2.84) for the 4th quartile (>7.9×10^{-4} $\mu g/m^3$-years) N not given Incidence: HR = 1.11 (0.82-1.49) for the 4th quartile (>7.9×10^{-4} $\mu g/m^3$-years) N=55 cases	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul); quartiles	Adjusted for age	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure Potential weaknesses: Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospective cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Prostate incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HR = 0.99 (0.93-1.04) for each log increase Similar results for 10- and 20-year lag Similar results for community members and workers	Subjects without the cancer of interest; log increase in PFOA	Adjusted for smoking, alcohol, educa-tion, and age	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) Potential weaknesses: Self-reported cancer Modeled exposures

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Prostate incidence	West Virginia and Ohio cancer registries	OR = 1.5 (0.9-2.5) for the highest category (30.8-109 ng/ml) of modeled serum levels N=31 cases ORs near 1.0 in all other categories	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, diagno-sis year, smoking, and insur-ance	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: yes Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontam-inated areas	Selection: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Prostate mortality	Death records for 1980-2013	RR = 1.00 (0.90-1.12) in the contaminated area N=401 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontam-inated areas	Age. Similar in SES and smoking	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA
Eriksen et al., 2009	Where: Denmark Years: 1993-2006	Nested case-control F/U: 12-13 years	Who: general population adults Ages: 50-64 N: 713 cases, 772 controls	Selection: all people ages 50-64, born in Denmark, no diagnosis of cancer and living in the Copenhagen, Frederiksberg, Aarhus, Hinnerup or Hørning municipalities Participation: approximately 40% (see Tjonneland et al., 2007) Equal groups: likely Blinded: yes Levels: median serum = 6.9 ng/ml	Serum PFOA collected at recruitment	Prostate incidence	Danish Cancer Registry 1993-2006 ("virtually complete ascertain-ment")	IRR by quartile all near 1.0 OR = 1.38 (0.99-1.93) for the upper 3 quartiles vs. lowest quartile, but no clear dose-response relationship (see notes)	Randomly sampled cohort members without cancer, matched on sex; quartiles	Adjusted for age, educa-tion, BMI, and diet	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Samples stored at -150º C Information on confounders from detailed questionnaires at baseline (i.e.,the beginning of the follow-up period) PFOA and PFOS serum concentrations highly correlated (R = 0.70) No effect modification by sex <u>Potential weaknesses:</u> Single measurement OR of 1.38 could be due to lower risk in lower quartile caused by chance

Table A7.27. Epidemiologic studies of PFOA and testicular cancer: all years

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Steen-land and Woskie, 2012	Where: DuPont plant, West Virginia Years: 1948-2008	Occupa-tional Retrospec-tive cohort F/U: mean 30 years	Who: worked at least one day 1948-2002 Ages: adults N: 5,791	Selection: all workers Participation: 5,791 of 6,027 (96%) with sufficient records Equal groups: likely Blinded: unclear Levels: mean serum = 0.35 µg/ml	Modeled serum levels based on serum from 1,308 workers (2004) and job categories	Testicular mortality	NDI, death certificates	SMR = 1.81 (0.05-10.03) for all exposed quartiles combined (0 to ≥2,700 ppm-years) N=1 case	Other DuPont workers in the Appalachian region; quartiles	Adjusted for age and gender	Large magnitude: yes Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: not given	Follow-up of Leonard et al., 2008 Mean employment: 19 years F/U through 2008 Incidence data not given for testicular cancer Peak PFOA usage in the 1990s 19% women and 5% non-whites Likely overlap with other C8 area studies <u>Potential weaknesses:</u> TFE exposure possible but levels unknown Mesothelioma SMRs were above 1.0 suggesting asbestos exposure Modeled exposures Limited information on confounding Possible short duration of exposure in some workers
Raleigh et al., 2014	Where: 3M plants in Minnesota Years: 1960-2008	Occupa-tional Retrospec-tive cohort F/U: 34 years (mean ages 29 to 63 years at start and end, respective-ly)	Who: all workers at two facilities who worked at least 1 year Ages: adults N: 4,668 (Cottage Grove), 4,359 (Saint Paul)	Selection: all workers Participation: follow-up of approximately 89% Equal groups: likely Blinded: unclear Levels: air levels up to 0.4 mg/m^3 (vs. 1 x 10^{-6} mg/m^3 in unexposed workers), geometric mean serum PFOA concentration of 2538 ng/ml (95% CI, 1626-3961 ng/ml) in those only working in the PFOA area	IH monitoring data and work records used to estimate air levels for all workers	Testicular incidence and mortality	Incidence: Minnesota and Wisconsin cancer registries (mandatory reporting) Mortality: Social Security, NDI	Few cases (N=5), SMRs and RRs not given	Minnesota cancer rates and unexposed workers (3M workers in Saint Paul)	Adjusted: NA	Large magnitude: no Statistical significance: no Dose-response: no Temporal association: no Subgroup only: no Adjustments: NA	Follow-up of Lundin et al., 2009 and Gilliland and Mandel, 1993 Cottage Grove plant used PFOA. The Saint Paul plant did not Saint Paul workers were 9 years older 21% women F/U: 1988 through 2008 (incidence) and 1960-2008 (mortality) No TFE exposure <u>Potential weaknesses:</u> Some out-migration (e.g., 31%) in lower exposed Cottage Grove workers Limited information on confounding
Barry et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1952-2011	Retrospec-tive cohort F/U: average 33 years	Who: living near DuPont plant and DuPont workers Ages: average age 53 N: 32,254	Selection: all current residents and all workers Participation: about 55% Equal groups: likely Blinded: unclear Levels: mean serum = 32.91 ng/ml (Frisbee et al., 2009)	Estimated yearly serum levels for 1952-2011 based on plant emissions, wind patterns, river and ground water flow, workplace exposure (see Steenland and Woskie, 2012), drinking water source, and water consumption	Testicular incidence	Self-reports ("Have you ever been told by a doctor…you had cancer…"), with confirmation by cancer registries or medical records	HRs = 1.00, 1.04, 1.91, and 3.17 by quartile of cumulative exposure HR = 3.17 (0.75-13.45) for the 4^{th} quartile N=17 cases overall p-trend = 0.04 Similar results for 10- and 20-year lag Only 2 cases in the worker cohort	Subjects without the cancer of interest; quartiles	Adjusted for smoking, alcohol, sex, educa-tion, and age	Large magnitude: yes Statistical significance: yes Dose-response: yes Temporal association: yes Subgroup only: no Adjustments: not given	Cohort assembled in 2005-6, and re-interviewed in 2008-11 Followed from age 20 or year 1952, whichever was later (no childhood exposure information) Likely overlap with other C8 area studies 30% of cancers reported could not be confirmed (but similar results when these were included) <u>Potential weaknesses:</u> Self-reported cancer Modeled exposures

Author Year	Location Years	Design	Who N	Factors related to bias	Exposure method	Outcome	Outcome method	Results	Compari-son	Confound-ing	Other aspects of causal inference	Notes
Vieira et al., 2013	Where: West Virginia and Ohio (DuPont) Years: 1996-2005	Cancer registry study	Who: cancer cases diagnosed 1996-2005 living near DuPont plant at time of diagnosis Ages: adults N: 25,107	Selection: all cancer cases in registry Participation: 100% Equal groups: unclear Blinded: unclear Levels: estimated mean serum by city = 5.3 to 125 ng/ml	Median serum levels by water district (both states) or individual modeled estimated serum levels (Ohio only) (see Shin et al., 2011 for details)	Testicular incidence	West Virginia and Ohio cancer registries	OR = 2.8 (0.8-9.2) for the highest category (30.8-109 ng/ml) of modeled serum levels N=6 cases ORs below 1.0 in all other categories OR = 5.1 (1.6-15.6) in the highest exposed water district	Other cancers except kidney, pancreas, testicular and liver; categorical	Adjusted for age, sex, diagno-sis year, smoking, and insur-ance	Large magnitude: yes Statistical significance: no for the analyses of modeled serum, yes for district water concentrations Dose-response: no Temporal association: yes Subgroup only: no Adjustments: not given	Some analyses assumed a 10-year latency Median residency duration of 17 years for participants of the C8 Health Project Model validation R = 0.82 Likely overlap with other C8 area studies <u>Potential weaknesses:</u> Only includes residence at the time of cancer diagnosis; some analyses assumed a 10-year residency Other cancer cases used as controls Partially ecologic
Mastran-tonio et al., 2017	Where: Veneto, Italy Years: 1980-2013	Ecologic F/U: 31 years	Who: all residents of non-urban areas in Veneto Region Participation: 100% Equal groups: the groups being compared were similar in terms of deprivation indices (socioeconomic status) and prevalence of smoking Blinded: unclear Levels: water levels >500 ng/L Ages: all N: 143,605 residents in the contami-nated areas, 588,012 in the uncontami-nated areas	Selection: all residents of non-urban areas in Veneto Region	Contaminated vs. non-contaminated areas. Contamination defined as at least one exceedance of 500 ng/L in 2013-2015 in water	Testicular mortality	Death records for 1980-2013	RR = 1.86 (0.81-4.27) for the contaminated area N=8 exposed cases	Communi-ties in the Veneto Region with water PFAS levels <10 ng/L. RR is the ratio of SMRs in the contami-nated vs. uncontami-nated areas	Age and sex stratified. Similar in SES and smoking	Large magnitude: yes Statistical significance: no Dose-response: not assessed Temporal association: unclear Subgroup only: no Adjustments: NA	Local water sources contaminated by a chemical manufacturing plant operating since 1964 Urban areas were excluded <u>Potential weaknesses:</u> Ecologic design Limited information on confounding Mortality only Multiple PFAS, but it appears the highest exposures were for PFOA

Excluded Studies

Table A7.28. Studies excluded after abstract or full article review and reasons for exclusion

Study reference	Health outcome	Reason for exclusion[1]
Bonefeld-Jorgensen EC, Long M, Bossi R, Ayotte P, Asmund G, Krüger T, Ghisari M et al. (2011). Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case control study. Environ Health. 10:88	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Cordiano V., Bai E., Crosignani P., Mastrantonio M. Increased mortality from diabetes mellitus, acute myocardial infarction, genitourinary tract diseases in a community heavily exposed since 1960s to drinking water contaminated with perfluorinated substances a class of endocrine disruptors and carcinogens in the Veneto Region. Italian Journal of Medicine 2016 10 Supplement 2 (30-)	Cancer	Overlap with Mastrantonio et al., 2017
Costa G, Sartori S, Consonni D (2009). Thirty years of medical surveillance in perfluooctanoic acid production workers. J Occup Environ Med. 51(3):364-72.	Cancer	No cancer data (cholesterol and exposure data)
Fry K., Power M.C. Persistent organic pollutants and mortality in the United States, NHANES 1999-2011. Environmental Health: A Global Access Science Source 2017 16:1	Cancer	No PFOS or PFOA and cancer data
Ghisari M, Eiberg H, Long M, Bonefeld-Jørgensen EC (2014). Polymorphisms in phase I and phase II genes and breast cancer risk and relations to persistent organic pollutant exposure: a case-control study in Inuit women. Environ Health. 13(1):19.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Girardi P and Merler E (2017). Mortality study of a cohort of chemical workers producing perfluorinated derivatives and other chemicals. Occupational and Environmental Medicine 74(Suppl 1): A12-A	Cancer	Abstract; overlap with Girardi and Merler, 2019
Giuliani J., Bonetti A. The exposure to perfluoroalkyl substances (PFAS) and the development of cancer. Recenti Progressi in Medicina 2019 110:7-8 (368-370)	Cancer	Review
Grandjean P. Health Status of Workers Exposed to Perfluorinated Alkylate Substances. J Occup Environ Med. 2018 Oct;60(10):e562.	Cancer	Letter to the Editor
Hardell, E., A. Kärrman, B. van Bavel, J. Boa, M. Carlberg, and L. Hardell. 2014. Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environment International 63:35–39.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Hocevar B.A., Kamendulis L.M. Promotion of pancreatic cancer by perfluorooctanoic acid (PFOA). Cancer Research 2018 78:13 Supplement 1	Cancer	Mice
Hurley S, Goldberg D, Wang M, Park JS, Petreas M, Bernstein L, Anton-Culver H, Nelson DO, Reynolds P. Breast cancer risk and serum levels of per- and poly-fluoroalkyl substances: a case-control study nested in the California Teachers Study. Environ Health. 2018 Nov 27;17(1):83.	Cancer	Serum collected 35 months after breast cancer diagnosis
Innes KE, Wimsatt JH, Frisbee S, Ducatman AM (2014). Inverse association of colorectal cancer prevalence to serum levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in a large Appalachian population. BMC Cancer. 14:45	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment

Study reference	Health outcome	Reason for exclusion[1]
Roswall N., Larsen S.B., Sørensen M., Tjønneland A., Raaschou-Nielsen O. Perfluorooctanoate and Perfluorooctanesulfonate plasma concentrations and survival after prostate and bladder cancer in a population-based study. Environmental Epidemiology 2018 2:3 Article Number e018	Cancer	Cancer survival
Tsai MS, Chang SH, Kuo WH, et al. A case-control study of perfluoroalkyl substances and the risk of breast cancer in Taiwanese women. Environ Int. 2020;142:105850.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Ubel FA, Sorenson SD, Roach DE. Health status of plant workers exposed to fluorochemicals--a preliminary report. Am Ind Hyg Assoc J. 1980 Aug;41(8):584-9.	Cancer	More detailed data in Raleigh et al., 2014
Vassiliadou, I., D. Costopoulou, A. Ferderigou, and L. Leondiadis. 2010. Levels of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in blood samples from different groups of adults living in Greece. Chemosphere 80:1199–1206.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Wielsøe M, Kern P, Bonefeld-Jørgensen EC. Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study. Environ Health. 2017 Jun 13;16(1):56.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Wielsøe M., Eiberg H., Ghisari M., Kern P., Lind O., Bonefeld-Jørgensen E.C. Genetic Variations, Exposure to Persistent Organic Pollutants and Breast Cancer Risk – A Greenlandic Case–Control Study. Basic and Clinical Pharmacology and Toxicology 2018 123:3 (335-346)	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Yeung LW, Guruge KS, Taniyasu S, Yamashita N, Angus PW, Herath CB. Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicol Environ Saf. 2013 Oct;96:139-46.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Yeung LW, Robinson SJ, Koschorreck J, Mabury SA. Part II. A temporal study of PFOS and its precursors in human plasma from two German cities in 1982-2009. Environ Sci Technol. 2013 Apr 16;47(8):3875-82.	Cancer	Exposure and half-life study
Yeung, L.W.Y., K.S. Guruge, S. Taniyasu, N. Yamashita, P.W. Angus, and C.B. Herath. 2013. Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicology & Environmental Safety 96:139–146.	Cancer	Cross-sectional: problems assessing temporal association; exposure data collected at or near the time of outcome diagnosis or assessment
Bjerregaard-Olesen C, Bach CC, Long M, Wielsøe M, Bech BH, Henriksen TB, et al. (2019). Associations of fetal growth outcomes with measures of the combined xenoestrogenic activity of maternal serum perfluorinated alkyl acids in danish pregnant women. Environ Health Perspect 127:17006-17006.	DART - fetal growth	PFAS mixtures (effects of PFOA and PFOS not separated)
Kishi R, Araki A, Minatoya M, Hanaoka T, Miyashita C, Itoh S, et al. (2017). The Hokkaido birth cohort study on environment and children's health: Cohort profile-updated 2017. Environ Health Prev Med 22:46-46.	DART - fetal growth	Review of Hokkaido Cohort studies
Liew Z, Goudarzi H, Oulhote Y. (2018). Developmental exposures to perfluoroalkyl substances (PFASs): An update of associated health outcomes. Curr Environ Health Rep 5:1-19.	DART - fetal growth	Review
Liu J, Liu G, Li Z. (2017). Importance of metabolomics analyses of maternal parameters and their influence on fetal growth. Experimental and Therapeutic Medicine 14:467-472.	DART - fetal growth	Review

Study reference	Health outcome	Reason for exclusion[1]
Ma RCW, Tam CHT, Cheung GPY, Lowe W, Metzger BE, Tam WH, et al. (2018). Maternal exposure to perfluorooctane sulfonate (PFOS) is associated with maternal hyperglycaemia and adverse neonatal and childhood outcomes. Diabetes 67:LB46.	DART - fetal growth	Conference abstract
Malits J, Blustein J, Trasande L, Attina TM. (2018). Perfluorooctanoic acid and low birth weight: Estimates of US attributable burden and economic costs from 2003 through 2014. Int J Hyg Environ Health 221:269-275.	DART - fetal growth	Attributable cost study
Negri E, Metruccio F, Guercio V, Tosti L, Benfenati E, Bonzi R, et al. (2017). Exposure to PFOA and PFOS and fetal growth: A critical merging of toxicological and epidemiological data. Crit Rev Toxicol 47:482-508.	DART - fetal growth	Review
Nicolle-Mir L. (2018). Perfluorinated compounds and child's health: Review of the epidemiological literature. Environnement, Risques et Sante 17:97-98.	DART - fetal growth	Review
Nicolle-Mir L. (2019). Influence of in utero exposure to organochlorines and perfluorochemicals on the risk of low birth weight: Pooled analysis of seven European cohorts. Environnement, Risques et Sante 18:14-15.	DART - fetal growth	Brief taken from Govarts et al. (2018)
Steenland K, Barry V, Savitz D. (2018). Serum perfluorooctanoic acid and birthweight: An updated meta-analysis with bias analysis. Epidemiology 29:765-776.	DART - fetal growth	Review
Tsai M-S, Chen M-H, Lin C-C, Ng S, Hsieh C-J, Liu C-Y, et al. (2017). Children's environmental health based on birth cohort studies of Asia. Sci Total Environ 609:396-409.	DART - fetal growth	Review
Tsuda S. (2016). Differential toxicity between perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). J Toxicol Sci 41:SP27-SP36.	DART - fetal growth	Discussion of US EPA and IARC documents
Vrijheid M, Casas M, Gascon M, Valvi D, Nieuwenhuijsen M. (2016). Environmental pollutants and child health-a review of recent concerns. Int J Hyg Environ Health 219:331-342.	DART - fetal growth	Review
Wang A, Padula A, Sirota M, Woodruff TJ. (2016). Environmental influences on reproductive health: The importance of chemical exposures. Fertil Steril 106:905-929.	DART - fetal growth	Review
Zlatnik MG. 2016. Endocrine-disrupting chemicals and reproductive health. J Midwifery Womens Health 61:442-455.	DART - fetal growth	Review
Bach CC, Vested A, Jørgensen KT, Bonde JPE, Henriksen TB, Toft G. (2016). Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: A systematic review. Crit Rev Toxicol 46:735-755.	DART- fertility and fecundity	Review
Karwacka A, Zamkowska D, Radwan M, Jurewicz J. (2019). Exposure to modern, widespread environmental endocrine disrupting chemicals and their effect on the reproductive potential of women: An overview of current epidemiological evidence. Human Fertility 22:2-25	DART- fertility and fecundity	Review
Avanasi R, Shin H-M, Vieira VM, Bartell SM. (2016a). Impacts of geocoding uncertainty on reconstructed pfoa exposures and their epidemiological association with preeclampsia. Environ Res 151:505-512.	DART - preeclampsia and gestational hypertension	Re-examination of previously published data
Avanasi R, Shin H-M, Vieira VM, Savitz DA, Bartell SM. (2016b). Impact of exposure uncertainty on the association between perfluorooctanoate and preeclampsia in the c8 health project population. Environ Health Perspect 124:126-132.	DART - preeclampsia and gestational hypertension	Re-examination of previously published data

Study reference	Health outcome	Reason for exclusion[1]
Casas M., Manzano-Salgado C.B., Granum B., Lopez-Espinosa M.-J., Ballester F., Iniguez C., Gascon M., Martinez D., Guxens M., Basterretxea M., Zabaleta C., Schettgen T., Sunyer J., Vrijheid M.Prenatal exposure to perfluoroalkyl substances and immune and respiratory outcomes. European Respiratory Journal 2018 52 Supplement 62	Immunotoxicity	Conference abstract (limited information)
Conway BN, Badders AN, Costacou T, Arthur JM, Innes KE. Perfluoroalkyl substances and kidney function in chronic kidney disease, anemia, and diabetes. Diabetes Metab Syndr Obes. 2018 Nov 15;11:707-716.	Immunotoxicity	Diabetes, CRP as a confounding variable only
DeWitt JC, Blossom SJ, Schaider LA. Exposure to per-fluoroalkyl and polyfluoroalkyl substances leads to immunotoxicity: epidemiological and toxicological evidence. J Expo Sci Environ Epidemiol. 2019 Mar;29(2):148-156	Immunotoxicity	Review
Hammer T, Lophaven SN, Nielsen KR, von Euler-Chelpin M, Weihe P, Munkholm P, Burisch J, Lynge E. Inflammatory bowel diseases in Faroese-born Danish residents and their offspring: further evidence of the dominant role of environmental factors in IBD development. Aliment Pharmacol Ther. 2017 Apr;45(8):1107-1114.	Immunotoxicity	No PFOA or PFOS data
Honda-Kohmo K., Hutcheson R., Innes K.E., Conway B.N. Perfluoroalkyl substances are inversely associated with coronary heart disease in adults with diabetes. Journal of Diabetes and its Complications 2019 33:6 (407-412)	Immunotoxicity	Conorary heart diseaese
Huang H, Wang Q, He X, Wu Y, Xu C. Association between polyfluoroalkyl chemical concentrations and leucocyte telomere length in US adults. Sci Total Environ. 2018 Oct 30;653:547-553.	Immunotoxicity	Telomere length
Kingsley S.L., Walker D.I., Calafat A.M., Chen A., Papandonatos G.D., Xu Y., Jones D.P., Lanphear B.P., Pennell K.D., Braun J.M. Metabolomics of childhood exposure to perfluoroalkyl substances: a cross-sectional study. Metabolomics 2019 15:7	Immunotoxicity	Metabolomics
Knudsen AS, Long M, Pedersen HS, Bonefeld-Jørgensen EC. Persistent organic pollutants and haematological markers in pregnant women: the ACCEPT sub-study. Int J Circumpolar Health. 2018 Dec;77(1):1456303.	Immunotoxicity	Only gives data for the sum of all PFAS
Lai KP, Ng AH, Wan HT, Wong AY, Leung CC, Li R, Wong CK. Dietary Exposure to the Environmental Chemical, PFOS on the Diversity of Gut Microbiota, Associated With the Development of Metabolic Syndrome. Front Microbiol. 2018 Oct 24;9:2552.	Immunotoxicity	Mice
Lee JK, Lee S, Baek MC, Lee BH, Lee HS, Kwon TK, Park PH, Shin TY, Khang D, Kim SH. Association between perfluorooctanoic acid exposure and degranulation of mast cells in allergic inflammation. J Appl Toxicol. 2017 May;37(5):554-562.	Immunotoxicity	In vitro study
Liu H, Chen Q, Lei L, Zhou W, Huang L, Zhang J, Chen D. Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances affects leukocyte telomere length in female newborns. Environ Pollut. 2018 Apr;235:446-452.	Immunotoxicity	Telomere length
Liu J, Liu S, Huang Z, et al. Associations between the serum levels of PFOS/PFOA and IgG N-glycosylation in adult or children. Environ Pollut. 2020;265(Pt A):114285	Immunotoxicity	IgG glycolsylation
Lowe AJ, Dharmage SC, Abramson MJ, Vijayasarathy S, Erbas B, Mueller JF, Lodge CJ. Cord-serum per- and poly-fluoroalkyl substances and atopy and eczema at 12-months. Allergy. 2019 Apr;74(4):812-815.	Immunotoxicity	Diagnosis of atopic eczema is unclear

Study reference	Health outcome	Reason for exclusion[1]
Luo Y, Deji Z, Huang Z. Exposure to perfluoroalkyl substances and allergic outcomes in children: A systematic review and meta-analysis [published online ahead of print, 2020 Aug 30]. Environ Res. 2020;191:110145.	Immunotoxicity	Meta-analysis
Oulhote Y, Shamim Z, Kielsen K, Weihe P, Grandjean P, Ryder LP, Heilmann C. Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants. Reprod Toxicol. 2017 Mar;68:207-214.	Immunotoxicity	Only gives data for the sum of all PFAS
Panikkar B, Lemmond B, Allen L, DiPirro C, Kasper S. Making the invisible visible: results of a community-led health survey following PFAS contamination of drinking water in Merrimack, New Hampshire. Environ Health. 2019 Aug 30;18(1):79.	Immunotoxicity	Multiple PFAS combined
Pennings JL, Jennen DG, Nygaard UC, Namork E, Haug LS, van Loveren H, Granum B. Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. J Immunotoxicol. 2016;13(2):173-80.	Immunotoxicity	Gene expression; mechanism
Rainieri S, Conlledo N, Langerholc T, Madorran E, Sala M, Barranco A. Toxic effects of perfluorinated compounds at human cellular level and on a model vertebrate. Food Chem Toxicol. 2017 Jun;104:14-25.	Immunotoxicity	In vitro
Salihovic S, Fall T, Ganna A, Broeckling CD, Prenni JE, Hyötyläinen T, Kärrman A, Lind PM, Ingelsson E, Lind L. Identification of metabolic profiles associated with human exposure to perfluoroalkyl substances. J Expo Sci Environ Epidemiol. 2018 Sep 5.	Immunotoxicity	Metabolomics
Steenland K, Kugathasan S, Barr DB. PFOA and ulcerative colitis. Environ Res. 2018 Aug;165:317-321.	Immunotoxicity	Overlap with previous study
Suo C, Fan Z, Zhou L, Qiu J. Perfluorooctane sulfonate affects intestinal immunity against bacterial infection. Sci Rep. 2017 Jul 12;7(1):5166.	Immunotoxicity	Mice
Wang J, Pan Y, Cui Q, Yao B, Wang J, Dai J. Penetration of PFASs Across the Blood Cerebrospinal Fluid Barrier and Its Determinants in Humans. Environ Sci Technol. 2018 Nov 20;52(22):13553-13561.	Immunotoxicity	Levels in cerebrospinal fluid
Wang X, Liu L, Zhang W, Zhang J, Du X, Huang Q, Tian M, Shen H. Serum metabolome biomarkers associate low-level environmental perfluorinated compound exposure with oxidative /nitrosative stress in humans. Environ Pollut. 2017 Oct;229:168-176.	Immunotoxicity	Metabolomics
Wikström S, Lin PI, Lindh CH, Shu H, Bornehag CG. Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatr Res. 2019 Dec 13. doi: 10.1038/s41390-019-0720-1.	Immunotoxicity	No immune, liver, thyroid
Yarahalli Jayaram V, Baggavalli S, Reddy D, Sistla S, Malempati R. Effect of endosulfan and bisphenol A on the expression of SUMO and UBC9. Drug Chem Toxicol. 2018 Nov 14:1-8.	Immunotoxicity	In vitro
Zeng XW, Bloom MS, Dharmage SC, Lodge CJ, Chen D, Li S, Guo Y, Roponen M, Jalava P, Hirvonen MR, Ma H, Hao YT, Chen W, Yang M, Chu C, Li QQ, Hu LW, Liu KK, Yang BY, Liu S, Fu C, Dong GH. Prenatal exposure to perfluoroalkyl substances is associated with lower hand, foot and mouth disease viruses antibody response in infancy: Findings from the Guangzhou Birth Cohort Study. Sci Total Environ. 2019 May 1;663:60-67.	Immunotoxicity	Antibodies to hand, foot, and mouth disease; exposure to hand, foot, and mouth disease antigen is unknown

Study reference	Health outcome	Reason for exclusion[1]
Zeng XW, Li QQ, Chu C, Ye WL, Yu S, Ma H, Zeng XY, Zhou Y, Yu HY, Hu LW, Yang BY, Dong GH. Alternatives of perfluoroalkyl acids and hepatitis B virus surface antibody in adults: Isomers of C8 Health Project in China. Environ Pollut. 2019 Dec 27;259:113857.	Immunotoxicity	Hepatitis B antibody levels
Zeng Z, Song B, Xiao R, Zeng G, Gong J, Chen M, Xu P, Zhang P, Shen M, Yi H. Assessing the human health risks of perfluorooctane sulfonate by in vivo and in vitro studies. Environ Int. 2019 May;126:598 610.	Immunotoxicity	Review
Zhong SQ, Chen ZX, Kong ML, Xie YQ, Zhou Y, Qin XD, Paul G, Zeng XW, Dong GH. Testosterone-Mediated Endocrine Function and TH1/TH2 Cytokine Balance after Prenatal Exposure to Perfluorooctane Sulfonate: By Sex Status. Int J Mol Sci. 2016;17(9).	Immunotoxicity	Mice
Zhou Y, Bao WW, Qian ZM, Dee Geiger S, Parrish KL, Yang BY, Lee YL, Dong GH. Perfluoroalkyl substance exposure and urine CC16 levels among asthmatics: A case-control study of children. Environ Res. 2017;159:158-163.	Immunotoxicity	Clara cell protein (CC16) levels
Alderete T.L., Jin R., Walker D.I., Valvi D., Chen Z., Jones D.P., Peng C., Gilliland F.D., Berhane K., Conti D.V., Goran M.I., Chatzi L. Perfluoroalkyl substances, metabolomic profiling, and alterations in glucose homeostasis among overweight and obese Hispanic children: A proof-of-concept analysis. Environment International 2019 126 (445-453)	Lipids	No lipid data
Ashley-Martin J, Dodds L, Arbuckle TE, Bouchard MF, Fisher M, Morriset AS, Monnier P, Shapiro GD, Ettinger AS, Dallaire R, Taback S, Fraser W, Platt RW. Maternal Concentrations of Perfluoroalkyl Substances and Fetal Markers of Metabolic Function and Birth Weight. Am J Epidemiol. 2017 Feb 1;185(3):185-193.	Lipids	Leptin and adiponectin
Bi X, Tey SL, Loo YT, Henry CJ. 2017. Central adiposity-induced plasma-free amino acid alterations are associated with increased insulin resistance in healthy Singaporean adults. Eur J Clin Nutr 71:1080-1087.	Lipids	Not PFOA or PFOS
Braun JM, Chen A, Romano ME, Calafat AM, Webster GM, Yolton K, Lanphear BP. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME study. Obesity (Silver Spring). 2016 Jan;24(1):231-7.	Lipids	No lipid data
Cardenas A, Gold DR, Hauser R, Kleinman KP, Hivert MF, Calafat AM, Ye X, Webster TF, Horton ES, Oken E. Plasma Concentrations of Per- and Polyfluoroalkyl Substances at Baseline and Associations with Glycemic Indicators and Diabetes Incidence among High-Risk Adults in the Diabetes Prevention Program Trial. Environ Health Perspect. 2017 Oct 2;125(10):107001.	Lipids	Diabetes
Cardenas A, Hauser R, Gold DR, Kleinman KP, Hivert MF, Fleisch AF, Lin PD, Calafat AM, Webster TF, Horton ES, Oken E. Association of Perfluoroalkyl and Polyfluoroalkyl Substances With Adiposity. JAMA Netw Open. 2018 Aug 3;1(4):e181493.	Lipids	No lipid data
Convertino M, Church TR, Olsen GW, Liu Y, Doyle E, Elcombe CR, et al. 2018. Stochastic Pharmacokinetic-Pharmacodynamic Modeling for Assessing the Systemic Health Risk of Perfluorooctanoate (PFOA). Toxicol Sci 163:293-306.	Lipids	PBPK modeling; cancer patients given extremely high PFOA doses
Conway B, Innes KE, Long D. Perfluoroalkyl substances and beta cell deficient diabetes. J Diabetes Complications. 2016 Aug;30(6):993-8.	Lipids	No lipid data

Study reference	Health outcome	Reason for exclusion[1]
Das KP, Wood CR, Lin MT, Starkov AA, Lau C, Wallace KB, et al. 2017. Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology 378:37-52	Lipids	Mice
Donat-Vargas C, Bergdahl IA, Tornevi A, Wennberg M, Sommar J, Kiviranta H, Koponen J, Rolandsson O, Åkesson A. Perfluoroalkyl substances and risk of type II diabetes: A prospective nested case-control study. Environ Int. 2019 Feb;123:390-398.	Lipids	No lipid data
Fai Tse WK, Li JW, Kwan Tse AC, Chan TF, Hin Ho JC, Sun Wu RS, Chu Wong CK, Lai KP. Fatty liver disease induced by perfluorooctane sulfonate: Novel insight from transcriptome analysis. Chemosphere. 2016 Sep;159:166-177.	Lipids	Zebrafish
Fleisch AF, Rifas-Shiman SL, Mora AM, Calafat AM, Ye X, Luttmann-Gibson H, Gillman MW, Oken E, Sagiv SK. Early-Life Exposure to Perfluoroalkyl Substances and Childhood Metabolic Function. Environ Health Perspect. 2017 Mar;125(3):481-487.	Lipids	No lipid data
Hartman TJ, Calafat AM, Holmes AK, Marcus M, Northstone K, Flanders WD, Kato K, Taylor EV. Prenatal Exposure to Perfluoroalkyl Substances and Body Fatness in Girls. Child Obes. 2017 Jun;13(3):222-230.	Lipids	No lipid data
Heffernan A.L., Cunningham T.K., Drage D.S., Aylward L.L., Thompson K., Vijayasarathy S., Mueller J.F., Atkin S.L., Sathyapalan T. Perfluorinated alkyl acids in the serum and follicular fluid of UK women with and without polycystic ovarian syndrome undergoing fertility treatment and associations with hormonal and metabolic parameters. Int J Hyg Environ Health 2018 221:7 (1068-1075)	Lipids	Polycystic ovarian syndrome patients only (used for thyroid)
Hui Z, Li R, Chen L. 2017. The impact of exposure to environmental contaminant on hepatocellular lipid metabolism. Gene 622:67-71.	Lipids	Mice
Hutcheson R, Innes K, Conway B. 2019. Perfluoroalkyl substances and likelihood of stroke in persons with and without diabetes. Diab Vasc Dis Res:1479164119892223.	Lipids	Stroke
Kingsley SL, Walker DI, Calafat AM, Chen A, Papandonatos GD, Xu Y, et al. 2019. Metabolomics of childhood exposure to perfluoroalkyl substances: a cross-sectional study. Metabolomics 15:95.	Lipids	Metabolomics
Lai K.P., Ng A.H.-M., Wan H.T., Wong A.Y.-M., Leung C.C.-T., Li R., Wong C.K.-C. Dietary exposure to the environmental chemical, PFOS on the diversity of gut microbiota, associated with the development of metabolic syndrome. Frontiers in Microbiology 2018 9:OCT	Lipids	Mice
Lind L, Salihovic S, Lampa E, Lind PM. Mixture effects of 30 environmental contaminants on incident metabolic syndrome-A prospective study. Environ Int. 2017 Oct;107:8-15.	Lipids	Metabolic syndrome; chemical mixtures
Lind PM, Salihovic S, Stubleski J, Karrman A, Lind L. 2018. Changes in plasma levels of perfluoroalkyl substances (PFASs) are related to increase in carotid intima-media thickness over 10 years - a longitudinal study. Environ Health 17:59.	Lipids	Intima media thickness
Lind PM, Salihovic S, van Bavel B, Lind L. Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery atherosclerosis. Environ Res. 2017 Jan;152:157-164.	Lipids	Carotid artery atherosclerosis
Liu W, Qin H, Pan Y, Luo F, Zhang Z. 2019. Low concentrations of perfluorooctane sulfonate repress osteogenic and enhance adipogenic differentiation of human mesenchymal stem cells. Toxicol Appl Pharmacol 367:82-91.	Lipids	In vitro

Study reference	Health outcome	Reason for exclusion[1]
Lu Y., Gao K., Li X., Tang Z., Xiang L., Zhao H., Fu J., Wang L., Zhu N., Cai Z., Liang Y., Wang Y., Jiang G.Mass Spectrometry-Based Metabolomics Reveals Occupational Exposure to Per-And Polyfluoroalkyl Substances Relates to Oxidative Stress, Fatty Acid β-Oxidation Disorder, and Kidney Injury in a Manufactory in China. Environmental Science and Technology 2019 53:16 (9800-9809)	Lipids	Metabolomics
Ma Y, Yang J, Wan Y, Peng Y, Ding S, Li Y, et al. 2018. Low-level perfluorooctanoic acid enhances 3T3-L1 preadipocyte differentiation via altering peroxisome proliferator activated receptor gamma expression and its promoter DNA methylation. J Appl Toxicol 38:398-407.	Lipids	In vitro
Mattsson K, Rignell-Hydbom A, Holmberg S, Thelin A, Jönsson BA, Lindh CH, Sehlstedt A, Rylander L. Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a population-based longitudinal study. Environ Res. 2015 Oct;142:148-54.	Lipids	Coronary heart disease
McGlinchey A, Sinioja T, Lamichhane S, et al. Prenatal exposure to perfluoroalkyl substances modulates neonatal serum phospholipids, increasing risk of type 1 diabetes [published online ahead of print, 2020 Jul 4]. Environ Int. 2020;143:105935	Lipids	Lipid metabolomics
Minatoya M, Itoh S, Miyashita C, Araki A, Sasaki S, Miura R, Goudarzi H, Iwasaki Y, Kishi R. Association of prenatal exposure to perfluoroalkyl substances with cord blood adipokines and birth size: The Hokkaido Study on environment and children's health. Environ Res. 2017 Jul;156:175-182.	Lipids	Adipokines
Mitro SD, Sagiv SK, Fleisch AF, et al. Pregnancy Per- and Polyfluoroalkyl Substance Concentrations and Postpartum Health in Project Viva: A Prospective Cohort. J Clin Endocrinol Metab. 2020;105(9):dgaa431.	Lipids	No lipid information
Mora AM, Oken E, Rifas-Shiman SL, Webster TF, Gillman MW, Calafat AM, Ye X, Sagiv SK. Prenatal Exposure to Perfluoroalkyl Substances and Adiposity in Early and Mid-Childhood. Environ Health Perspect. 2017 Mar;125(3):467-473.	Lipids	No lipid data
More VR, Campos CR, Evans RA, Oliver KD, Chan GN, Miller DS, et al. 2017. PPAR-alpha, a lipid-sensing transcription factor, regulates blood-brain barrier efflux transporter expression. J Cereb Blood Flow Metab 37:1199-1212.	Lipids	Rats
Parikh A, Vacek TP. 2018. PFO closure in high-risk patient with paradoxical arterial embolism, deep vein thrombosis, pulmonary embolism and factor V Leiden genetic mutation. Oxf Med Case Reports 2018:omx105.	Lipids	Case report
Qi W, Clark JM, Timme-Laragy AR, Park Y. 2018. Perfluorobutanesulfonic acid (PFBS) potentiates adipogenesis of 3T3-L1 adipocytes. Food Chem Toxicol 120:340-345.	Lipids	In vitro
Qiu T, Chen M, Sun X, Cao J, Feng C, Li D, et al. 2016. Perfluorooctane sulfonate-induced insulin resistance is mediated by protein kinase B pathway. Biochem Biophys Res Commun 477:781-785.	Lipids	In vitro
Raymond M.R., Christensen K.Y., Thompson B.A., Anderson H.A. Associations between fish consumption and contaminant biomarkers with cardiovascular conditions among older male anglers in Wisconsin. Journal of Occupational and Environmental Medicine 2016 58:7 (676-682)	Lipids	Overlap with Christensen et al., 2016

Study reference	Health outcome	Reason for exclusion[1]
Rosen MB, Das KP, Rooney J, Abbott B, Lau C, Corton JC. 2017. PPARalpha-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology 387:95-107.	Lipids	Mice
Sakuma A, Wasada Ochi H, Yoshioka M, Yamanaka N, Ikezawa M, Guruge KS. 2019. Changes in hepato-renal gene expression in microminipigs following a single exposure to a mixture of perfluoroalkyl acids. PLoS One 14:e0210110.	Lipids	Pigs
Salihovic S., Fall T., Ganna A., Broeckling C.D., Prenni J.E., Hyötyläinen T., Kärrman A., Lind P.M., Ingelsson E., Lind L. Identification of metabolic profiles associated with human exposure to perfluoroalkyl substances. [In Process] Journal of exposure science & environmental epidemiology 2019 29:2 (196-205)	Lipids	Metabolomics
Shapiro GD, Dodds L, Arbuckle TE, Ashley-Martin J, Ettinger AS, Fisher M, Taback S, Bouchard MF, Monnier P, Dallaire R, Morisset AS, Fraser W. Exposure to organophosphorus and organochlorine pesticides, perfluoroalkyl substances, and polychlorinated biphenyls in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC Study. Environ Res. 2016 May;147:71-81.	Lipids	No lipid data
Su TC, Kuo CC, Hwang JJ, Lien GW, Chen MF, Chen PC. Serum perfluorinated chemicals, glucose homeostasis and the risk of diabetes in working-aged Taiwanese adults. Environ Int. 2016 Mar;88:15-22.	Lipids	No lipid data
Sunderland EM, Hu XC, Dassuncao C, Tokranov AK, Wagner CC, Allen JG. 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J Expo Sci Environ Epidemiol 29:131-147.	Lipids	Review
Tang W., He X., Liu Y., Xu B., Gu L. Blood perfluorooctanoate is associated with diabetes and metabolic alteration. Diabetes/Metabolism Research and Reviews 2017 33 Supplement 1	Lipids	Abstract (poster) only
Valvi D, P Weihe, P Grandjean. Cardiometabolic Risk in Young Adults Exposed to Perfluoroalkyl Substances during Critical Developmental Periods - ISEE Conference Abstracts, 2016	Lipids	No lipid data
Wang X, Liu L, Zhang W, Zhang J, Du X, Huang Q, Tian M, Shen H. Serum metabolome biomarkers associate low-level environmental perfluorinated compound exposure with oxidative /nitrosative stress in humans. Environ Pollut. 2017 Oct;229:168-176.	Lipids	Metabolomics
Yang C., Lee H.K., Kong A.P.S., Lim L.L., Cai Z., Chung A.C.K. Early-life exposure to endocrine disrupting chemicals associates with childhood obesity. Ann Ped Endocrinol Metabol 2018 23:4 182-195	Lipids	Review
Zeng Z., Song B., Xiao R., Zeng G., Gong J., Chen M., Xu P., Zhang P., Shen M., Yi H. Assessing the human health risks of perfluorooctane sulfonate by in vivo and in vitro studies. Environment International 2019 126 (598-610)	Lipids	Review
Abe T, Takahashi M, Kano M, Amaike Y, Ishii C, Maeda K, Kudoh Y, Morishita T, Hosaka T, Sasaki T, Kodama S, Matsuzawa A, Kojima H, Yoshinari K. Activation of nuclear receptor CAR by an environmental pollutant perfluorooctanoic acid. Arch Toxicol. 2017 91(6):2365-2374.	Liver	Mice
Attanasio R. Association between perfluoroalkyl acids and liver function: Data on sex differences in adolescents. Data Brief. 2019 Oct 5;27:104618.	Liver	Same as Attanasio, 2019

Study reference	Health outcome	Reason for exclusion[1]
Beggs KM, McGreal SR, McCarthy A, Gunewardena S, Lampe JN, Lau C, Apte U. The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction. Toxicol Appl Pharmacol. 2016 304:18-29.	Liver	In vitro
Chen Y, Hu W, Huang C, Hua S, Wei Q, Bai C, Chen J, Norris MB, Winn R, Yang D, Dong Q. Subchronic perfluorooctanesulfonate (PFOS) exposure induces elevated mutant frequency in an in vivo λ transgenic medaka mutation assay. Sci Rep. 2016 6:38466.	Liver	Fish
Das KP, Wood CR, Lin MT, Starkov AA, Lau C, Wallace KB, Corton JC, Abbott BD. Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology. 2017 378:37-52.	Liver	Mice
Deierlein AL, Rock S, Park S. Persistent endocrine-disrupting chemicals and fatty liver disease. Curr Environ Health Rep. 2017 4(4):439-449.	Liver	Review
Du G, Sun J, Zhang Y. Perfluorooctanoic acid impaired glucose homeostasis through affecting adipose AKT pathway. Cytotechnology. 2018 70(1):479-487.	Liver	Mice
Fai Tse WK, Li JW, Kwan Tse AC, Chan TF, Hin Ho JC, Sun Wu RS, Chu Wong CK, Lai KP. Fatty liver disease induced by perfluorooctane sulfonate: Novel insight from transcriptome analysis. Chemosphere. 2016 159:166-177.	Liver	Zebrafish
Fleisch AF, Rifas-Shiman SL, Mora AM, Calafat AM, Ye X, Luttmann-Gibson H, Gillman MW, Oken E, Sagiv SK. Early-life exposure to perfluoroalkyl substances and childhood metabolic function. Environ Health Perspect. 2017 125(3):481-487.	Liver	No liver outcomes
Foulds CE, Treviño LS, York B, Walker CL. Endocrine disrupting chemicals and fatty liver disease. Nat Rev Endocrinol. 2017 Aug;13(8):445-457.	Liver	Review
Gomis MI, Vestergren R, Borg D, Cousins IT. Comparing the toxic potency in vivo of long-chain perfluoroalkyl acids and fluorinated alternatives. Environ Int. 2018 113:1-9.	Liver	Rats
Han R, Hu M, Zhong Q, Wan C, Liu L, Li F, Zhang F, Ding W. Perfluorooctane sulphonate induces oxidative hepatic damage via mitochondria-dependent and NF-κB/TNF-α -mediated pathway. Chemosphere. 2018 Jan;191:1056-1064.	Liver	Rats
Jin R, McConnell R, Catherine C, Xu S, Walker DI, Stratakis N, Jones DP, Miller GW, Peng C, Conti DV, Vos MB, Chatzi L. Perfluoroalkyl substances and severity of nonalcoholic fatty liver in Children: An untargeted metabolomics approach. Environ Int. 2020 Jan;134:105220.	Liver	Only includes children with non-alcoholic fatty liver disease
Lai KP, Li JW, Cheung A, Li R, Billah MB, Chan TF, Wong CKC. Transcriptome sequencing reveals prenatal PFOS exposure on liver disorders. Environ Pollut. 2017 223:416-425.	Liver	Transcriptome sequencing
Liu WS, Chan HL, Lai YT, Lin CC, Li SY, Liu CK, Tsou HH, Liu TY. Dialysis membranes influence perfluorochemical concentrations and liver function in patients on hemodialysis. Int J Environ Res Public Health. 2018 15(11) (no page numbers)	Liver	Associations before and after dialysis
Liu, W. S.,Lai, Y. T.,Chan, H. L.,Li, S. Y.,Lin, C. C.,Liu, C. K.,Tsou, H. H.,Liu, T. Y.. Associations between perfluorinated chemicals and serum biochemical markers and performance status in uremic patients under hemodialysis. PLoS One. 2018. 13:e0200271	Liver	Dialysis patients

Study reference	Health outcome	Reason for exclusion[1]
Rantakokko P, Männistö V, Airaksinen R, Koponen J, Viluksela M, Kiviranta H, Pihlajamäki J. Persistent organic pollutants and non-alcoholic fatty liver disease in morbidly obese patients: a cohort study. Environ Health. 2015 Sep 29;14:79.	Liver	Bariatric surgery patients
VoPham T. Environmental risk factors for liver cancer and nonalcoholic fatty liver disease. Curr Epidemiol Rep. 2019 Mar;6(1):50-66.	Liver	Review
Yamakado M, Tanaka T, Nagao K, Imaizumi A, Komatsu M, Daimon T, Miyano H, Tani M, Toda A, Yamamoto H, Horimoto K, Ishizaka Y. Plasma amino acid profile associated with fatty liver disease and co-occurrence of metabolic risk factors. Sci Rep. 2017 7(1):14485.	Liver	No data on PFAS
Yan S, Zhang H, Guo X, Wang J, Dai J. High perfluorooctanoic acid exposure induces autophagy blockage and disturbs intracellular vesicle fusion in the liver. Arch Toxicol. 2017 91(1):247-258.	Liver	Mice
Berg V, Nøst TH, Pettersen RD, Hansen S, Veyhe AS, Jorde R, Odland JØ, Sandanger TM. Persistent Organic Pollutants and the Association with Maternal and Infant Thyroid Homeostasis: A Multipollutant Assessment. Environ Health Perspect. 2017 Jan;125(1):127-133.	Thyroid	Overlap with Berg et al., 2015
Deng M, Wu Y, Xu C, Jin Y, He X, Wan J, Yu X, Rao H, Tu W. Multiple approaches to assess the effects of F-53B, a Chinese PFOS alternative, on thyroid endocrine disruption at environmentally relevant concentrations. Sci Total Environ. 2018 May 15;624:215-224.	Thyroid	Zebrafish and rat study
Gaberščček S, Zaletel K. Epidemiological trends of iodine-related thyroid disorders: an example from Slovenia. Arh Hig Rada Toksikol. 2016 Jun 1;67(2):93-8.	Thyroid	Review
Gaudino R., Beccherle F., Cavarzere P., Lauriola S., Camilot M., Teofoli F., Vincenzi M., Rizzoli C., Antoniazzi F. Neonatal screening for congenital hypothyroidism: Analysis of a large cohort of affected patients (1987-2017) and relationship with perfluoroalkylated substances (PFAS) in Northeastern Italy. Hormone Research in Paediatrics 2019 91 Supplement 1 (94-)	Thyroid	Abstract only, limited information
Goudarzi H, Araki A, Itoh S, Sasaki S, Miyashita C, Mitsui T, Nakazawa H, Nonomura K, Kishi R. The Association of Prenatal Exposure to Perfluorinated Chemicals with Glucocorticoid and Androgenic Hormones in Cord Blood Samples: The Hokkaido Study. Environ Health Perspect. 2017 Jan;125(1):111-118.	Thyroid	No thyroid hormone data
Kim H.Y., Kim K.-N., Lee Y.A., Lim Y.-H., Kim J.I., Kim B.-N., Oh S.-Y., Hong Y.-C., Shin C.H. The relationship between perfluoroalkyl compounds concentrations at ages 2, 4, and 6 years and thyroid function in early childhood: A prospective cohort study. Hormone Research in Paediatrics 2019 91 Supplement 1 (343-)	Thyroid	Abstract only, limited information
Kishi R, Araki A, Minatoya M, Hanaoka T, Miyashita C, Itoh S, Kobayashi S, Ait Bamai Y, Yamazaki K, Miura R, Tamura N, Ito K, Goudarzi H; members of The Hokkaido Study on Environment and Children's Health. The Hokkaido Birth Cohort Study on Environment and Children's Health: cohort profile-updated 2017. Environ Health Prev Med. 2017 May 18;22(1):46.	Thyroid	Review
Liew Z, Goudarzi H, Oulhote Y. Developmental Exposures to Perfluoroalkyl Substances (PFASs): An Update of Associated Health Outcomes. Curr Environ Health Rep. 2018 Mar;5(1):1-19.	Thyroid	Review

Study reference	Health outcome	Reason for exclusion[1]
Lopez-Espinosa MJ, Mondal D, Armstrong BG, Eskenazi B, Fletcher T. Perfluoroalkyl Substances, Sex Hormones, and Insulin-like Growth Factor-1 at 6-9 Years of Age: A Cross-Sectional Analysis within the C8 Health Project. Environ Health Perspect. 2016 Aug;124(8):1269-75.	Thyroid	No thyroid hormone data
Shrestha S, Bloom MS, Yucel R, Seegal RF, Rej R, McCaffrey RJ, Wu Q, Kannan K, Fitzgerald EF. Perfluoroalkyl substances, thyroid hormones, and neuropsychological status in older adults. Int J Hyg Environ Health. 2017 Jun;220(4):679-685.	Thyroid	Same as Shrestha et al., 2015 reviewed and cited in US EPA 2016a and 2016b
Silva AV, Ringblom J, Lindh C, Scott K, Jakobsson K, Öberg M. A Probabilistic Approach to Evaluate the Risk of Decreased Total Triiodothyronine Hormone Levels following Chronic Exposure to PFOS and PFHxS via Contaminated Drinking Water [published correction appears in Environ Health Perspect. 2020 Aug;128(8):89001]. Environ Health Perspect. 2020;128(7):76001.	Thyroid	Risk assessment
Yu N, Wang X, Zhang B, Yang J, Li M, Li J, Shi W, Wei S, Yu H. Distribution of perfluorooctane sulfonate isomers and predicted risk of thyroid hormonal perturbation in drinking water. Water Res. 2015 Jun 1;76:171-80.	Thyroid	Risk assessment

Studies Identified After Initial Literature Review

Table A7.29. Studies identified after OEHHAs initial literature review (published or identified between January 2, 2020 and December 31, 2020)

Reference	Major finding	Risk assessment implications
Abraham K, Mielke H, Fromme H, et al (2020). Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Arch Toxicol 94(6):2131-47.	PFOA associated with decreases in antibody response to tetanus, diphtheria, and influenza vaccine in 1 year old children; further described in the main PHG document	Used to develop PODs for PFOA
Aimuzi R, Luo K, Huang R, et al. (2020). Perfluoroalkyl and polyfluroalkyl substances and maternal thyroid hormones in early pregnancy. Environ Pollut 264:114557	1,885 pregnant women from the Shanghai Birth Cohort; cross-sectional analyses of serum PFAS and serum thyroid hormones collected prior to 16 weeks gestation; PFOA was positively associated with FT4; no association with TSH or FT3. No associations seen for PFOS overall, although some decrease in TSH in TPO antibody positive women	No change
Ait Bamai Y, Goudarzi H, Araki A, et al. (2020). Effect of prenatal exposure to per- and polyfluoroalkyl substances on childhood allergies and common infectious diseases in children up to age 7 years: The Hokkaido study on environment and children's health [published online ahead of print, 2020 Jul 24]. Environ Int 143:105979	Continuation of the Hokkaido study (Goudarzi et al., 2016; Goudarzi et al., 2017; Kishi et al., 2015); prospective study; 2,689 mother-child pairs; maternal serum PFAS and allergy or immune-related symptoms at age 7 (questionnaire); PFOA and PFOS inversely associated with eczema. PFOS inversely associated with respiratory syncytial virus. Possible association between PFOA and respiratory syncytial virus and pneumonia. No association with rhinoconjunctivitis, wheeze, or chickenpox.	Some evidence to support immune-related effects of PFOA and PFOS, but results are mixed overall
Chen Z, Yang T, Walker DI, et al. (2020). Dysregulated lipid and fatty acid metabolism link perfluoroalkyl substances exposure and impaired glucose metabolism in young adults [published online ahead of print, 2020 Sep 3]. Environ Int 145:106091	102 mostly overweight or obese young adults from Southern California; cross-sectional analysis of serum PFAS and serum lipids; no association between PFOA and TC, LDL or TG; inverse association between PFOA and HDL; no associations with PFOS.	Small study, mixed results overall
Cohn BA, La Merrill MA, Krigbaum NY, et al. (2020). In utero exposure to poly- and perfluoroalkyl substances (PFASs) and subsequent breast cancer. Reprod Toxicol 92:112-9	Perinatal PFAS and breast cancer risk; 54 year follow-up; nested case-control (102 cases and 310 controls); elevated maternal N-ethyl-perfluorooctane sulfonamido acetic acid, a PFOS precursor, in combination with high maternal TC associated with a 3.6-fold increased risk of breast cancer (p-interaction<0.05); PFOS associated with decreased breast cancer risk; no associations seen for PFOA.	No change
Dzierlenga MW, Moreau M, Song G, et al. (2020). Quantitative bias analysis of the association between subclinical thyroid disease and two perfluoroalkyl substances in a single study. Environ Res 182:109017	Quantitative analysis of bias using physiologically-based pharmacokinetic modeling and simulations; results suggest that some links between PFOA or PFOS and hypothyroidism may be due to reverse causality (i.e., the effects of hypothyroidism on GFR and PFAS excretion).	No change

Reference	Major finding	Risk assessment implications
Fan Y, Li X, Xu Q, et al. (2020). Serum albumin mediates the effect of multiple per- and polyfluoroalkyl substances on serum lipid levels. Environ Pollut 266(Pt 2):115138	1,067 adults from NHANES 2001-2014; cross-sectional analyses of serum lipids and serum PFAS; associations seen between PFOA, PFOS, and PFNA and increased LDL and TC; effect for PFOS less at higher exposure levels when adjusted for other PFAS; PFNA and PFOS highly correlated; in weighted quantile sum regression analyses PFNA and PFOS appear to have the greatest impacts on LDL and TC; evidence of some modest mediation (<30%) of these associations by serum albumin.	Some findings support an association between PFOS and TC and LDL
Huang H, Yu K, Zeng X, et al. (2020). Association between prenatal exposure to perfluoroalkyl substances and respiratory tract infections in preschool children [published online ahead of print, 2020 Aug 29]. Environ Res 110156	344 children from the Shanghai Prenatal Cohort; cord blood PFAS and respiratory tract infections (face to face interviews/medical records, yearly follow-up) in the first five years of life; no association between PFOA or PFOS and respiratory tract infections or serum IgE levels; similar results when stratified by age and sex.	No change
Jackson-Browne MS, Eliot M, Patti M, Spanier AJ, Braun JM (2020). PFAS (per- and polyfluoroalkyl substances) and asthma in young children: NHANES 2013-2014. Int J Hyg Environ Health 229:113565	607 children ages 3-11 in the 2013-14 US NHANES; serum PFAS and parent-reported, doctor-diagnosed, asthma using a standardized questionnaire; no association between PFOA or PFOS and asthma overall; OR = 1.7 (1.0-3.0) for PFOS in children age 3-5.	Possible effect modification of PFOS and asthma association by age
Jensen RC, Andersen MS, Larsen PV, et al. (2020). Prenatal exposures to perfluoroalkyl acids and associations with markers of adiposity and plasma lipids in infancy: an Odense Child Cohort Study. Environ Health Perspect 128(7):77001	Maternal PFAS serum levels and non-fasting lipids in the offspring at ages 3 months (n=262) and 18 months (n=198); dropout rate >50%; no associations seen between PFOA or PFOS and TC and HDL at 3 or 18 months; association between PFOA and decreased LDL and increased TG in boys at 18 months but not in girls and not at 3 months.	Mostly null results; in children
Kim HY, Kim KN, Shin CH, et al. (2020). The relationship between perfluoroalkyl substances concentrations and thyroid function in early childhood: a prospective cohort study [published online ahead of print, 2020 Jun 2]. Thyroid 10.1089/thy.2019.0436	Approximately 600 children from South Korea; serum PFAS and thyroid hormones measured at ages 2, 4, and 6; no association between PFOA or PFOS and TSH after adjustment for iodine intake; association between PFOA and fT4 at age 6 in boys; association between PFOS and triiodothyronine (T3) at age 6 in boys.	Mixed results
Lebeaux RM, Doherty BT, Gallagher LG, et al. (2020). Maternal serum perfluoroalkyl substance mixtures and thyroid hormone concentrations in maternal and cord sera: The HOME Study. Environ Res. 185:109395	Pregnant women and their children from the greater Cincinnati, Ohio region; maternal (n=185) and cord blood (n=256) PFAS and thyroid hormone measurements; no clear association between PFOA or PFOS and TSH or FT4 overall; PFOS associated with increased TSH and decreased FT4 in maternal samples, although a large number of comparisons.	Mixed results
Li Y, Barregard L, Xu Y, et al. (2020). Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. Environ Health. 2020;19(1):33	1,945 adults aged 20–60 from Ronneby, Sweden, a municipality with environmental contamination to PFOS (median serum level = 157 ng/ml) and PFHxS (median =136 ng/ml); these two chemicals were highly correlated (R=0.9); associations were reported between PFOS and PFHxS with increased TC and LDL; these associations were seen using both serum levels as well as exposure estimates based on residential history; some dose-response data.	Supports an association between PFOS and TC and LDL; similarity of findings based on serum levels and residential history argues against reverse causality related to bile acids

Reference	Major finding	Risk assessment implications
Lin TW, Chen MK, Lin CC, et al. (2020). Association between exposure to perfluoroalkyl substances and metabolic syndrome and related outcomes among older residents living near a Science Park in Taiwan [published online ahead of print, 2020 Sep 9]. Int J Hyg Environ Health 230:113607	397 adults living near an industrial park in Taiwan with environmental PFAS contamination; cross-sectional analysis of serum lipids and serum PFAS; for PFOS, ORs for an elevated LDL are above 1.0 but plateau after quartile 2 (p-trend = 0.03); corresponding ORs are also elevated for PFOA but are not statistically significant.	Supports an association between PFOS and LDL
Liu G, Zhang B, Hu Y, et al. (2020). Associations of perfluoroalkyl substances with blood lipids and apolipoproteins in lipoprotein subspecies: the POUNDS-lost study. Environ Health 19(1):5	326 participants in a weight loss trial; cross-sectional and prospective (2-year follow-up); serum PFOS associated with 5-6% increase in TC at baseline, but not statistically significant (p=0.21); no association seen for PFOA; PFOA associated with 15-20% increase in TG but not statistically significant (p=0.06); no association between TG and PFOS; PFOA associated with subspecies of intermediate density lipoprotein, LDL, and HDL that contain apolipoprotein C-III.	Supports an association between PFOS and TC. Similar effect size as Steenland et al., 2009.
Preston EV, Webster TF, Claus Henn B, et al. (2020). Prenatal exposure to per- and polyfluoroalkyl substances and maternal and neonatal thyroid function in the Project Viva Cohort: A mixtures approach. Environ Int 139:105728	726 mothers and 465 neonates in Boston, Massachusetts area; PFAS and thyroid hormones measured in maternal plasma samples, and T4 in neonatal heel stick samples; statistical analyses involved weighted quantile sum (WQS) regression and Bayesian kernel machine regression (BKMR); PFAS mixture associated with lower maternal fT4, with 2-(N-ethylperfluorooctane sulfonamido) acetate, and 2-(N-methyl-perfluorooctane sulfonamido) acetate, PFOA, and PFHxS contributing most to the overall mixture effect; no association with T4 or TSH; in infants, PFAS mixture was associated with lower T4 levels, primarily in males, with PFHxS and MeFOSAA contributing most in the WQS regression, and PFHxS contributing most in the BKMR analysis.	Possible mixture effects with thyroid hormone
Salihović S, Dickens AM, Schoultz I, et al. (2020). Simultaneous determination of perfluoroalkyl substances and bile acids in human serum using ultra-high-performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 412(10):2251-9	A new method was used to simultaneously measure PFAS and bile acids in plasma; in 20 participants, PFAS were negatively associated with most bile acids.	Findings are consistent with other research showing that several PFAS, including PFOS, can suppress CYP7A1, an enzyme that controls the rate-limiting step in bile acid formation from cholesterol. These findings argue against the hypothesis that PFOS-TC associations are due to similarities in enterohepatic circulation.

Reference	Major finding	Risk assessment implications
Salihovic S, Lind L, Larsson A, Lind PM (2020). Plasma perfluoroalkyls are associated with decreased levels of proteomic inflammatory markers in a cross-sectional study of an elderly population [published online ahead of print, 2020 Sep 8]. Environ Int 145:106099	N=965, all age 70 from Sweden; proteomic analysis; PFOA and PFOS associated with several protein markers of inflammation; not associated with CRP after adjustments for sex, sample storage time, smoking, exercise, education, energy and alcohol intake, BMI, GFR, corticoid and COX-inhibitor treatment.	May provide some biologic plausibility or mechanistic information for associations between PFAS and immune function
Stratakis N, Conti DV, Jin R, et al. (2020). Prenatal exposure to perfluoroalkyl substances associated with increased susceptibility to liver injury in children [published online ahead of print, 2020 Aug 1]. Hepatology 10.1002/hep.31483	1,105 mothers and their children (median age 8 years) in Europe; PFAS measured in maternal blood and liver enzymes in child serum; analyses based on BKMR; PFAS mixture during pregnancy was associated with higher liver enzyme levels (ALT, AST, GGT) in children; effects seem to be primarily due to PFOA and PFNA.	Supports the association between PFOA and increased liver enzymes
Timmermann CAG, Jensen KJ, Nielsen F, et al. (2020). Serum perfluoroalkyl substances, vaccine responses, and morbidity in a cohort of Guinea-Bissau children. Environ Health Perspect 128(8):87002	PFOA and PFOS associated with reductions in measles antibody levels in 237 children age 9 months from Guinea-Bissau, West Africa. ORs elevated for some respiratory/infectious symptoms	Supports a causal association between PFOA and PFOS and decreases in antibody response
Xiao C, Grandjean P, Valvi D, et al. (2020). Associations of exposure to perfluoroalkyl substances With thyroid hormone concentrations and birth size. J Clin Endocrinol Metab 105(3):735-45	Faroe Islands; PFAS measured in maternal serum at 34 weeks gestation in 172 mother-child pairs; thyroid hormones measured in maternal and cord serum; maternal serum PFOS and PFOA associated with a 53% (95% CI, 18-99%) and 40% (95% CI, 8-81%) increase in cord TSH; most other results null or not statistically significant	No change
Yang Q, Guo X, Chen Y, et al. (2020). Blood levels of perfluoroalkyl substances (PFASs), elements and their associations with metabolic syndrome (MetS) in Chinese male adults mediated by metabolic-related risk factors. Sci Total Environ 742:140595	80 adult males with metabolic syndrome and 64 males without; cross-sectional analysis of serum PFAS and thyroid hormones; authors report that no associations seen with thyroid hormones although actual results are not presented	No change
Zeng XW, Li QQ, Chu C, et al. (2020). Alternatives of perfluoroalkyl acids and hepatitis B virus surface antibody in adults: Isomers of C8 Health Project in China. Environ Pollut. 259:113857	Association between PFOA and PFOS and lower hepatitis B antibody levels in 605 adults from the Isomer of C8 Health Project in China	Supports a causal association between PFOA and PFOS and decreased antibody response to vaccines
BA Cohn, MA La Merrill, NY Krigbaum, M Wang, JS Park, M Petreas, G Yeh, RC Hovey, L Zimmermann, PM Cirillo (2020). In utero exposure to poly- and perfluoroalkyl substances (PFASs) and subsequent breast cancer. Reproductive toxicology (Elmsford, N.Y.), 92	Maternal perinatal serum levels measured in 1959-67; 102 cases in daughters and 310 controls; interaction between a PFOS precursor and cholesterol reported; decreased risk with maternal PFOS. PFAS levels not measured in daughters	Mixed results overall

Reference	Major finding	Risk assessment implications
R Jin, R McConnell, C Catherine, S Xu, DI Walker, N Stratakis, DP Jones, GW Miller, C Peng, DV Conti, MB Vos, L Chatzi (2020). Perfluoroalkyl substances and severity of nonalcoholic fatty liver in Children: An untargeted metabolomics approach. Environment international, 134	74 children with non-alcoholic fatty liver disease; no healthy comparison group.; increased odds of nonalcoholic steatohepatitis compared to children with steatosis alone with increased PFOS; no association with PFOA	PFOS may be associated with increased liver disease severity; no change
P Dufour, C Pirard, P Petrossians, A Beckers, C Charlier (2020). Association between mixture of persistent organic pollutants and thyroid pathologies in a Belgian population. Environmental research, 181	Belgian adults; case-control study of hypo- and hyper- thyroidism; 79 cases and 160 controls; PFOS and PFOA associated with lower ORs of both hypo- and hyper- thyroidism	Mixed results
G Liu, B Zhang, Y Hu, J Rood, L Liang, L Qi, GA Bray, L DeJonge, B Coull, P Grandjean, JD Furtado, Q Sun (2020). Associations of Perfluoroalkyl substances with blood lipids and Apolipoproteins in lipoprotein subspecies: the POUNDS-lost study. Environmental health : a global access science source, 19(1)	326 men and women from the Prevention of Obesity Using Novel Dietary Strategies Lost randomized trial; serum PFOS associated with some increase in TC (p=0.21); no association with PFOA	Supports association between PFOS and lipid alterations
RM Lebeaux, BT Doherty, LG Gallagher, RT Zoeller, AN Hoofnagle, AM Calafat, MR Karagas, K Yolton, A Chen, BP Lanphear, JM Braun, ME Romano (2020). Maternal serum perfluoroalkyl substance mixtures and thyroid hormone concentrations in maternal and cord sera: The HOME Study. Environmental research, 185	468 pregnant women and their children in the greater Cincinnati, Ohio region; serum PFAS concentrations during pregnancy and maternal (n = 185) and cord (n = 256) thyroid hormone levels; generally null results although some indication of effect modification by anti-thyroid antibodies for both PFOA and PFOS and decreased free T4	No change
EV Preston, TF Webster, B Claus Henn, MD McClean, C Gennings, E Oken, SL Rifas-Shiman, EN Pearce, AM Calafat, AF Fleisch, SK Sagiv (2020). Prenatal exposure to per- and polyfluoroalkyl substances and maternal and neonatal thyroid function in the Project Viva Cohort: A mixtures approach. Environment international, 139	726 mothers and 465 neonates from the Boston, Massachusetts area; maternal plasma PFAS and thyroid hormones collected during early pregnancy, and neonatal postpartum heel stick thyroxine levels; overlap with Preston et al (2018); focus on PFAS mixtures; overall mixed results	No change
HY Kim, KN Kim, CH Shin, YH Lim, JI Kim, BN Kim, YC Hong, YA Lee (2020). The Relationship Between Perfluoroalkyl Substances Concentrations and Thyroid Function in Early Childhood: A Prospective Cohort Study. Thyroid : official journal of the American Thyroid Association, 30(11),	Serum PFAS and TSH in 660 children at 2, 4, or 6 years of age; inverse association between PFOA and TSH in boys although somewhat reduced after adjustment for iodine intake	No change
R Aimuzi, K Luo, R Huang, X Huo, M Nian, F Ouyang, Y Du, L Feng, W Wang, J Zhang, (2020). Perfluoroalkyl and polyfluroalkyl substances and maternal thyroid hormones in early pregnancy. Environmental pollution (Barking, Essex : 1987), 264	1885 pregnant women in the Shanghai Birth Cohort; PFAS and thyroid hormones in maternal blood collected prior to 16 weeks of gestation; PFOA positively associated with free T4; no clear associations for PFOS	No change

Reference	Major finding	Risk assessment implications
RC Jensen, MS Andersen, PV Larsen, D Glintborg, C Dalgård, CAG Timmermann, F Nielsen, MB Sandberg, HR Andersen, HT Christesen, P Grandjean, TK Jensen (2020). Prenatal Exposures to Perfluoroalkyl Acids and Associations with Markers of Adiposity and Plasma Lipids in Infancy: An Odense Child Cohort Study. Environmental health perspectives, 128(7)	649 women and children from the Odense Cohort study; serum lipids measured at 3 and 18 months of age; maternal PFAS measured at various times in pregnancy; mostly no associations	Study in children; no change
Y Fan, X Li, Q Xu, Y Zhang, X Yang, X Han, G Du, Y Xia, X Wang, C Lu (2020). Serum albumin mediates the effect of multiple per- and polyfluoroalkyl substances on serum lipid levels. Environmental pollution (Barking, Essex : 1987), 266(Pt 2)	NHANES 2011-14; 1,067 adults; PFOS and PFOA associated with HDL, LDL, and TC with associations greater for PFOS and PFHxS; evidence of some mediation (16-27%) by serum albumin	Supports association between PFOS and serum lipids; no new data for dose-response
C Canova, G Barbieri, M Zare Jeddi, M Gion, A Fabricio, F Daprà, F Russo, T Fletcher, G Pitter (2020). Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region. Environment international, 145	Serum PFAS and lipids in 15,720 adults from a regional health study in Veneto region, Italy, an area with water contamination by PFOA; associations identified between PFOA and PFOS with TC, LDL, and HDL; greater effects for PFOS than PFOA	Supports PFOS and lipid associations; dose response data by deciles are available although no obvious major advantage over Steenland et al. (2009); evidence that associations for PFOS not solely due to PFOA
H Gardener, Q Sun, P Grandjean (2021). PFAS concentration during pregnancy in relation to cardiometabolic health and birth outcomes. Environmental research, 192	433 pregnant women enrolled in the US Vanguard Pilot Study of the National Children's Study; PFAS, TC, and TG in third trimester serum; PFOS associated with increased TC and TG; PFOA associated with increased TG but not TC	Supports PFOS-TC association; dose-response data in figure form only
J Yang, H Wang, H Du, H Fang, M Han, L Xu, S Liu, J Yi, Y Chen, Q Jiang, G He (2020). Serum perfluoroalkyl substances in relation to lipid metabolism in Chinese pregnant women. Chemosphere	436 pregnant women in Tangshan City, North China; serum levels of PFAS in first term, serum lipids in third term; possible decrease in LDL/HDL ratio for PFOA otherwise no clear associations	Findings in pregnant women, no change
OE Omoike, RP Pack, HM Mamudu, Y Liu, S Strasser, S Zheng, J Okoro, L Wang (2020). Association between per and polyfluoroalkyl substances and markers of inflammation and oxidative stress. Environmental research	NHANES 2005-2012; 6,652 adults; associations between PFOS and PFOA with some inflammatory markers including neutrophil and lymphocyte count but not CRP	Supports association between PFOA and PFOS and immune toxicity; no change
P Grandjean, CAG Timmermann, M Kruse, F Nielsen, PJ Vinholt, L Boding, C Heilmann, K Mølbak (2020). Severity of COVID-19 at elevated exposure to perfluorinated alkylates. medRxiv : the preprint server for health sciences	Plasma PFAS and health registry data in 323 adults from Denmark with known Covid-19 infection; no increase in infection severity with increased PFOS or PFOA	No change
Y Tian, M Miao, H Ji, X Zhang, A Chen, Z Wang, W Yuan, H Liang (2021). Prenatal exposure to perfluoroalkyl substances and cord plasma lipid concentrations. Environmental pollution (Barking, Essex : 1987), 268(Pt A)	Maternal plasma PFAS at 12-16 weeks and cord blood lipid levels; PFOS associated with decreased TC; no clear association with PFOA	Findings are in neonates; no change

Reference	Major finding	Risk assessment implications
H Liang, Z Wang, M Miao, Y Tian, Y Zhou, S Wen, Y Chen, X Sun, W Yuan (2020). Prenatal exposure to perfluoroalkyl substances and thyroid hormone concentrations in cord plasma in a Chinese birth cohort. Environmental health : a global access science source, 19(1)	300 mother-infant pairs from the Shanghai-Minhang Birth Cohort Study; PFAS and thyroid hormones in maternal plasma at 12-16 weeks gestation; mixed and inconsistent effects overall	No change
M van Gerwen, N Alpert, M Alsen, K Ziadkhanpour, E Taioli, E Genden (2020). The Impact of Smoking on the Association between Perfluoroalkyl Acids (PFAS) and Thyroid Hormones: A National Health and Nutrition Examination Survey Analysis. Toxics, 8(4)	NHANES 2011-12; 1,325 adults; positive association between PFOS and free T4, primarily in non-smokers; no associations for PFOA	No change
Y Li, Y Xu, T Fletcher, K Scott, C Nielsen, D Pineda, CH Lindh, DS Olsson, EM Andersson, K Jakobsson (2020). Associations between perfluoroalkyl substances and thyroid hormones after high exposure through drinking water. Environmental research, 194	3,297 participants from Ronneby, a municipality with drinking water highly contaminated by PFAS and a reference group (n=226) from a nearby municipality with non-contaminated drinking water supply; overall no clear associations except PFOS and PFOA associated with increased free T4 in males over 50 years old	No change
T Dalla Zuanna, DA Savitz, G Barbieri, G Pitter, M Zare Jeddi, F Daprà, ASC Fabricio, F Russo, T Fletcher, C Canova (2021). The association between perfluoroalkyl substances and lipid profile in exposed pregnant women in the Veneto region, Italy. Ecotoxicology and environmental safety, 209	319 pregnant women from Veneto region, Italy, an area with PFOA drinking water contamination; serum PFAS and lipids collected at various times during pregnancy; fairly large increases in TC and LDL from first to third trimester; PFOS was positively associated with TC in the first trimester, mixed results otherwise; an inverse relationship seen between PFOA both TC and LDL-C in the third trimester	Findings in pregnant women; no change
N Li, Y Liu, GD Papandonatos, AM Calafat, CB Eaton, KT Kelsey, KM Cecil, HJ Kalkwarf, K Yolton, BP Lanphear, A Chen, JM Braun (2021). Gestational and childhood exposure to per- and polyfluoroalkyl substances and cardiometabolic risk at age 12 years. Environment international, 147	PFAS in serum during pregnancy, at birth, and at ages 3, 8, and 12 years from 221 mother-child pairs in the HOME Study; 2003-06, Cincinnati, Ohio; HDL, TGs and other biomarkers measured in children at age 12 years old; positive association between PFOS at 8 and 12 years old and HDL; no clear associations with TGs or with PFOA	Findings are in children; TC or LDL not measured; no change
M Averina, J Brox, S Huber, AS Furberg (2021). Exposure to perfluoroalkyl substances (PFAS) and dyslipidemia, hypertension and obesity in adolescents. The Fit Futures study. Environmental research, 195	940 adolescents in Norway; serum PFAS and lipids; positive association between PFOS and TC and LDL; overlap with Averina et al (2019)	Supports association between PFOS and lipid alterations
J Guo, J Zhang, Z Wang, L Zhang, X Qi, Y Zhang, X Chang, C Wu, Z Zhou (2021). Umbilical cord serum perfluoroalkyl substance mixtures in relation to thyroid function of newborns: Findings from Sheyang Mini Birth Cohort Study. Chemosphere, 273	490 mother-newborn pairs from the Sheyang Mini Birth Cohort Study, recruited between June 2009 and January 2010; PFAS and thyroid hormones measured in cord blood; PFOS associated with increased total T4 and contributed 46% to the PFAS mixture effect; PFOA also associated with free T4 and contributed 29% to the PFAS mixture effect	PFOS associated with increased total and free T4 in newborns; no change

Reference	Major finding	Risk assessment implications
L Dalsager, N Christensen, U Halekoh, CAG Timmermann, F Nielsen, HB Kyhl, S Husby, P Grandjean, TK Jensen, HR Andersen (2021). Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Child Cohort. Environment international, 149	PFAS serum levels in first trimester pregnant women from the Odense Child Cohort (Denmark) were measured in 2010-2012; data on child hospitalizations at ages 0-4 years old for infectious disease obtained from the Danish National Patient Register; appears to be an expansion of Dalsager et al (2016); 1,503 mother-child pairs; a doubling in maternal PFOS was associated with a 23% increase in the risk of hospitalization due to any infection (hazard ratio (HR): 1.23 (95% CI: 1.05, 1.44); a doubling of PFOA or PFOS increased the risk of lower respiratory tract infection by 27% (HR: 1.27 (1.01, 1.59)) and 54% (HR: 1.54 (1.11, 2.15)), respectively	Supports the association between PFOS and PFOA and immune toxicity
AL Bjorke-Monsen, K Varsi, M Averina, J Brox, S Huber (2020). Perfluoroalkyl substances (PFASs) and mercury in never-pregnant women of fertile age: association with fish consumption and unfavorable lipid profile. BMJ nutrition, prevention & health, 3(2)	158 Norwegian women (not pregnant) ages 18-39 years old; serum PFAS and lipids; PFOS associated with increased TC and LDL; no associations seen for PFOA	Supports the association between PFOS and lipid alterations; no new data for dose-response
CM Bulka, V Avula, RC Fry (2021). Associations of exposure to perfluoroalkyl substances individually and in mixtures with persistent infections: Recent findings from NHANES 1999-2016. Environmental pollution (Barking, Essex : 1987), 275	8,778 individuals (3,189 adolescents; 5,589 adults) in NHANES 1999-2016; serum concentrations of PFAS and antibodies to cytomegalovirus, Epstein Barr virus, hepatitis C and E, herpes simplex 1 and 2, HIV, T. gondii, and Toxocara spp; seropositivity summed to calculate a pathogen burden score; both PFOA and PFOS associated with a higher pathogen burden scores	Supports the association between PFOS and PFOA and immune toxicity

APPENDIX 8. KEY CHARACTERISTICS OF CARCINOGENS

The key characteristics (KCs) of carcinogens (Smith et al., 2016; IARC, 2020) were used to organize the mechanistic data relevant to carcinogenicity from studies of PFOA and PFOS. OEHHA utilized the KCs concept to systematically identify, organize, and summarize mechanistic information. Human carcinogens often share one or more KCs, and act through multiple mechanisms. Therefore, the KCs approach allows for a broader consideration of possible mechanistic pathways and hypotheses based on the available evidence. In the case of the mechanistic data currently available for PFOA and PFOS, OEHHA reviewed the evidence identified through literature searches on five of the KCs (2, 5, 7, 8, and 10).

KC2: Is genotoxic

Genotoxicity refers to the ability of a chemical or other type of agent or biological process to damage DNA or induce changes in the DNA sequence. The link between genotoxicity and carcinogenesis is well established (Smith et al., 2016; Smith et al., 2020). Changes in the DNA sequence include gene or point mutations, such as base substitutions, frameshifts and small deletions or insertions, and chromosomal effects, such as chromosomal aberrations, micronuclei, and aneuploidy. Examples of DNA damage include DNA adducts, DNA strand breaks, and DNA-DNA and DNA-protein crosslinks.

Genotoxicity studies of PFOA and PFOS have been reviewed and summarized in detail by IARC (2017a) for PFOA and by the European Food Safety Authority (EFSA, 2008; EFSA, 2018) for PFOA and PFOS. IARC (2017a) stated that “PFOA is not DNA-reactive,” based on negative findings in a large number of assays assessing direct genotoxic activity, while noting that “some studies [of PFOA] indicate that indirect DNA damage may result from induction of oxidative stress.” In reviewing the genotoxicity studies for PFOA and PFOS, EFSA (2018) concluded “the available data are inconclusive,” noting that there is “some evidence that the observed effects [genotoxicity] are related to oxidative stress” and “[f]rom in vitro and in vivo genotoxicity studies, there is no evidence for a direct genotoxic mode of action for both PFOS and PFOA, however, genotoxicity cannot be excluded.”

OEHHA identified additional studies relevant to the genotoxicity of PFOA that were not included in the IARC (2017a) and EFSA (2008, 2018) evaluations, and additional studies relevant to the genotoxicity of PFOS that were not included in the EFSA (2008, 2018) assessments. These additional studies are discussed separately below, first for PFOA and then for PFOS. Findings from all of the studies relevant to genotoxicity are summarized in Tables A8.1, A8.2, and A8.3 for PFOA, and in Tables A8.4, A8.5, and A8.6 for PFOS.

PFOA

OEHHA identified seven additional PFOA studies and reports that were not included in the IARC (2017a) and EFSA reviews (Governini et al., 2015; Lu et al., 2016; Franken et al., 2017; Peropadre et al., 2018; Crebelli et al., 2019; Li et al., 2019; NTP, 2019a). Two of these studies are of PFOA exposed humans (Governini et al., 2015; Franken et al., 2017), and a third is in a human cell line (Peropadre et al., 2018). One study in exposed humans examined associations between a number of chemicals and reported a statistically significant association between serum PFOA concentrations and increased DNA damage (single- and double-strand breaks and alkali-labile sites, measured in the alkaline comet assay) in the peripheral blood cells of Flemish teenagers (14-15 years old) (Franken et al., 2017). This association was no longer significant

after correction for multiple hypothesis testing. No association was observed between serum PFOA and oxidative damage to DNA bases in peripheral blood cells, measured by the formamidopyrimidine DNA glycosylase (FPG)-modified comet assays, or between serum PFOA and urinary levels of the oxidized DNA base 8-OHdG. Regression models were adjusted for sex, age, smoking status, maximum body temperature seven days before sample collection (alkaline comet assay only), BMI (FPG-modified comet assay only), and highest education level of family (FPG-modified comet assay only).

The second additional genotoxicity study in exposed humans, Governini et al. (2015), examined chromosomal effects, namely disomy and aneuploidy of chromosomes 18, X and Y in human sperm obtained from patients at a fertility clinic in Italy, associated with the presence of PFOA and/or PFOS in seminal plasma and whole blood. Study participants with detectable levels of either PFOA or PFOS in blood and seminal plasma (58% of the study population) were considered "PFC [perfluorinated compound] positive." Limits of detection for PFOA and PFOS were reported as 3 ng/g and 1.5 ng/g, respectively. In men with detectable levels of these compounds in seminal plasma, mean values were 7.68 ± 0.78 ng/g for PFOA and 5.37 ± 0.45 ng/g for PFOS. In men with detectable levels of these compounds in whole blood, mean values were 8.03 ± 1.04 ng/g for PFOA and 7.07 ± 0.66 ng/g for PFOS. The authors reported increased aneuploidy, chromosome 18 disomy, and total disomies in the "PFC positive" group, compared to the PFC negative group. Because this study did not report results specific to either PFOA or PFOS, it is not included in Table A8.1.

In a study using a p53-deficient human skin cell line (HaCaT keratinocytes), PFOA at 50 µM induced DNA damage, as detected by immunofluorescence staining of γ-H2AX foci. Increased staining for γ-H2AX was observed immediately after the 24-hour exposure period, and persisted for 8 days post-exposure, during which time the cells were cultured in PFOA-free medium (Peropadre et al., 2018). Although several other studies (see Tables A8.1 and A8.2) have reported that PFOA may induce DNA damage as a result of increased oxidative stress, Peropadre et al. (2018) noted, "Our results were not entirely consistent with this assumption because DNA damage [increased staining for γ-H2AX] found in HaCaT cells immediately after a moderate treatment with PFOA was not concomitant with significant oxidative stress, as determined by 8-OHdG immunostaining." An increase in 8-OHdG staining, a biomarker of oxidative damage to DNA, was observed 8 days post-exposure, but not immediately after the 24-hour exposure period (Peropadre et al., 2018).

Three additional publications report genotoxicity findings observed in rodents exposed to PFOA (Crebelli et al., 2019; Li et al., 2019; NTP, 2019a). In the NTP studies, a statistically significant increase in micronucleated reticulocytes was observed in the peripheral blood of male Sprague Dawley rats treated with PFOA via gavage, at a dose range of 0.625 to 10 mg/kg-day for 28 days (dose-related trend, $p \leq 0.025$), but no effects were observed in female Sprague Dawley rats (NTP, 2019a). The lack of effects in female rats might be attributed to the much faster PFOA clearance rate compared to male rats (see Table A6.4). No effect was observed in immature erythrocytes of either sex. The authors note that the increase in micronucleated reticulocytes is significant compared to concurrent controls, but within historical control data. NTP (2019a) also reported that PFOA did not induce bacterial reverse mutations in *S. typhimurium* TA98, TA100, or in *E. coli* (WP2 uvRA pKM101). A second publication reported that gavage exposure of pregnant Kunming mice to PFOA at daily doses of 0, 1, 2.5, 5, or 10 mg/kg body weight from GD 1-17 resulted in a dose-dependent increase in 8-OHdG in the liver of female offspring, when assessed on PND 21 (Li et al., 2019). A third publication reported negative genotoxicity results in mice given PFOA in drinking water for five weeks (Crebelli et al.,

2019). Specifically, no increases in MN formation were observed in splenocytes or reticulocytes, and no increases in DNA strand breaks (assessed by the comet assay) were observed in the liver or testis.

The seventh additional genotoxicity study used a cell-free system to measure DNA charge transfer in 15-base pair double-stranded (ds) DNA oligonucleotides in the presence and absence of PFOA (Lu et al., 2016). PFOA increased the DNA charge transfer resistance in this system, and the authors interpreted this as resulting from a loosening of the duplex DNA structure and a change in the DNA base pair stacking by PFOA. The authors postulate that the perfluorinated alkyl carbon chain of PFOA binds to the groove in the DNA double helix and disrupts the hydrogen binding of G to C and A to T by forming hydrogen bonds with fluorine (F) (Lu et al., 2016).

Findings from studies relevant to the genotoxicity of PFOA, including those reviewed by IARC and EFSA (see IARC (2017a): Table 4.2 on pages 78-79, EFSA (2018): Table 18 on pages 118-119, and EFSA (2008): pages 88-89) are presented in Tables A8.1, A8.2, and A8.3 below.

Table A8.1. Genotoxicity studies of PFOA in humans and other mammals

Test endpoint	Species assayed	Route, duration, dosing regimen	Results	Reference
Micronuclei	Mouse	5,000 mg/kg-day	No significant changes in polychromatic erythrocytes in bone marrow	Murli (1995) as reported by IARC (2017a)[1]
Micronuclei	Mouse	A single dose of 950 mg/kg via oral gavage	No significant changes in polychromatic erythrocytes in bone marrow	Murli (1996) as reported by IARC (2017a) and (EFSA, 2008)[2]
Micronuclei	Mouse: male C57BL/6 (6-8/dose)	0, 0.55, 5.5, or 28 mg/L in drinking water for 5 weeks (0, 0.1, 1, or 5 mg/kg-day)	No significant changes in reticulocytes or splenocytes	Crebelli et al. (2019)
Micronuclei	Rat: female Sprague Dawley (10/dose)	0, 6.25, 12.5, 25, 50, or 100 mg/kg-day via oral gavage for 28 days	No significant changes in reticulocytes or immature erythrocytes	NTP (2019a)
Micronuclei	Rat: male Sprague Dawley (10/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day via oral gavage for 28 days	Increased micronucleated reticulocytes in a dose-dependent manner; no significant changes in immature erythrocytes	NTP (2019a)

Test endpoint	Species assayed	Route, duration, dosing regimen	Results	Reference
DNA strand breaks (alkaline comet assay, and FPG-modified comet assay)	Human: 14- to 15-year-old Flemish subjects (N=196 for alkaline comet assay; N=193 for FPG-modified comet assay)	Exposure effect estimate in blood: 1.090 (95% CI, 1.015-1.170) µg/L for alkaline comet assay; 1.012 (95% CI, 0.871-1.175) µg/L for FPG-modified comet assay	Increased serum PFOA levels associated with increased DNA damage in peripheral blood cells, as measured by alkaline comet assay; not significant after correction for multiple hypothesis testing. No association with breaks caused by oxidative DNA damage, as measured by FPG-modified comet assay.	Franken et al. (2017)
DNA strand breaks (comet assay)	Mouse: male C57BL/6 (6-8/dose)	0, 0.55, 5.5, or 28 mg/L in drinking water for 5 weeks (0, 0.1, 1, or 5 mg/kg-day)	No significant changes in liver or testis	Crebelli et al. (2019)
Oxidative damage to DNA (8-OHdG in urine)	Human: 14- to 15-year-old Flemish subjects (N=195)	Exposure effect estimate in blood: 1.053 (95% CI, 0.968-1.147) µg/L	No significant association with PFOA	Franken et al. (2017)
Oxidative damage to DNA (8-OHdG in liver)	Mice: female Kunming offspring (6/dose)	Pregnant dams treated with 0, 1, 2.5, 5, or 10 mg/kg via gavage from GD 1-17; offspring assessed at PND 21	Dose-dependent and significant increases in liver 8-OHdG in 2.5, 5 and 10 mg/kg groups	Li et al. (2019)
Oxidative damage to DNA (8-OHdG in liver)	Rat: male Fischer 344 (5/dose/time point)	A single i.p. injection of 100 mg/kg; samples were collected after 1, 3, 5, and 8 days	Significant increases of 8-OHdG in liver after 3, 5, and 8 days	Takagi et al. (1991), also reviewed in IARC (2017a) and EFSA (2008)
Oxidative damage to DNA (8-OHdG in kidney)	Rat: male Fischer 344 (5/dose/time point)	A single i.p. injection of 100 mg/kg; samples were collected after 1, 3, 5, and 8 days	No increase of 8-OHdG in kidney	Takagi et al. (1991), also reviewed in IARC (2017a)
Oxidative damage to DNA (8-OHdG in liver)	Rat: male Fischer 344 (5/dose)	0.02% in the diet for 2 weeks	Significant increases of 8-OHdG in liver	Takagi et al. (1991), also reviewed in IARC (2017a) and EFSA (2008)
Oxidative damage to DNA (8-OHdG in kidney)	Rat: male Fischer 344 (5/dose)	0.02% in the diet for 2 weeks	No increase of 8-OHdG in kidney	Takagi et al. (1991), also reviewed in IARC (2017a)

8-OHdG, 8-hydroxydeoxyguanosine; GD, gestation day; i.p., intraperitoneal; PND, postnatal day

[1] OEHHA has no access to Murli (1995), which was summarized by IARC (2017a). The IARC (2017a) summary of the study did not include information on study duration or the number of animals per treatment group.
[2] OEHHA has no access to Murli (1996), which was summarized by EFSA (2008) and IARC (2017a). Neither the EFSA (2008) summary nor the IARC (2017a) summary of the study included information on the number of animals per treatment group.

Table A8.2. Genotoxicity studies of PFOA in mammalian cell lines

Test endpoint	Species/ cell line	Concentration/ duration	Results/comments	Reference
Mutation (CD59 gene locus)	Human-hamster hybrid (A_L) cells	0-200 µM, 16 days	Positive at 200 µM for 16 days; negative at 0-200 µM for 1-8 days	Zhao et al. (2011), also reviewed in IARC (2017a) and EFSA (2018)
Mutation (CD59 gene locus)	Mitochondrial DNA-deficient human-hamster hybrid (ρ^0A_L) cells	0-200 µM, 16 days	Negative	(Zhao et al., 2011), also reviewed in IARC (2017a) and EFSA (2018)
Mutation (*Hprt* locus)	K-1 Chinese hamster ovary cells	Up to 94 µM (-S9, +S9)	Negative with or without S9	Sadhu (2002), as reported by IARC (2017a) and EFSA (2008)[1]
Micronuclei	Human hepatoma HepG2 cells	0, 50-400 µM, 24 hours	Positive at ≥100 µM	Yao and Zhong (2005), also reviewed in IARC (2017a) and EFSA (2018)
Micronuclei	Human hepatoma HepG2 cells	0, 50-400 µM, 1 or 24 hours	Negative; significant cytotoxicity at ≥200 µM	Florentin et al. (2011), also reviewed in IARC (2017a) and EFSA (2018)
Micronuclei	Chinese hamster V79 lung cells	10 µM (-S9, +S9), 3 hours (+21 hours post-incubation)	Negative	Buhrke et al. (2013), also reviewed in IARC (2017a) and EFSA (2018)
Chromosomal aberrations	Human lymphocytes	Up to 3,640 µM (-S9, +S9)	Negative with or without S9	Murli (1996a), as reported by IARC (2017a)[2]

Test endpoint	Species/ cell line	Concentration/ duration	Results/comments	Reference
Chromosomal aberrations	Chinese hamster ovary cells	Up to 2,000 µM	Positive at 2,000 µM in 3-hour treatment with S9 and harvest time 20 hours after initiation of treatment	Murli (1996b), as reported by IARC (2017a) and EFSA (2008)[3]
Chromosomal aberrations	Chinese hamster ovary cells	Up to 3,740 µM	Positive at 3,740 µM in 3-hour treatment with S9 and harvest time 20 hours after initiation of treatment	Murli (1996c), as reported by IARC (2017a) and EFSA (2008)[4]
Polyploidy	Chinese hamster ovary cells	Up to 2,000 µM	Positive at 2,000 µM in 3-hour treatment with S9 and harvest time 44 hours after initiation of treatment	Murli (1996b), as reported by IARC (2017a) and EFSA (2008)[3]
Polyploidy	Chinese hamster ovary cells	Up to 3,740 µM (-S9); 4,970 µM (+S9)	Positive at 3,740 µM in 3-hour treatment without S9 (4,970 µM (+S9)) and harvest time 44 hours after initiation of treatment	Murli (1996c), as reported by IARC (2017a) and EFSA (2008)[4]
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 50-400 µM, 1 hour	Positive at ≥50 µM	Yao and Zhong (2005), also reviewed in IARC (2017a) and EFSA (2018)
DNA strand breaks and FPG-sensitive sites (comet assay)	Human hepatoma HepG2 cells	0, 100, 400 µM, 24 hours	Negative; all tested doses were not cytotoxic	Eriksen et al. (2010), also reviewed in IARC (2017a) and EFSA (2018)
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 50-400 µM, 1 or 24 hours	Negative; significant cytotoxicity at ≥200 µM	Florentin et al. (2011), also reviewed in IARC (2017a) and EFSA (2018)
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 0.2-20 µM, 24 hours	Positive at ≥10 µM	Wielsoe et al. (2015), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Human lymphoblastoid (TK6) cells	0, 125, 250, 500 µg/ml, 2 hours	Positive at ≥250 µg/ml; the authors stated that cells were viable (as measured by trypan blue)	Yahia et al. (2014), also reviewed in EFSA (2018)

Test endpoint	Species/ cell line	Concentration/ duration	Results/comments	Reference
DNA strand breaks (comet assay)	Syrian hamster embryo (SHE) cells	0, 0.00037-300 µM (0-124 µg/ml), 5 or 24 hours	Negative	Jacquet et al. (2012b), also reviewed in EFSA (2018)
DNA damage (γ-H2AX)	Human epidermal p53-deficient (HaCaT) keratinocytes	50 µM for 24 hours	Positive at 50 µM for 24 hours; increased staining for γH2AX persisted for 8 days	Peropadre et al. (2018)
Oxidative damage to DNA (8-OHdG)	Human hepatoma HepG2 cells	0, 50-400 µM, 3 hours	Positive at ≥100 µM	Yao and Zhong (2005), also reviewed in (IARC, 2017a) and EFSA (2018)
Oxidative damage to DNA (8-OHdG)	Human lymphoblastoid (TK6) cells	0, 125, 250, 500 µg/ml, 2 hours	Positive at ≥250 µg/ml; the authors stated that cells were viable (as measured by trypan blue)	Yahia et al. (2014), also reviewed in EFSA (2018)
Oxidative damage to DNA (8-OHdG)	Human epidermal p53-deficient (HaCaT) keratinocytes	50 µM for 24 hours	Negative at 24 hours; positive at 8 days	Peropadre et al. (2018)

[1] OEHHA has no access to Sadhu (2002), which was summarized by EFSA (2008) and IARC (2017a). Neither the EFSA (2008) summary nor the IARC (2017a) summary of the study included information on treatment duration.
[2] OEHHA has no access to Murli (1996a), which was summarized by IARC (2017a). The IARC (2017a) summary of the study did not include information on treatment duration.
[3] OEHHA has no access to Murli (1996b), which was summarized by EFSA (2008) and IARC (2017a). Neither the EFSA (2008) summary nor the IARC (2017a) summary of the study included information on treatment duration.
[4] OEHHA has no access to Murli (1996c), which was summarized by EFSA (2008) and IARC (2017a). Neither the EFSA (2008) summary nor the IARC (2017a) summary of the study included information on treatment duration.

Table A8.3. Genotoxicity studies of PFOA in non-mammalian systems

Test endpoint	Test system	Concentration	Results/comments	Reference
Mutation	*Saccharomyces cerevisiae*	Up to 500 µg/plate	Negative with or without S9	Griffith and Long (1980), also reviewed in IARC (2017a)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 1,000 µg/plate	Negative with or without S9	Griffith and Long (1980), also reviewed in IARC (2017a)

Test endpoint	Test system	Concentration	Results/comments	Reference
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Up to 5,000 µg/plate	Negative with or without S9	Lawlor (1995, 1996), as reported by IARC (2017a) and EFSA (2008)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	5 µmol/plate	Negative with or without S9	Buhrke et al. (2013), also reviewed in IARC (2017a) and EFSA (2018)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA102, TA104	100, 500 µM (up to 207 µg/plate)	Negative with or without S9	Fernández Freire et al. (2008), also reviewed in IARC (2017a) and EFSA (2018)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98 and TA100	0-1,000 µg/plate without 10% rat liver S9; 0-5,000 µg/plate with 10% rat liver S9	Negative in TA100 with or without S9; negative in TA98 with S9; equivocal in TA98 without S9	NTP (2019a)
Reverse mutation assay (*umu* test)	*S. typhimurium* TA1535/pSK1002 (*hisG46, rfa, uvrB*)	Up to 414 µg/plate	Negative with or without S9	Oda et al. (2007), as reported by IARC (2017a)
Reverse mutation assay	*E. coli* (WP2 *uvrA*)	Up to 5,000 µg/plate	Negative with or without S9	Lawlor (1995, 1996), as reported by IARC (2017a) and EFSA (2008)
Reverse mutation assay	*E. coli* (WP2 uvRA pKM101)	0-1,000 µg/plate without 10% rat liver S9; 0-10,000 µg/plate with 10% rat liver S9	Negative with or without S9	NTP (2019a)
DNA strand breaks (alkali-labile sites, apurinic/ apyrimidinic (AP) sites) (comet assay, pH ≥13)	*Paramecium caudatum*	0, 10, 30, 100 µM (1, 3, 24 hours); 0, 100 µM (6, 12 hours)	Positive at 100 µM for 12 and 24 hours	Kawamoto et al. (2010), also reviewed in IARC (2017a) and EFSA (2018)

Test endpoint	Test system	Concentration	Results/comments	Reference
DNA strand breaks (comet assay, pH=12.1)	*Paramecium caudatum*	0, 10, 30, 100 µM (24 hours)	Negative	Kawamoto et al. (2010), also reviewed in IARC (2017a) and EFSA (2018)
DNA strand breaks (comet assay)	Green mussel (*Perna viridis*)	0, 0.01-1,000 µg/L (7 days)	Positive at ≥1,000 µg/L	Liu et al. (2014), also reviewed in EFSA (2018)
Oxidative damage to DNA (8-OHdG)	*Paramecium caudatum*	0, 10, 30, 100 µM (1, 3 hours); 0, 10, 30 µM (24 hours)	Negative	Kawamoto et al. (2010), also reviewed in IARC (2017a) and EFSA (2018)
Altered DNA structure (DNA charge transfer)	Cell-free; synthesized double-stranded (ds) DNA oligonucleotides (15-mers)	1.00×10^{-8} µM to 100 µM	Increased DNA charge transfer resistance with a positive linear dose-response (R=0.996) between 10^{-8} to 100 µM. Authors interpret this as an indication of the loosening of duplex DNA structure and a change in DNA base pair stacking.	Lu et al. (2016)

As shown in Table A8.3, PFOA is not mutagenic in bacterial assays conducted in multiple strains of *S. typhimurium* and *E. coli*, or in the yeast *S. cerevisae*. PFOA induced mutations at the *CD59* gene locus in human-hamster hybrid (A_L) cells (with normal levels of mitochondrial DNA) after long-term (16 days) exposure (Zhao et al., 2011), but not in K-1 Chinese hamster ovary (CHO) cells at the *Hprt* locus (Table A8.2).

In studies detecting chromosomal effects (Tables A8.2 and A8.3), PFOA increased micronuclei (MN) in vivo in male rat reticulocytes (NTP, 2019a), but not in female rat reticulocytes or in mouse reticulocytes, splenocytes, or bone marrow. PFOA increased MN in human hepatoma HepG2 cells in one study (Yao and Zhong, 2005), but not another (Florentin et al., 2011) and no increase was observed in Chinese hamster lung V79 cells (Buhrke et al., 2013; IARC, 2017a). PFOA induced chromosomal aberrations (CA) (and polyploidy) in CHO cells in two studies, and did not increase CA in human lymphocytes exposed in vitro (IARC, 2017a).

In several studies, PFOA induced DNA damage, measured as increases in DNA strand breaks, γ-H2AX, and 8-OHdG (IARC, 2017a; EFSA, 2018). In one study in humans, increased serum levels of PFOA were associated with increased levels of DNA strand breaks as measured in the alkaline comet assay, although the authors reported that the association was no longer significant after correction for multiple hypothesis testing (Franken et al., 2017). No increase in

DNA strand breaks was observed in the liver or testis of mice exposed to PFOA (Crebelli et al., 2019). Among four studies conducted in human hepatoma HepG2 cells, DNA strand breaks were assessed at 1 hour in one study and at 24 hours in three studies (Yao and Zhong, 2005; Eriksen et al., 2010; Florentin et al., 2011; Wielsoe et al., 2015). Increases were observed in the study that assessed DNA strand breaks in HepG2 cells at 1 hour, and in one of the three studies that assessed DNA strand breaks in HepG2 cells at 24 hours. The lowest concentration tested in the two studies that did not observe increases in DNA strand breaks at 24 hours was higher than the highest concentration tested in the study that did observe increases. PFOA also increased DNA strand breaks in human lymphoblastoid (TK6) cells (Yahia et al., 2014), but not in Syrian hamster embryo cells (Jacquet et al., 2012b). In non-mammalian systems, PFOA induced DNA strand breaks in green mussels and *Paramecium caudatum* (Kawamoto et al., 2010; Liu et al., 2014). PFOA increased staining for γ-H2AX, another marker of DNA damage, in HaCaT keratinocytes, and this effect persisted for 8 days following cessation of exposure (Peropadre et al., 2018). With regard to 8-OHdG, no association was observed between PFOA serum levels and urinary levels of 8-OHdG in one study in humans (Franken et al., 2017). In rodents exposed to PFOA, levels of 8-OHdG were increased in the liver of female Kunming mice exposed in utero (Li et al., 2019), and in the liver but not the kidney of rats exposed either via a single i.p. injection, or via the diet for two weeks (Takagi et al., 1991). PFOA increased 8-OHdG levels in human HepG2 and TK6 cells (Yao and Zhong, 2005; Yahia et al., 2014). 8-OHdG was not increased in human HaCaT keratinocytes immediately after a 24-hour PFOA exposure, but levels were increased 8 days later (Peropadre et al., 2018). No increase in 8-OHdG was observed in *Paramecium caudatum* (Liu et al., 2014).

PFOS

OEHHA identified four genotoxicity publications on PFOS that were not included in the EFSA (2008, 2018) reviews (Lu et al., 2012; Chen et al., 2016; Eke et al., 2017; NTP, 2019b). One fish study showed that a 30-day exposure to PFOS, followed by a 15-day exposure to clean water induced mutations in a target gene present in the liver of transgenic medaka fish (Chen et al., 2016). These authors noted that PFOS induced “a distinct mutational spectrum dominated by +1 frameshift mutations” in the target gene in the liver of exposed fish. One rat study reported that PFOS administered at doses of 0, 0.6, 1.25, or 2.5 mg/kg every 48 hours via oral gavage over a four-week period increased MN and DNA damage (DNA strand breaks, as measured by the comet assay) in a dose-dependent manner in liver hepatocytes of male rats (Eke et al., 2017). A set of studies conducted by NTP reported findings from bacterial mutagenicity assays and 28-day MN studies in rats exposed by gavage (NTP, 2019b). PFOS was not mutagenic in the two strains of *Salmonella* in which it was tested (TA98, TA100) or in the *E. coli* strain WP2 *uvrA*/pkM101, in either the presence or absence of metabolic activation (S9). A statistically significant increase in MN was observed in polychromatic erythrocytes in the peripheral blood of female rats in the high dose group, with a significant dose-related trend, following exposure to PFOS (NTP, 2019b). However, the increases in MN were within the historical control range, and thus NTP considered the findings in female rats to be equivocal. No increases in MN were observed in similarly exposed male rats (NTP, 2019b). Dose dependent decreases in the percentages of polychromatic erythrocytes in peripheral blood were observed in rats of both sexes following 28-day administration of PFOS, suggesting that the bone marrow is a target of PFOS cytotoxicity (NTP, 2019b). In addition, one electrochemical study of the interaction of PFOS with calf thymus DNA immobilized on a specially prepared carbon electrode found that PFOS binds to the groove in the DNA double helix, intercalates into the DNA, and forms hydrogen bonds with DNA bases, perturbing base pair stacking and reducing DNA charge transport (Lu et al., 2012).

Findings from studies relevant to the genotoxicity of PFOS, including those reviewed by EFSA (see EFSA (2008): page 73, and EFSA (2018): Table 17 on pages 116-117) are presented in Tables A8.4, A8.5, and A8.6 below.

Table A8.4. Genotoxicity studies of PFOS in mammals

Test endpoint	Species assayed	Route, duration, dosing regimen	Results	Reference
Mutation	Mouse: male *gpt* delta transgenic (6/dose)	0, 1.5, 4, or 10 mg/kg via gavage for 28 days	Increase of the *red/gam* locus mutation frequencies in the liver at ≥4 mg/kg	Wang et al. (2015), also reviewed in EFSA (2018)
Micronuclei	Mouse: male and female	A single oral dose of 237.5, 450, or 950 mg/kg, with sampling at 24, 48, or 72 hours	Negative	Corning Hazleton, Inc. (1993), as reported by EFSA (2008)[1]
Micronuclei	Mouse: male *gpt* delta transgenic (6/dose)	0, 1.5, 4, or 10 mg/kg via gavage for 28 days	Non-significant increase of MN frequency in the liver at ≥4 mg/kg	Wang et al. (2015), also reviewed in EFSA (2018)
Micronuclei	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased MN frequency of polychromatic erythrocytes in bone marrow at ≥1.25 mg/kg	Celik et al. (2013), also reviewed in EFSA (2018)
Micronuclei	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased MN frequency in peripheral blood cells at ≥0.6 mg/kg	Eke and Celik (2016), also reviewed in EFSA (2018)
Micronuclei	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Dose-dependent increases in MN frequency in hepatocytes at ≥0.6 mg/kg	Eke et al. (2017)
Micronuclei	Rat: female Sprague Dawley (10/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	Dose-dependent increases in MN in polychromatic erythrocytes in peripheral blood; increases are within historical control range. Also reported a dose-dependent decrease in percentage of polychromatic erythrocytes in peripheral blood.	NTP (2019b)
Micronuclei	Rat: male Sprague Dawley (10/dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via gavage for 28 days	No increase in MN in polychromatic erythrocytes in peripheral blood; dose-dependent decrease in percentage of polychromatic erythrocytes in peripheral blood	NTP (2019b)
DNA strand breaks (comet assay)	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased DNA damage (strand breaks) in bone marrow at ≥0.6 mg/kg	Celik et al. (2013), also reviewed in EFSA (2018)

Test endpoint	Species assayed	Route, duration, dosing regimen	Results	Reference
DNA strand breaks (comet assay)	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased DNA damage (strand breaks) in peripheral blood cells at ≥0.6 mg/kg	Eke and Celik (2016), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Rat: male Swiss albino (Wistar) (6/dose)	0, 0.6, 1.25, or 2.5 mg/kg via gavage every 48 hours over a 4-week period	Increased DNA damage (strand breaks) in hepatocytes at ≥0.6 mg/kg	Eke et al. (2017)

[1] OEHHA has no access to Corning Hazleton, Inc. (1993), which was summarized by EFSA (2008). The EFSA (2008) summary of the study did not include information on the number of animals per treatment group.

Table A8.5. Genotoxicity studies of PFOS in mammalian cell lines

Test endpoint	Species/cell line	Concentration/ duration	Results/comments	Reference
Mutation (*redBA/gam* gene locus (Spi$^-$ assay))	*gpt* delta transgenic mouse embryonic fibroblasts	0, 0-20 µM, 24 hours	Positive at ≥10 µM	Wang et al. (2015), also reviewed in EFSA (2018)
Micronuclei	Human hepatoma HepG2 cells	0, 5-300 µM, 24 hours	Negative	Florentin et al. (2011), also reviewed in EFSA (2018)
Chromosomal aberrations	Human peripheral blood lymphocytes	Up to 599 µg/ml (-S9); up to 449 µg/ml (+S9)	Negative with or without S9	Cifone (1999), as reported by EFSA (2008)[1]
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 5-300 µM, 24 hours	Negative	Florentin et al. (2011), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Human hepatoma HepG2 cells	0, 0.2-20 µM, 24 hours	Positive at ≥0.02 µM	Wielsoe et al. (2015), also reviewed in EFSA (2018)
DNA strand breaks and FPG-sensitive sites (comet assay)	Human hepatoma HepG2 cells	0, 100, 400 µM, 24 hours	Negative	Eriksen et al. (2010), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Syrian hamster embryo cells	0, 0.00037-93 µM, 5 or 24 hours	Negative	Jacquet et al. (2012a), also reviewed in EFSA (2018)
DNA damage (γ-H2AX)	*gpt* delta transgenic mouse embryonic fibroblasts	0, 0-20 µM, 24 hours	Positive at 20 µM	Wang et al. (2015), also reviewed in EFSA (2018)

Test endpoint	Species/cell line	Concentration/ duration	Results/comments	Reference
Unscheduled DNA synthesis	Primary cultured rat liver cells	Up to 4,000 µg/ml	Negative	Cifone (1999), as reported by EFSA (2008)[1]

[1] OEHHA has no access to Cifone (1999), which was summarized by EFSA (2008). The EFSA (2008) summary of the study did not report the numeric results for chromosomal aberrations or unscheduled DNA synthesis observed at each treatment concentration.

Table A8.6. Genotoxicity studies of PFOS in non-mammalian systems

Test endpoint	Test system	Concentration	Results/comments	Reference
Mutation (c*II* gene locus)	Fish: λ transgenic medaka (7-12/conc.)	0, 6.7, 27.6, or 87.6 µg/L in water for 30 days	Dose-dependent increase in mutations in liver, as measured in the *cII* transgene, with a distinct mutational spectrum dominated by +1 frameshift mutations at ≥6.7 µg/L	Chen et al. (2016)
Reverse mutation assay (Ames test)	*S. typhimurium* TA98, TA100, TA1535, TA1537	Up to 5,000 µg/plate	Negative with or without S9	Mecchi (1999), as reported by EFSA (2008)[1]
Reverse mutation assay (Ames test)	*S. typhimurium* TA09, TA100, TA1535, TA1537, TA1538	0.01-500 µg/plate (-S9); 0.1-500 µg/plate (+S9)	Negative with or without S9	Litton Bionetics, Inc. (1978), as reported by EFSA (2008)[2]
Reverse mutation assay (Ames test)	*S. typhimurium* TA98 and TA100	0-10,000 µg/plate with or without 10% rat liver S9	Negative with or without S9	NTP (2019b)
Reverse mutation assay	*E. coli (WP2 uvRA pKM101)*	0-5,000 µg/plate with or without 10% rat liver S9	Negative with or without S9	NTP (2019b)
Reverse mutation assay	*E. coli* (WP2 uvRA)	Up to 5,000 µg/plate	Negative with or without S9	Mecchi (1999), as reported by EFSA (2008)[1]
Mitotic recombination	*Saccharomyces cerevisiae* (D4)	Not reported	Negative	Litton Bionetics, Inc. (1978), as reported by EFSA (2008)[2]
Micronuclei	Zebrafish	0, 0.4-1.6 mg/L, 30 days incubation of embryos	Positive in peripheral blood cells at ≥0.8 mg/L	Du et al. (2014), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Zebrafish	0, 0.4-1.6 mg/L, 30 days incubation of embryos	Positive in peripheral blood cells at ≥0.4 mg/L	Du et al. (2014), also reviewed in EFSA (2018)

Test endpoint	Test system	Concentration	Results/comments	Reference
DNA strand breaks (comet assay)	Gull eggs (*Larus michahellis*)	0, 100, 200 ng/g egg (injected)	Negative	Parolini et al. (2016), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Green mussel (*Perna viridis*)	0, 0.01-1,000 µg/L for 7 days	Positive at ≥1,000 µg/L	Liu et al. (2014), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	Earthworms (*E. foetida*)	0, 0.25-8 µg/cm^3, 48 hours	Positive at ≥0.25 µg/cm^3	Xu et al. (2013), also reviewed in EFSA (2018)
DNA strand breaks (comet assay)	*Paramecium caudatum*	0, 10, 30, 100 µM (1, 3 hours); 0, 10, 30 µM (24 hours)	Negative	Kawamoto et al. (2010), also reviewed in EFSA (2018)
DNA damage (Hus-1: GFP Focus)	*Caenorhabditis elegans*	0, 0.25-25µM, 12-60 hours	Positive at ≥0.25 µM, 24 hours, in germ cell nuclei	Guo et al. (2016), also reviewed in EFSA (2018)
Altered DNA structure (DNA charge transfer)	Cell-free; calf thymus DNA	10 µmol/L, 30 min, 37^{o}C	Positive; increased DNA charge transfer resistance. Authors interpret this as an indication of the loosening of duplex DNA structure and change in DNA base pair stacking	Lu et al. (2012)

[1] OEHHA has no access to Mecchi (1999), which was summarized by EFSA (2008). The EFSA (2008) summary of the studies did not report the numeric results for mutations observed at each treatment concentration.
[2] OEHHA has no access to Litton Bionetics, INC. (1978), which was summarized by EFSA (2008). The EFSA (2008) summary of the studies did not report the numeric results for mutations or recombination events at each treatment concentration.

As shown in Tables A8.6, PFOS was not mutagenic in bacterial assays conducted in multiple strains of *S. typhimurium* and *E. coli*. PFOS induced mutations in the liver of gpt delta transgenic mice at the *redBA/gam* locus (Wang et al., 2015) and transgenic fish at the c*II* gene locus (Chen et al., 2016) after long-term exposure (28 and 30 days, respectively), and at the *redBA/gam* locus in *gpt* delta transgenic mouse embryonic fibroblast cells in vitro (Wang et al., 2015).

With regard to chromosomal effects, a number of studies in rodents and one study in fish (Table A8.4) have reported increases in MN following long-term exposure (28 or 30 days) to PFOS. A 28-day exposure to PFOS increased MN in male Wistar rat bone marrow polychromatic erythrocytes (Celik et al., 2013), peripheral blood cells (Eke and Celik, 2016), and hepatocytes (Eke et al., 2017), and in female Sprague Dawley rat polychromatic erythrocytes (in a dose-dependent manner, although the levels were within the historical control range) but not in male

Sprague Dawley rat polychromatic erythrocytes (NTP, 2019b). A 28-day exposure to PFOS increased MN in hepatocytes of male transgenic mice, although the increase did not reach statistical significance (Wang et al., 2015), whereas a single oral dose of PFOS did not increase MN in mice (EFSA, 2008). A 30-day exposure to PFOS increased MN in peripheral blood cells of zebrafish (Du et al., 2014). PFOS did not increase MN in human hepatoma HepG2 cells (Yao and Zhong, 2005), CA in human peripheral blood lymphocytes exposed in vitro (Buhrke et al., 2013; IARC, 2017a), or mitotic recombination in *S. cerevisae*.

In several studies, PFOS induced DNA damage, measured as increases in DNA strand breaks, γ-H2AX, and foci of Hus-1 (Eke et al., 2017; EFSA, 2018). In male Wistar rats exposed for 28 days, PFOS significantly increased DNA strand breaks as measured in the comet assay in bone marrow (Celik et al., 2013), peripheral blood cells (Eke and Celik, 2016), and hepatocytes (Eke et al., 2017). PFOS also increased DNA strand breaks as measured in the comet assay in the peripheral blood cells of zebrafish following a 30-day exposure, and in green mussels and earthworms, but not in gull eggs or *Paramecium caudatum* (EFSA, 2018). In vitro, PFOS increased DNA strand breaks in one of three studies conducted in human hepatoma HepG2 cells (Table A8.5). The lowest concentration tested in the two HepG2 studies (Eriksen et al., 2010; Florentin et al., 2011) that did not observe increases in DNA strand breaks was higher than the highest concentration tested in the one HepG2 study (Wielsoe et al., 2015) that did observe increases. PFOS did not increase DNA strand breaks in Syrian hamster embryo cells (Jacquet et al., 2012a). PFOS increased γ-H2AX, a marker for DNA damage, in transgenic mouse embryonic fibroblasts (Wang et al., 2015), and increased the number of foci of the DNA damage checkpoint protein Hus-1 in germ cells of *C. elegans* (strain *hus-1:gfp*) (Guo et al., 2016). No increase in unscheduled DNA synthesis was observed in primary liver cell cultures (EFSA, 2008).

Summary of evidence

PFOA has been tested in many genotoxicity test systems that have assessed numerous endpoints indicative of either mutagenicity, chromosomal effects, or DNA damage. Several studies provide evidence that PFOA causes DNA damage, measured as increases in DNA strand breaks (in human cell lines and in non-mammalian species), γ-H2AX (in a human cell line), and 8-OHdG (in human cell lines and in rodent liver). Some studies provide evidence that PFOA may have chromosomal effects, while others do not, and several studies provide evidence that PFOA is not mutagenic.

PFOS has also been tested in many genotoxicity test systems that have assessed numerous endpoints indicative of either mutagenicity, chromosomal effects, or DNA damage. Some studies provide evidence that PFOS is mutagenic (in transgenic mice and fish and transgenic mouse cells), several studies provide evidence of chromosomal effects (e.g., induction of MN in rodents and zebrafish), and several studies provide evidence of DNA damage (e.g., induction of DNA strand breaks in rats, zebrafish, and other non-mammalian species).

KC5: Induces oxidative stress

Oxidative stress refers to a condition of an imbalance between the production and elimination of reactive oxygen and nitrogen species (ROS, RNS). Oxidative stress may contribute to carcinogenic processes by causing DNA mutations, chromosomal damage, genomic instability, and altered cell cycle regulation (Reuter et al., 2010).

There are a number of studies conducted in either whole animals or a variety of in vitro systems that have investigated whether PFOA and PFOS induce oxidative stress. Findings from several of these studies are briefly summarized here, including studies that have looked at 8-OHdG, a marker of oxidative damage to DNA that is linked to mutagenesis and carcinogenesis, and other oxidative damage to DNA, ROS or RNS production, malondialdehyde (MDA, a marker of lipid peroxidation), total antioxidant capacity (TAC), antioxidant enzyme activities (e.g., superoxide dismutase (SOD), catalase (CAT)), and glutathione status (e.g., reduced glutathione (GSH), glutathione disulfide (GSSG), GSH/GSSG ratios). Studies reporting on PFOA and PFOS induced oxidative DNA damage, e.g., 8-OHdG formation, have also been discussed under KC2.

PFOA

Studies assessing the effect of PFOA on levels of 8-OHdG provide evidence relevant to both KC5 and KC2 and are included in Tables A8.1, A8.2, and A8.3 (see discussion of KC2, above). A brief summary of the findings from these studies of PFOA on 8-OHdG levels follows. In one study in humans, no associations were observed between PFOA serum levels and either urinary levels of 8-OHdG or oxidative damage to DNA in peripheral blood cells (measured in the FPG-modified comet assay) (Franken et al., 2017). Increased levels of 8-OHdG were observed in the liver of PND 21 female Kunming mice exposed in utero from GD 1-17 at maternal PFOA doses of 2.5 mg/kg-day and above (Li et al., 2019) and in the liver, but not the kidney of rats exposed either via a single i.p. injection of 100 mg/kg, or via the diet (0.02%) for two weeks (Takagi et al., 1991). PFOA increased 8-OHdG levels in human liver HepG2 cells following exposure to 250 µg/ml and above for two hours (Yahia et al., 2014) and human lymphoblastoid (TK6) cells following exposure to 100 µM and above for three hours (Yao and Zhong, 2005). 8-OHdG was not increased in human epidermal p53-deficient keratinocytes immediately after a 24-hour PFOA exposure, but levels were increased eight days later (Peropadre et al., 2018). No increase in 8-OHdG was observed in *Paramecium caudatum* (Liu et al., 2014).

PFOA significantly increased hepatic levels of ROS, measured as hydrogen peroxide, in male Kunming mice exposed to 5 or 10 mg/kg-day PFOA for 14 days (Yang et al., 2014). The ability of PFOA to significantly increase ROS in HepG2 cells was observed in four studies (at concentrations as low as 0.2 µM in one study and 0.4 µM in another) (Panaretakis et al., 2001; Hu and Hu, 2009; Eriksen et al., 2010; Wielsoe et al., 2015), but not in a fifth (at concentrations up to 400 µM) (Florentin et al., 2011). Exposure of human-hamster hybrid (A_L) cells to PFOA for one day at 100 µM and above increased intracellular levels of ROS and RNS, and similar increases were also observed after longer exposures (4 and 16 days) (Zhao et al., 2011). Significant increases in intracellular ROS were also observed in mouse Leydig tumor cells (mLTC-1) after exposure to PFOA at 50 µM and above for 24 hours (Zhao et al., 2017) and in *Paramecium caudatum* after exposure at 100 µM for one hour and 10 µM for 24 hours (Kawamoto et al., 2010).

PFOA has been observed to increase lipid peroxidation, measured as MDA, in the liver of male Kunming mice exposed orally for 14 consecutive days at doses of 1.5, 5, or 10 mg/kg-day (Yang et al., 2014). An increase in MDA was observed in human erythrocytes exposed for 3 hours to 100 µM PFOA (Pan et al., 2018). In addition, PFOA was observed to increase MDA in rat PC12 cells (an adrenal pheochromocytoma-derived, neuronotypic cell line) at a concentration of 10 µM in undifferentiated cells, and at concentrations of 10 µM and 250 µM in differentiating cells (Slotkin et al., 2008; Pan et al., 2018).

Increased levels of the antioxidant enzymes SOD and CAT were observed in the liver of PND 21 female Kunming mice exposed in utero from GD 1-17 at maternal doses of 2.5 mg/kg-day and above for SOD, and 5 mg/kg-day and above for CAT (Li et al., 2019). In human erythrocytes exposed for 3 hours, GSH levels were decreased at PFOA concentrations of 10 µM and above and CAT and glutathione peroxidase activities were decreased at 100 µM, while no change in SOD activity was observed (Pan et al., 2018). The effect of PFOA on antioxidant enzyme activity in HepG2 cells has been reported in two studies. In the first, PFOA exposure for 48 hours resulted in decreases in GSH levels and glutathione peroxidase activity at concentrations of 100 µM and above, and increases in SOD, CAT, and glutathione reductase activities at concentrations of 150 µM and above (Hu and Hu, 2009). In the second, PFOA exposure for 24 hours decreased measures of TAC at concentrations of 0.02 µM and above (Wielsoe et al., 2015). Exposure of male Japanese medaka fish *Oryzias latipes* to PFOA at concentrations of 50 or 100 mg/L for 7 days decreased hepatic CAT activity, but had no effect on SOD or glutathione peroxidase activity (Yang, 2010).

PFOS

PFOS increased intracellular hepatic levels of ROS in a dose-dependent manner in male Sprague Dawley rats exposed to 1 or 10 mg/kg-day PFOS for 28 days (Han et al., 2018). The ability of PFOS to significantly increase ROS in HepG2 cells was observed in three studies (at concentrations as low as 0.2 µM in one study and 0.4 µM in another) (Hu and Hu, 2009; Eriksen et al., 2010; Wielsoe et al., 2015). In a study of isolated rat hepatocytes, ROS formation was increased after incubation with 25 µM PFOS for one hour (Khansari et al., 2017). Similar to PFOA, significant increases in intracellular ROS were observed in mLTC-1 (mouse Leydig tumor) cells after exposure to PFOS at 50 µM and above for 24 hours (Zhao et al., 2017). Additionally, ROS levels were increased in a dose-dependent manner in *C. elegans* exposed to PFOS concentrations of 0.25, 2.5, and 25 µM (Guo et al., 2016).

PFOS has been observed to increase lipid peroxidation, measured as MDA, in the liver of male Sprague Dawley rats exposed to 1 or 10 mg/kg-day PFOS for 28 days (Han et al., 2018). PFOS also increased levels of MDA (or thiobarbituric acid-reactive substances) in undifferentiated and differentiating rat PC12 cells at concentrations of 10 µM and above, and 50 µM and above, respectively (Slotkin et al., 2008) and in isolated rat hepatocytes following a 3-hour incubation with 25 µM PFOS (Khansari et al., 2017).

In male Sprague Dawley rats exposed to PFOS orally (1 or 10 mg/kg-day) for 28 days, alterations in the liver content and form of glutathione were observed, as well as alterations in liver antioxidant enzyme activities (Han et al., 2018). Specifically, decreases in GSH, increases in GSSG, and decreases in the ratio of GSH:GSSG were observed in both the 1 and 10 mg/kg-day dose groups, along with decreases in CAT activity in both dose groups and decreases in SOD activity in the 10 mg/kg-day dose group (Han et al., 2018). The effect of PFOS on antioxidant enzyme activity in HepG2 cells has been investigated in two studies. In the first, PFOS exposure for 48 hours resulted in decreases in GSH and glutathione peroxidase activity at concentrations of 100 µM and above, decreases in glutathione-S-transferase activity at 200 µM, and increases in SOD, CAT, and glutathione reductase activities at concentrations of 150 µM and above (Hu and Hu, 2009). In the second, PFOS exposure for 24 hours slightly and non-statistically significantly decreased measures of TAC at concentrations of 0.2-20 µM (Wielsoe et al., 2015).

Summary of Evidence

A number of studies indicate that PFOA may cause oxidative stress. Two studies in rodents and two studies in human cells have shown that PFOA led to increased 8-OHdG, a marker of oxidative DNA damage, while one study in human cells reported mixed results and two studies (one in exposed humans and one in a unicellular organism) found no effect. Several studies, including one in mice, four in human HepG2 cells, and one in a mouse cell line, have shown that PFOA increased intracellular production of ROS, while a study in human-hamster hybrid cells showed increased intracellular production of both ROS and RNS. Increased lipid peroxidation was observed in mice, in human erythrocytes exposed in vitro, and in a rat cell line. PFOA also has been shown to alter TAC, antioxidant enzyme content or activity, and glutathione levels in mice, fish, and in human erythrocytes and HepG2 cells.

A number of studies indicate that PFOS may cause oxidative stress. Several studies, including one in rats, three in human HepG2 cells, two in rodent cells or cell lines, and one in *C. elegans*, have shown that PFOS increased intracellular production of ROS. Increased lipid peroxidation was observed in one study in rats and two studies in rat cells or cell lines. PFOS also has been shown to alter antioxidant enzyme activity and glutathione levels in one study in rats and two studies in human HepG2 cells.

KC7: Is immunosuppressive

Immunosuppression can result in a reduction in the capacity of the immune system to respond effectively to tumor cells. Immunosuppression may allow neoplastic cells to escape immune surveillance and permit the survival and replication of these cells to form tumors (Smith et al., 2020). Both the innate and adaptive parts of the immune system participate in immune surveillance, i.e., recognition and removal of malignant cells. The innate immune system is the first line of defense, and key components of the innate (or natural) immune system include natural immunoglobulin M (IgM) antibody-producing B1 or $CD5^+$ cells, macrophages, mast cells, dendritic cells, and natural killer (NK) cells (Vollmers and Brandlein, 2009). The adaptive immune system consists of a heterogeneous population of infiltrating lymphocytes such as T cells and other immune cells to modulate the anti-tumor response (Neeve et al., 2019).

Both natural IgM (produced by B1 cells and marginal zone cells) and adaptive IgM (synthesized by B2 cells) play important roles in the cancer immune response. Natural IgM eliminates tumor cells when they begin to transform; adaptive IgM eliminates tumor cells during growth (Diaz-Zaragoza et al., 2015). Natural IgM antibodies recognize and bind to tumor-specific surface antigens and induce apoptosis via induction of cellular stress, for example by cross-linking of modified anti-complement receptors, blocking of growth-factor receptors, or by increasing the intracellular level of neutral lipids (Vollmers and Brandlein, 2009). NK cells are effector lymphocytes that control several types of tumors and microbial infections by limiting their spread and subsequent tissue damage. Functions of NK cells, including the control of tumor development, can be dependent on their interaction with dendritic cells, macrophages, T cells and endothelial cells (Vivier et al., 2008). NK cells have been observed to induce tumor cell apoptosis through interferon gamma (IFN-γ) and perforin release (Neeve et al., 2019). Dendritic cells are antigen-presenting cells (i.e., they capture tumor antigen) and are capable of activating naive T cells to differentiate into tumor antigen-specific $CD4^+$ helper T cells or to $CD8^+$ cytotoxic T cells. Neutrophils are key cellular mediators of the innate immune response (Qazi et al., 2009a). Results from various studies suggest that tumor-associated neutrophils have anti-tumor properties, including the ability to induce cytotoxicity and inhibit metastasis. Conversely,

other studies point to a tumor-supporting role of neutrophils (Uribe-Querol and Rosales, 2015; Shaul and Fridlender, 2019).

This section summarizes data for immunosuppressive effects of PFOA and PFOS that are relevant to carcinogenesis. Some studies discussed below have also been summarized earlier in Section 5.1 of this document.

PFOA

Effects on T cell dependent and independent antibody response (TDAR/TIAR)

The T cell dependent antibody response (TDAR) assesses immune function in rodents. TDAR focuses on the humoral arm of adaptive immunity and a response requires antigen recognition and presentation, T and B cell signaling, and class switching (DeWitt et al., 2012). TDAR can detect immunosuppression across a range of cell types and signals, usually measured as changes in levels of IgM or IgG production (DeWitt et al., 2012).

The response of IgM, which peaks at days 7 to 14, precedes the response of IgG, which peaks at days 14 to 21 (Lebrec et al., 2011). Assessing IgM TDAR to an antigen is a sensitive measure of immune function, as it requires T cells, B cells, and antigen-presenting cells to function properly to elicit an antibody response (DeWitt et al., 2008). T cell dependent release of IgM (also known as adaptive IgM) has been associated with recognition of breast cancer antigens and priming of the subsequent adaptive immune response (Diaz-Zaragoza et al., 2015). While TDAR measures antibody production resulting from the combined action of T and B cells, another assay, T cell independent IgM antibody response (TIAR), assesses B cell-specific antibody production (DeWitt et al., 2012).

Here, relevant results from animal studies using TDAR and TIAR to assess immune function after PFOA administration are briefly described.

- Suppression of TDAR: Reductions of IgM (but not IgG) titers in adult female C57BL/6J mice (30 mg/kg-day PFOA for 10 or 15 days via gavage), and dose-dependent reduction of IgM by 7-29% in adult female C57BL/6N mice (3.75, 7.5, 15 or 30 mg/kg-day PFOA for 15 days in drinking water) in the presence of antigen challenge with sheep red blood cells (SRBCs) (DeWitt et al., 2008).
- Suppression of TDAR: Reductions of IgM (but not IgG) titers in adult female C57BL/6N wild-type and PPARα knockout mice exposed to 30 mg/kg-day PFOA for 15 days (no effect at 7.5 mg/kg-day) and antigen challenge with SRBCs (DeWitt et al., 2016).
- Suppression of TIAR: Reductions of IgM in female BALB/c mice following gavage treatment with PFOA (20 mg/kg-day) and antigen challenge with trinitrophenol (TNP), and inhibition of general immunoglobulin formation following challenge with ovalbumin (Vetvicka and Vetvickova, 2013).
- Suppression of TIAR: Suppression of IgM in all but the lowest dose in female C57BL/6N mice exposed to 0.94, 1.88, 3.75, or 7.5 mg/kg-day PFOA for 15 days and immunized with a T cell independent antigen dinitrophenyl-ficoll (DNP) on day 11, with sera collected 7 days later (DeWitt et al., 2016).

Effects on T cell and B cell cellularity or proliferation

PFOA has been shown to reduce the number and proliferation of thymocytes and splenocytes in mice in multiple studies. Reduced spleen and thymus weights can be reflective of reductions in splenocytes and thymocytes, respectively, although moderate caloric restriction is also known to reduce thymic and splenic weight and cellularity (Qazi et al., 2009a; Qazi et al., 2009b).

- Reduction of thymocytes in female BALB/c mice following gavage treatment with PFOA (20 mg/kg-day for 7 days) and reduced mitogen-stimulated proliferation of T cells and B cells (Vetvicka and Vetvickova, 2013).
- Reduction of thymocytes and splenocytes by 85% and 80%, respectively, in male C57BL/6 mice exposed via diet (0.02% PFOA weight/weight) for 7 days; altered ratio of thymocyte subpopulations, with 95% decrease in the number of $CD4^+CD8^+$ cells; 64% and 72% decrease in the number of $CD4^+CD8^-$ and $CD4^-CD8^+$ cells, respectively; and 57% decrease in the number of $CD4^-CD8^-$ cells (Yang et al., 2000).
- Decreased relative spleen (17%) and thymus (14%) weights in female C57BL/6N mice following exposure to 7.5 mg/kg-day PFOA in drinking water for 15 days (DeWitt et al., 2016).
- Decreased absolute and relative spleen weight in male Sprague Dawley rats at 16 weeks following post-weaning exposure to 150 or 300 ppm PFOA in the diet (NTP, 2020).

Effects on Neutrophils

- Reduced numbers of circulating neutrophils in male C57BL/6 mice following 10 days of dietary exposure to 0.02% (weight/weight) PFOA (Qazi et al., 2009a).

PFOS

Effects on T cell dependent and independent antibody response (TDAR/TIAR)

TDAR-IgM and TIAR-IgM responses were suppressed in several studies following administration of PFOS and subsequent antigen challenge. One study reported IgM suppression without antigen challenge.

- Suppression of TDAR: Suppression (by 53%) of SRBC-specific IgM production in male B6C3F1 pups following maternal exposure to PFOS (5 mg/kg-day) during gestation and challenge with SRBCs; no suppression was observed in female pups (Keil et al., 2008).
- Suppression of TDAR/TIAR: Dose-dependent suppression of IgM production in male and female B6C3F1 mice exposed to PFOS daily via gavage for 28 days (0, 0.005, 0.05, 0.1, 0.5, 1, or 5 mg/kg) and challenged with SRBC (males responded at lower doses), and suppression of TNP-specific IgM following a challenge with TNP conjugated to LPS (T cell independent) (Peden-Adams et al., 2008).
- Suppression of TIAR: Suppression of total immunoglobulin formation (measured as response to ovalbumin) and IgM formation (measured as TNP response) in female BALB/c mice exposed to PFOS via gavage (20 mg/kg-day) and challenged with ovalbumin or TNP (Vetvicka and Vetvickova, 2013).

- IgM suppression without antigen challenge: Dose-dependent suppression of total IgM in adult male C57BL/6 mice (oral gavage for 7 days; 0, 5, or 20 mg/kg-day); no consistent effect on IgG levels (Zheng et al., 2011).

Effects on T cell and B cell cellularity or proliferation

PFOS has been shown to reduce the number and proliferation of thymocytes and splenocytes in mice in multiple studies.

- Reduced total number of thymocytes and splenocytes, as well as the ratio of thymocytes expressing both CD4 and CD8 to total thymocytes in male C57BL/6 mice following exposure via diet to PFOS (0.02%) (Qazi et al., 2009b).
- Reduced proliferation of T cells and decreased cellularity in the thymus (by more than 50%) and in the spleen in BALB/c mice following exposure to PFOS via gavage (20 mg/kg-day) (Vetvicka and Vetvickova, 2013).
- Reductions in splenic and thymic cellularity by up to 51% and 61%, respectively, in male C57BL/6 mice treated with 20 and 40 mg/kg-day PFOS; and decreases of 28% and 21%, respectively, in the relative $CD4^+CD8^-$ and $CD4^-CD8^+$ populations in the highest dose group (Zheng et al., 2009).
- Modulation of T cell subpopulations in male B6C3F1 mice, with increased $CD4^-CD8^-$ subpopulation at all doses; minimal alteration of splenic T cell immune phenotypes in females and no alteration of lymphocyte proliferation in either sex following exposure up to 5 mg/kg-day PFOS via gavage (Peden-Adams et al., 2008).
- Decreased splenic B220 cells at 4 weeks (but not 8 weeks) in female (but not male) B6C3F1 pups exposed to 5 mg/kg-day PFOS in utero; however, male pups exposed to this dose in utero had a 25% decrease in $CD3^+$ and a 28% decrease in $CD4^+$ thymocytes at 8 weeks), and a reduction in thymocyte CD4:CD8 ratio to 3.5 (as compared to 4.2 in controls) (Keil et al., 2008).

Additional evidence of PFOS's effects on immune system cellularity is described in the immunotoxicity section of this document, and includes decreased absolute spleen weight (Xing et al., 2016; Zhong et al., 2016) and decreased absolute thymus weight and cellularity (Zhong et al., 2016) in C57BL/6 mice, decreased relative thymus weight in Sprague Dawley rats (NTP, 2019b), and decreased white blood cells, neutrophils, and eosinophils in male Sprague Dawley rats (NTP, 2019b).

Effects on NK cells

NK cell activity was assessed in one human in vitro study and in four animal studies. The human study and three animal studies reported decreases in NK cell activity following exposure to PFOS. The fourth animal study reported an increase in NK cell activity in male but not female mice.

- Decreased NK cell activity in human peripheral blood mononuclear cells cultured for 24 hours in the presence of PFOS (Brieger et al., 2011).
- Suppressed NK cell activity in 8-week-old male and female B6C3F1 pups following maternal oral exposure to PFOS at the two highest doses (1 and 5 mg/kg-day) (Keil et al., 2008).

- Decreased NK cell activity in adult male C57BL/6 mice at the two highest doses (20 and 40 mg/kg-day) (Zheng et al., 2009).
- Decreased splenic NK cell activity in C57BL/6 mice of both sexes exposed in utero *(*1.0 and 5.0 mg/kg-day maternal dose) (Zhong et al., 2016).
- A 2–2.5-fold increase in splenic NK activity in male (but not female) B6C3F1 mice treated via gavage (at 0.5, 1, and 5 mg/kg total administered dose); no change in lymphocyte proliferation in either sex (Peden-Adams et al., 2008).

Summary of evidence

Several animal studies have shown that PFOA suppresses TDAR/TIAR IgM production, reduces cellularity and proliferation of T cells and B cells, and reduces the number of neutrophils. Similarly, several animal studies have shown that PFOS suppresses TDAR/TIAR IgM production and reduces cellularity of T cells and B cells. PFOS also suppresses NK cell activity, including one study in cultured human blood cells and three studies in mice, although one other mouse study reported an increase in NK cell activity. The preponderance of evidence shows that PFOA and PFOS can suppress the immune system in ways that would allow neoplastic cells to escape immune surveillance, survive, and replicate to form tumors.

KC8: Modulates receptor-mediated effects

Chemicals may modulate receptor-mediated effects in a variety of ways, including binding to and either activating or inactivating a receptor, altering receptor levels or function, altering levels of endogenous ligands that are available to bind to the receptor, or otherwise altering receptor-mediated gene expression or intracellular signaling. Many cellular receptors regulate critical cellular pathways, such as those involved in differentiation and proliferation, the disruption of which can contribute to carcinogenic processes. For example, activation of certain growth factor receptors, or estrogen, androgen, or progesterone receptors can lead to the development of various types of cancer in humans and animals. As another example, activation of the aryl hydrocarbon receptor (AhR) is involved in the development of a number of different types of cancers in humans and animals. Activation of other nuclear receptors, including peroxisome proliferator activated receptor alpha (PPARα) and the constitutive androstane receptor (CAR), also has been associated with carcinogenesis (Smith et al., 2020).

PFOA

Estrogen receptor

One study compared the gene expression profiles in liver of wild-type mice treated with 3 mg/kg PFOA by gavage for seven days to those of wild-type or ERα -null mice treated with a known ERα agonist. The gene expression profile in the liver of PFOA-treated wild-type mice was similar to that of wild-type mice treated with a known ERα agonist, and different from the gene expression profile in the liver of ERα-null mice treated with the known ERα agonist, indicating that PFOA's effects on gene expression in the liver are mediated through ERα (Rosen et al., 2017).

One study reported that PFOA increased ERα reporter activity in human embryonic kidney (HEK293T) cells transfected with a human ERα reporter gene and two studies reported that PFOA induced estrogenic effects in human breast cancer (MCF-7) cells (Maras et al., 2006;

Benninghoff et al., 2011; Henry and Fair, 2013). PFOA induced dose-dependent activation of human ERα reporter gene transcription at concentrations ranging from 1-1,000 nM in HEK293T cells, with a 2- to 2.5-fold induction above control levels at concentrations of 100 and 1,000 nM (Benninghoff et al., 2011). Analyses using an in silico computational model indicate that PFOA can efficiently dock with human ERα in the ligand-binding domain and form a hydrogen bond at residue Arg394 in a manner similar to that of estrogens (Benninghoff et al., 2011). Henry and Fair (2013) reported that PFOA induced significantly positive estrogenic responses in human MCF-7 cells (E-SCREEN) at all concentrations tested (0.01 to 30 µg/ml, or 0.024 to 72.5 µM), and anti-estrogenic activity when cells were co-exposed to PFOA and estradiol (E2). E-SCREEN is an assay designed to use the estrogen sensitivity of phosphate-buffered saline pre-incubated MCF-7 cells to determine effects of exogenous agents on estrogen receptor dependent-cell proliferation. Also using E-SCREEN, Maras et al. (2006) reported that while PFOA did not induce proliferation in MCF-7 cells at a concentration of 50 µM, it induced a small but significant up-regulation in gene expression of two estrogen-responsive genes, trefoil peptide gene (*TFF1*) and estrogen receptor α gene (*ESR1*), and a small but significant downregulation of another estrogen-responsive gene, erb-b2 receptor tyrosine kinase 2 (*ERBB2*).

ER-mediated effects of PFOA have been observed in studies in rainbow trout in vivo and in vitro (Benninghoff et al., 2011). Specifically, a significantly dose-dependent increase in the level of the estrogen-responsive biomarker protein, vitellogenin (Vtg) in plasma was observed in fish administered diets containing 0, 5, 50, or 250 ppm of PFOA for 2 weeks (5 days/week). PFOA competitively binds to trout liver ER, albeit weakly, with a half-maximal inhibitory concentration (IC_{50}) value of 1.82 mM in liver cytosol homogenates. In toxicogenomic studies in rainbow trout, the hepatic gene expression profile of fish exposed to PFOA (2,000 ppm) for two weeks in the diet was similar overall to that of fish exposed to E2 for two weeks (Benninghoff et al., 2012).

In addition, a liver tumor promotion study in rainbow trout suggests that PFOA may cause effects that are mediated through the ER, since PFOA and E2 had similar tumor promoting activity in fish exposed to the liver tumor initiator aflatoxin B1 (Benninghoff et al., 2012).

PPARα

Several rodent studies have shown that PFOA alters gene expression in the liver through PPARα mediated effects (Rosen et al., 2008a; Rosen et al., 2008b; Elcombe et al., 2010; Rosen et al., 2017; NTP, 2019a). The gene expression profiles of livers from wild-type or PPARα-null mice exposed to PFOA (1 or 3 mg/kg-day) by gavage for seven days were compared to those exposed to 50 mg/kg-day Wy-14,643, a PPARα agonist (Rosen et al., 2008a; Rosen et al., 2008b; Rosen et al., 2017). The data showed that the expression of most genes was altered by PFOA through PPARα mediated pathways in wild-type mice. However, in PPARα-null mice, the expression of a subset of genes appeared to be altered through PPARα-independent pathways. In two other studies, male Sprague Dawley rats exposed to PFOA (up to 5 mg/kg-day) by gavage (NTP, 2019a) or 300 ppm in the diet (Elcombe et al., 2010) for 28 days exhibited significant increases in the expression of *Acox1* and *Cyp4a1 (NTP, 2019a)* or just *Cyp4a1* (Elcombe et al., 2010), which are markers of PPARα activity.

Bjork et al. (2011) showed that PFOA at a concentration of 25 µM can induce a number of PPARα-related genes in primary rat hepatocytes and to a lesser extent in primary human hepatocytes. In human HepG2 cells, PFOA at concentrations of 30 and 100 µM induced a significant increase in activity in a PPARα reporter gene assay (Rosenmai et al., 2018).

Additionally, Corsini et al. (2012) reported that PFOA at a concentration of 25 µM significantly increased PPARα-driven transcription in a human promyelocytic cell line (THP-1 cells) transiently transfected with a luciferase reporter plasmid construct containing the ligand binding domain of PPARα.

Other nuclear receptors, i.e., PPARγ, PXR, CAR, and PPARβ/δ

Besides effects on PPARα, several studies show that PFOA can modulate other receptors such as PPARγ, pregnane X receptor (PXR), CAR, and possibly PPARβ/δ, by measuring increased expression of target gene and/or protein levels (cytochrome P450 isoforms) from translation of downstream regulated genes in rodents (Takacs and Abbott, 2007; Rosen et al., 2008a; Rosen et al., 2008b; Elcombe et al., 2010; Rosen et al., 2017; NTP, 2019a). In both PPARα-null mice and wild-type mice treated with PFOA, microarray data revealed increased gene expression of PPARγ and CAR (as well as ERα) transcriptional targets, as compared to mice that received no treatment (Rosen et al., 2017). In other studies in PPARα-null mice, PFOA increased expression of genes regulated by either PPARγ, PXR, or CAR (Rosen et al., 2008a; Rosen et al., 2008b). Additionally, male Sprague Dawley rats exposed to PFOA by gavage (NTP, 2019a) or 300 ppm in diet (Elcombe et al., 2010) for 28 days exhibited significant increases in the expression of *Cyp2b1*, and *Cyp2b2*, which are markers of CAR activity (NTP, 2019a) or significantly increased expression of *Cyp3a1*, which is the marker of PXR activity (Elcombe et al., 2010). Finally, in studies conducted in Cos-1 cells (a monkey kidney fibroblast-like cell line) transfected with reporter genes, PFOA transactivated mouse PPARβ/δ, but not human PPARβ/δ or mouse or human PPARγ (Takacs and Abbott, 2007).

Modulation of endogenous hormones and other receptor ligands

Estradiol and progesterone in females

Exposure of female rodents to PFOA during critical periods of development has been observed to alter serum levels of either estradiol or progesterone. In one study in female Sprague Dawley rats, neonatal PFOA exposure (0.1 and 1 mg/kg-day via subcutaneous injection during PND 1-5) increased serum estradiol levels, while exposure during PND 26-30 had no effect (Du et al., 2019). In a study in C57BL/6 mice, prenatal exposure to PFOA (0.01 to 1.0 mg/kg-day between GD 1-17) increased serum estradiol in female offspring (assessed on PND 61), but had no effect on serum progesterone (Tucker et al., 2015). In another study in female C57BL/6 mice, peripubertal (3-7 weeks of age) exposure to PFOA (5 mg/kg-day for 4 weeks via gavage) significantly increased serum progesterone levels about three-fold, but had no effect on serum estradiol levels (Zhao et al., 2010).

In female mice, peripubertal (3-7 weeks of age) exposure to PFOA has been found to alter development of reproductive tissues, including the mammary gland (e.g., the growth of terminal end buds (TEBs) and terminal ducts (TDs)) and uterus (e.g., relative uterine weight) (Yang et al., 2009; Zhao et al., 2010; White et al., 2011). In general, TEBs are sensitive to chemical carcinogen treatment and persistent proliferation of TEBs has been positively associated with the development of mammary tumors in rodent bioassays. The peripubertal period, when TEBs are proliferating, may be an important window of mammary gland susceptibility to environmental exposures that may affect breast cancer risk later in life (Russo and Russo, 1996; Fenton, 2006). In one study, Yang et al. (2009) treated 21-day-old female C57BL/6 and Balb/c mice with PFOA by oral gavage at 0, 1, 5, or 10 mg/kg-day, 5 days per week for 4 weeks, and examined developmental effects in the mammary gland and uterus. Yang et al. (2009) reported

that exposure to PFOA induced stimulatory effects on the development of the mammary gland (measured as increased number of TEBs and stimulated TDs) and the uterus at the lower dose (5 mg/kg) and inhibitory effects at the higher dose (10 mg/kg) in female C57BL/6 mice. However, in female Balb/c mice PFOA exposure inhibited development of both organs at the two higher doses (5 and 10 mg/kg). The basis for the strain-specific differences in these organs' responses to PFOA is unknown.

Zhao et al. (2010) compared the stimulatory effects of peripubertal exposure to PFOA (5 mg/kg-day, 5 days/week for 4 weeks) via gavage on the development of the mammary gland in female C57BL/6 wild-type and PPARα-null mice. Besides effects on TEBs and TDs, Zhao et al. (2010) also reported significant increases in the levels of the steroid hormone synthetic enzymes in the ovaries, serum progesterone, and growth factors in the mammary glands of PFOA treated C57BL/6 wild-type mice. A subset of these effects were also observed in PFOA treated PPARα-null mice. These effects of PFOA were not seen in ovariectomized mice. Therefore, the authors hypothesized the underlying mechanism by which PFOA stimulates mammary gland development might be through indirect ER-mediated effects by promoting steroid hormone production in ovaries and increasing the levels of growth factors.

Estradiol and testosterone in males

In one experiment, Biegel et al. (1995) treated adult male CD rats with PFOA (ammonium salt) by oral gavage at 0 or 25 mg/kg-day for 14 days, and observed a statistically significant 4.5-fold increase in hepatic aromatase activity in treated rats, compared to pair-fed controls. Aromatase converts testosterone to estradiol. In another experiment, the same treatment of adult male CD rats with PFOA (ammonium salt) resulted in an increase in serum and testicular interstitial fluid estradiol levels and an increase in transforming growth factor α (TGF α) in testicular interstitial fluid, all compared to pair-fed controls (Biegel et al., 1995). Increased levels of serum estradiol were also observed in male CD rats fed 300 ppm PFOA (ammonium salt) in the diet during the first 12 months of a two-year study; increases in Leydig cell hyperplasia and adenoma were also observed in the PFOA treatment group, compared to pair fed and ad libitum controls, respectively (Biegel et al., 2001). An earlier study of adult male CD rats employing a broader range of PFOA (ammonium salt) doses (0, 1, 10, 25, or 50 mg/kg-day for 14 days) resulted in significant increases in serum estradiol levels in the 10, 25 and 50 mg/kg-day dose groups, compared to either pair-fed or ad libitum controls (Cook et al., 1992). A decrease in serum testosterone, which was significant by trend (using ad libitum, but not pair-fed controls), was also observed by Cook et al. (1992). PFOA-treated male CD rats had a significantly reduced response (i.e., an increase in serum testosterone) to a human chorionic gonadotropin (hCG) challenge as compared to untreated controls (Cook et al., 1992). These investigators proposed that PFOA's induction of hepatic aromatase activity increases estradiol levels in the serum and in the testicular interstitial fluid, which can lead to altered function and responses of Leydig cells, resulting in effects on testosterone production and testicular TGF α levels, all of which may play a role in the induction of Leydig cell tumors by PFOA (Cook et al., 1992; Biegel et al., 1995).

Thyroid hormones

The effect of PFOA on thyroid hormones has been discussed in detail in Section 5.4 of this document. In the general human population, while several studies report statistically significant findings, OEHHA did not identify consistent associations between PFOA in serum and levels of TSH or thyroid hormones. However, in pregnant women with anti-TPO antibodies, an association between PFOA and TSH has been observed (Webster et al., 2014). In animals

exposed to PFOA, changes in levels of thyroid hormones have been observed in rodents (Martin et al., 2007; NTP, 2019a) and in non-human primates (Butenhoff et al., 2002). Overall, there are consistent trends showing PFOA can decrease thyroid hormone levels in animals, although the study numbers are limited.

PFOS

Estrogen receptor

One study compared the gene expression profiles in liver of wild-type mice treated with 10 mg/kg PFOS by gavage for seven days to those of wild-type or ERα-null mice treated with a known ERα agonist. The gene expression profile in the liver of PFOS-treated wild-type mice was similar to that of wild-type mice treated with a known ERα agonist, and different from the gene expression profile in the liver of ERα-null mice treated with the known ERα agonist, indicating that the effects of PFOS on gene expression in the liver are mediated through ERα (Rosen et al., 2017).

One study reported that PFOS increased ERα reporter activity in human embryonic kidney (HEK293T) cells transfected with a human ERα reporter gene and multiple studies reported that PFOS induced estrogenic effects in human breast cell lines (Maras et al., 2006; Benninghoff et al., 2011; Henry and Fair, 2013; Pierozan and Karlsson, 2018). PFOS induced dose-dependent activation of human ERα reporter gene transcription at concentrations ranging from 1-1,000 nM in HEK293T cells, with a greater than 2-fold induction above control levels at concentrations of 100 and 1,000 nM (Benninghoff et al., 2011). Analyses using an in silico computational model indicate that PFOS can efficiently dock with human ERα in the ligand-binding domain and form a hydrogen bond at residue Arg394 in a manner similar to that of estrogens (Benninghoff et al., 2011). PFOS increased cell proliferation and cell cycle progression in human breast epithelial MCF-10A cells at concentrations of 1 or 10 µM after 72 hours of treatment, and had no effect on cellular protein levels of either ERα or ERβ (Pierozan and Karlsson, 2018). PFOS-induced cell proliferation was partially blocked by the ER antagonist ICI 182,780, indicating that the effect of PFOS on cell proliferation was partially due to activation of ER (Pierozan and Karlsson, 2018). Henry and Fair (2013) reported that PFOS induced significantly positive estrogenic responses in human MCF-7 cells (E-SCREEN) at 0.01 µg/ml (~0.025 µM) and 30 µg/ml (~75 µM), but not at concentrations in between, and anti-estrogenic activity at all concentrations tested when cells were co-exposed to PFOS and E2. Also using E-SCREEN, Maras et al. (2006) reported that while PFOS did not induce proliferation in MCF-7 cells at a concentration of 50 µM, it induced a small but significant downregulation in gene expression of two estrogen-responsive genes, *TFF1* and *ESR1*.

In studies with rainbow trout, PFOS in the diet at 250 ppm for two weeks (5 days/week) had no effect on plasma levels of Vtg, the estrogen-responsive biomarker protein. However, PFOS competitively binds, albeit weakly (IC_{50} value of 1.82 mM) to trout liver ER in studies with liver cytosol homogenates (Benninghoff et al., 2011). In toxicogenomic studies in rainbow trout, the hepatic gene expression profile of fish exposed to PFOS (200 ppm) for two weeks in the diet was similar overall to that of fish exposed to E2 for two weeks (Benninghoff et al., 2012).

Other studies in rats and rainbow trout suggest that PFOS causes effects that are mediated through the ER. In female Sprague Dawley rats, daily administration of PFOS (at doses of 1 or 10 mg/kg bw by i.p. injection for two weeks) resulted in dose-dependent changes in estrous cyclicity (irregular cycles/persistent diestrus) that were statistically significant at the high dose

(Austin et al., 2003). In liver tumor promotion studies in rainbow trout initiated with either aflatoxin B1 or N-methyl-N'-nitrosoguanidine, PFOS promoted liver tumors in a manner similar to that of E2 (Benninghoff et al., 2012).

Androgen receptor (AR)

PFOS significantly antagonized dihydrotestosterone-induced AR activity in a concentration-dependent manner in a Chinese hamster ovary cell line (CHO-K1) transfected with AR and a reporter vector (Kjeldsen and Bonefeld-Jorgensen, 2013).

PPARα

Several rodent studies have shown that PFOS alters gene expression in the liver through PPARα mediated effects (Elcombe et al., 2012a; Elcombe et al., 2012b; Rosen et al., 2017; NTP, 2019b). The gene expression profiles of livers from wild-type or PPARα-null mice exposed to PFOS (10 mg/kg-day) by gavage for seven days were compared to those exposed to 50 mg/kg-day Wy-14,643, a PPARα agonist (Rosen et al., 2017). The data showed that the expression of most genes was altered by PFOS through PPARα mediated pathways in wild-type mice. However, in PPARα-null mice, the expression of a subset of genes appeared to be altered through PPARα-independent pathways. In another study, male Sprague Dawley rats exposed to PFOS (up to 5 mg/kg-day) by gavage for 28 days exhibited significant increases in the expression of *Acox1* and *Cyp4a1*, which are markers of PPARα activity (NTP, 2019b). In a study of male Sprague Dawley rats exposed to PFOS in the diet at concentrations of 0, 20 or 100 ppm for 1, 7 or 28 days, significant increases in liver palmitoyl CoA oxidase (ACOX) activity, a marker of PPARα activation, were observed in animals exposed to 20 ppm for 28 days and in animals exposed to 100 ppm for 1 or 28 days. Increases in hepatic lauric acid 12-hydroxylation activity (a marker for CYP4A, another indicator of PPARα activation) were observed in animals exposed to 20 or 100 ppm for 7 days and increases in hepatic CYP4A1 protein levels were observed in both dose groups at each time point (Elcombe et al., 2012a). In another study, male Sprague Dawley rats were exposed to PFOS in the diet at concentrations of 0, 20 or 100 ppm for 7 days, and then fed control diet for up to 84 days. Increases in ACOX and lauric acid 12-hydroxylase activities were observed in the 100 ppm PFOS dose group through recovery day 56 (no increase at recovery day 84) (Elcombe et al., 2012b). No increases were seen in the 20 ppm dose group at any recovery time point (Elcombe et al., 2012b). Increased expression of PPARα was observed in the liver of male adult Sprague Dawley rats treated with 100 mg/kg PFOS by i.p. injection, while no increase was seen in the liver of neonatal rats exposed to PFOS in utero (dams were treated at 3 mg/kg-day via gavage on GD 2-20) (Bjork et al., 2008).

In human HepG2 cells transfected with a reporter gene, PFOS at concentrations up to 100 µM did not induce PPARα activity (Rosenmai et al., 2018). However, cellular uptake of PFOS by the HepG2 cells was less than 0.05% (approximately 6-fold lower than the cellular uptake of PFOA in the same study), thus the lack of PPARα activity observed with PFOS may be due to the limited uptake by the cells.

A difference regarding the effect of PFOS on the expression of PPARα-dependent genes was observed between human and rat liver cells (Bjork and Wallace, 2009). PFOS at a concentration of 25 µM did not induce increased expression of PPARα-dependent genes in human primary hepatocytes or human hepatoma HepG2/C3A cells, whereas rat hepatocytes did respond at this concentration (Bjork and Wallace, 2009).

Other nuclear receptors, i.e., PPARγ, PXR, CAR, and PPARβ/δ

Besides effects on PPARα, several studies show that PFOS can modulate other receptors such as PPARγ, PXR, CAR, and possibly PPARβ/δ, by measuring receptor activation-induced luciferase reporter activity, and increased expression of target gene and/or protein levels (cytochrome P450 isoforms) from translation of downstream regulated genes in rodents (Takacs and Abbott, 2007; Elcombe et al., 2012a; Elcombe et al., 2012b; Rosen et al., 2017; NTP, 2019b). As was seen with PFOA, mice treated with PFOS had increased gene expression of PPARγ, CAR, and ERα transcriptional targets (Rosen et al., 2017).

Male and female Sprague Dawley rats exposed to PFOS by gavage for 28 days exhibited significant increases in expression of *Cyp2b1* and *Cyp2b2* compared to controls, an indication of increased CAR activity (NTP, 2019b). In a study of male Sprague Dawley rats exposed to PFOS in the diet at concentrations of 0, 20 or 100 ppm for 1, 7 or 28 days, increases in liver activity and protein levels of CYP2B and CYP3A, target cytochrome P450 isoforms of CAR and PXR activation, respectively, were observed (Elcombe et al., 2012a). In another study, male Sprague Dawley rats were exposed to PFOS in the diet at concentrations of 0, 20 or 100 ppm for 7 days, and then fed control diet for up to an additional 84 days. Increases in liver activity and protein levels of CYP3A, a marker of PXR activation, persisted throughout the additional 84-day period on control diet in the 100 ppm dose group, whereas liver activity and protein levels of CYP2B, a marker of CAR activation, remained elevated after 1 and 28 days on control diet, were equivalent to levels in untreated animals after 56 days, and were decreased compared to untreated animals after 84 days (Elcombe et al., 2012b). In studies conducted in Cos-1 cells (a monkey kidney fibroblast-like cell line) transfected with plasmids containing various human or murine PPAR genes and a luciferase reporter, PFOS transactivated mouse PPARβ/δ, but not human PPARβ/δ or mouse or human PPARγ (Takacs and Abbott, 2007).

Modulation of endogenous ligands and hormones

Estradiol

The effect of PFOS neonatal or post-weaning exposure (0.1, 1, and 10 mg/kg-day via subcutaneous injection for five days) on serum estradiol levels was investigated in female Sprague Dawley rats (Du et al., 2019). In rats exposed on PND 1-5, PFOS at 0.1, 1 and 10 mg/kg significantly increased serum estradiol, with the estradiol level being the highest at the lowest dose of PFOS. In rats exposed on PND 26-30, only the low and medium doses of PFOS significantly increased serum estradiol levels.

Thyroid Hormones

The effect of PFOS on thyroid hormones has been discussed in detail in Section 5.4 of this document. In the general human population, OEHHA did not find consistent trends across the different studies reviewed by US EPA (2016d) and studies published after this review. In pregnant women, US EPA (2016d) identified three studies that reported positive associations between PFOS and TSH. However, the four studies published since US EPA's review (US EPA, 2016d) (or otherwise not included in that review) reported essentially opposite findings. In animals, the overall body of evidence from the literature, including studies summarized by (US EPA, 2016d) and recent studies newly identified and reviewed in Section 5.4 of this document, suggests that PFOS decreases thyroid hormone levels. Recent mechanistic studies suggest that PFOS may interact with thyroid hormone transporters and receptors in animals, which is

similar to results reported in mechanistic studies with human thyroid hormone transporters and receptors (US EPA, 2016d).

Summary of evidence

Several animal studies have shown that PFOA alters gene expression in the liver, and that these effects are mediated through ERα, PPARα, PPARγ, PXR, and CAR. Evidence that PFOA can bind to or activate ERα, PPARα, and possibly PPARβ/δ comes from in silico modeling studies (human ERα), in vitro studies in human cells or cell lines (ERα, PPARα), and in vitro studies in animal tissue preparations or cell lines (ERα, PPARβ/δ). There is also evidence from studies in animals that PFOA can modulate levels of endogenous hormones, including estradiol, progesterone, testosterone and thyroid hormones, and possibly levels of growth factors in the testis and mammary gland.

Several animal studies have shown that PFOS alters the expression of genes that are regulated by ERα, PPARα, PPARγ, PXR, and CAR, and one reporter gene study shows PFOS activates murine PPARβ/δ in vitro. The evidence for the estrogenic effect of PFOS also comes from increased ER reporter activity in human cell lines, increased proliferation of estrogen-responsive human breast cancer cell lines in several studies, weak binding to ER in fish, and similar gene expression patterns between PFOS and E2 in fish. One reporter gene study indicates PFOS inhibited AR activation by DHT. There is also evidence from animal studies that PFOS can decrease thyroid hormone levels and increase estradiol levels.

KC10: Alters cell proliferation, cell death or nutrient supply

Examples of the types of effects indicative of KC10 have been described by Smith et al. (2016), and include increased cell proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, and increased angiogenesis.

This section summarizes data on the effects of PFOA and PFOS on cell proliferation, growth factors, and signaling pathways related to cellular replication and cell cycle control.

PFOA

In Sprague Dawley rats treated with PFOA via gavage for 28 days, hyperplasia of the olfactory and respiratory epithelium were observed (NTP, 2019a). The study authors noted that the pattern of nasal pathology in this study was not suggestive of gavage-related reflux and should be considered treatment-related. Another animal study showed that peripubertal 28-day gavage exposure of female C57BL/6 wild-type and PPARα knockout mice to PFOA (5 mg/kg-day, 5 days/week) stimulated mammary gland development, as evidenced by increased numbers of mammary gland TEBs and duct ends (Zhao et al., 2010). An earlier study from the same laboratory had seen a similar stimulation of mammary gland development and increase of proliferation index at 5 mg/kg-day PFOA in C57BL/6 mice, but inhibition of mammary gland development at 10 mg/kg-day; in Balb/c mice, inhibition of mammary gland development was observed at 5 or 10 mg/kg-day and decreased proliferation index was observed at 5 mg/kg-day (Yang et al., 2009). It is noteworthy that TEBs are sensitive to chemical carcinogen treatment and persistent proliferation of TEBs has been positively associated with development of mammary tumors in rodent bioassays (Russo and Russo, 1996; Fenton, 2006).

After a 72-hour treatment, PFOA increased cell proliferation in a human breast epithelial cell line (MCF-10A) at concentrations of 50 and 100 µM (Pierozan et al., 2018). Furthermore, 100 µM PFOA increased the level of regulatory cell cycle proteins, such as cyclin D1 and cyclin-dependent kinases in MCF-10A cells. Another two studies using the MCF-7 human breast cancer cell line (Maras et al., 2006; Henry and Fair, 2013) demonstrated that PFOA (1 to 100 µM) increased cell proliferation via an E-SCREEN assay. However, in the presence of estradiol (E2), PFOA significantly decreased cell proliferation in the E-SCREEN assay compared with E2 alone (Henry and Fair, 2013). Additionally, PFOA at concentrations ranging from 0.2 ng/ml to 2 µg/ml increased cell proliferation in two human ovarian granulosa cell tumor cell lines, COV434 and KGN, after 72-hour treatments (Gogola et al., 2019). This effect was abolished with pre-treatment of an insulin-like growth factor 1 receptor (IGF1R) antagonist, indicating a possible role of IGF1R in PFOA-induced cell proliferation. Studies have shown that IGF1 signaling regulates cell proliferation, malignant cell invasion, and metastasis in ovarian cancers (Beauchamp et al., 2010).

PFOS

PFOS (20 or 100 ppm in the diet) increased the liver proliferative index and decreased the liver apoptotic index in Sprague Dawley rats. The effects were observed after only 1 day of exposure at 100 ppm and after 7 or 28 days of exposure at both concentrations (Elcombe et al., 2012a). In a follow-up study, in which Sprague Dawley rats were similarly fed PFOS in the diet for 7 days, a sustained decrease in liver apoptotic index was observed 84 days after cessation of exposure (20 or 100 ppm) (Elcombe et al., 2012b).

In studies with a human fetal hepatic cell line (HL-7702), 50 µM PFOS stimulated cell proliferation and altered expression of 27 proteins associated with cell proliferation, including hepatoma-derived growth factor (HdGF), the proliferation biomarker Ki67, cyclin D1, cyclin E2, cyclin A2, cyclin B1, c-Myc, and p53 (Cui et al., 2015).

In human breast epithelial cells (MCF-10A), PFOS increased cell proliferation and cell-cycle progression at concentrations of 1 or 10 µM after a 72-hour treatment (Pierozan and Karlsson, 2018). The ER blocker, ICI 182, 780, partially blocked PFOS-induced cell proliferation, indicating stimulation of proliferation was at least in part driven by ER activation (Pierozan and Karlsson, 2018). Additionally, PFOS at 0.01 µg/ml (~0.025 µM) and 30 µg/ml (~75 µM) was shown to be estrogenic and to induce cell proliferation in the E-SCREEN assay in estrogen-sensitive MCF-7 cells (Henry and Fair, 2013). Similar to PFOA, in the presence of E2, PFOS significantly decreased cell proliferation in the E-SCREEN assay compared with E2 alone. Also similar to PFOA, PFOS increased cell proliferation in the COV434 and KGN human ovarian granulosa cell tumor cell lines after 72-hour treatment with concentrations as low as 0.08 ng/ml, and this effect was abolished by pretreatment with an IGF1 receptor antagonist (Gogola et al., 2019).

Summary of evidence

Several animal studies provide evidence that PFOA increases cell proliferation, based on respiratory tissue hyperplasia observed in rats, and stimulated mammary gland development in mice. In addition, PFOA increased levels of regulatory cell cycle proteins in a human breast epithelial cell line, and increased cell proliferation in multiple studies of human breast and ovarian cell lines.

Two studies in rats provide evidence that PFOS increases cell proliferation and inhibits apoptosis in the liver, with the latter effect being long-lived. In multiple studies of human fetal liver, breast and ovarian cell lines, PFOS increased cell proliferation. In addition, PFOS altered the expression of proteins linked to cell proliferation, including increasing levels of regulatory cell cycle proteins and growth factors in a human fetal liver cell line, and increased cell proliferation in multiple studies of human breast, ovarian, and fetal liver cell lines.

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APPENDIX 9. TOXCAST DATA

OEHHA has organized the ToxCast HTS data for PFOA and PFOS (accessed on May 3, 2021), including:

- Table A9.1. 81 Active ToxCast assays for PFOA
- Table A9.2. 58 Active ToxCast assays for PFOA ammonium salt
- Table A9.3. 260 Active ToxCast assays for PFOS
- Table A9.4. 179 Active ToxCast assays for PFOS potassium salt
- Table A9.5. 26 Active ToxCast assays for PFOS lithium salt

Table A9.1. 81 Active ToxCast assays[1] for PFOA

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_CAT_up	CAT	human	HepaRG	catalase	28.2
BSK_KF3CT_ICAM1_down	ICAM1	human	keratinocytes and foreskin fibroblasts	cell adhesion molecules	40.0
BSK_LPS_Eselectin_up	SELE	human	umbilical vein endothelium and peripheral blood mononuclear cells	cell adhesion molecules	40.0
LTEA_HepaRG_CDKN1A_dn	CDKN1A	human	HepaRG	cell cycle	0.890
TOX21_HRE_BLA_Agonist_viability	NA	human	cervis ME-180	cell cycle	23.4
TOX21_RT_HEPG2_GLO_00hr_ctrl_viability	NA	human	HepG2	cell cycle	42.0
TOX21_ERb_BLA_Agonist_viability	NA	human	HEK293T	cell cycle	22.7
TOX21_ERb_BLA_Antagonist_viability	NA	human	HEK293T	cell cycle	32.6
APR_HepG2_MicrotubuleCSK_72h_up	NA	human	HepG2	cell morphology	109
APR_HepG2_MitoMembPot_72h_dn	NA	human	HepG2	cell morphology	116
LTEA_HepaRG_CYP1A1_up	CYP1A1	human	HepaRG	cyp	36.1
LTEA_HepaRG_CYP3A7_up	CYP3A7	human	HepaRG	cyp	33.9
LTEA_HepaRG_CYP4A11_up	CYP4A11	human	HepaRG	cyp	12.9
LTEA_HepaRG_CYP4A22_up	CYP4A22	human	HepaRG	cyp	5.58
LTEA_HepaRG_CYP2B6_up	CYP2B6	human	HepaRG	cyp	23.8
CLD_CYP2B6_24hr	CYP2B6	human	primary hepatocyte	cyp	0.499
LTEA_HepaRG_CYP2C8_up	CYP2C8	human	HepaRG	cyp	10.8
LTEA_HepaRG_CYP2C19_up	CYP2C19	human	HepaRG	cyp	22.7
LTEA_HepaRG_CYP2E1_up	CYP2E1	human	HepaRG	cyp	28.5
NVS_ADME_rCYP2A2	Cyp2a2	rat	NA	cyp	1.52
NVS_ADME_rCYP2C11_Activator	Cyp2c11	rat	NA	cyp	0.903

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
BSK_3C_uPAR_down	PLAUR	human	umbilical vein endothelium	cytokine	10.0
BSK_LPS_TNFa_down	TNF	human	umbilical vein endothelium and peripheral blood mononuclear cells	cytokine	10.0
LTEA_HepaRG_IL6R_up	IL6R	human	HepaRG	cytokine receptor	168
LTEA_HepaRG_DDIT3_up	DDIT3	human	HepaRG	dna binding	44.7
ATG_AP_1_CIS_up	FOS	human	HepG2	dna binding	81.8
LTEA_HepaRG_FOXO3_up	FOXO3	human	HepaRG	dna binding	71.3
ATG_NRF2_ARE_CIS_up	NFE2L2	human	HepG2	dna binding	47.9
TOX21_ARE_BLA_agonist_ratio	NFE2L2	human	HepG2	dna binding	29.1
LTEA_HepaRG_NFE2L2_up	NFE2L2	human	HepaRG	dna binding	42.0
TOX21_RXR_BLA_Agonist_ratio	RXRA	human	HEK293T	dna binding	17.3
ATG_Sp1_CIS_up	SP1	human	HepG2	dna binding	32.3
LTEA_HepaRG_XBP1_up	XBP1	human	HepaRG	dna binding	29.7
NVS_GPCR_hAdoRA2a	ADORA2A	human	NA	gpcr	16.0
NVS_GPCR_hLTB4_BLT1	LTB4R	human	NA	gpcr	32.9
NVS_GPCR_gLTB4	Ltb4r	guinea pig	NA	gpcr	16.5
LTEA_HepaRG_TGFA_up	TGFA	human	HepaRG	growth factor	82.9
LTEA_HepaRG_THRSP_dn	THRSP	human	HepaRG	growth factor	28.0
NVS_ENZ_hPI3Ka	PIK3CA	human	NA	kinase	14.6
LTEA_HepaRG_PDK4_up	PDK4	human	HepaRG	kinase	24.8
NVS_ENZ_hTie2	TEK	human	NA	kinase	18.2
LTEA_HepaRG_ACLY_up	ACLY	human	HepaRG	lyase	32.8
LTEA_HepaRG_FASN_up	FASN	human	HepaRG	lyase	26.1
LTEA_HepaRG_HMGCS2_up	HMGCS2	human	HepaRG	lyase	5.91
CLD_HMGCS2_48hr	HMGCS2	human	primary hepatocyte	lyase	35.0
LTEA_HepaRG_GADD45B_up	GADD45B	human	HepaRG	mutagenicity response	39.8
LTEA_HepaRG_GADD45G_up	GADD45G	human	HepaRG	mutagenicity response	229
ATG_ERE_CIS_up	ESR1	human	HepG2	nuclear receptor	33.8
ATG_ERa_TRANS_up	ESR1	human	HepG2	nuclear receptor	47.2
TOX21_ERa_BLA_Antagonist_ratio	ESR1	human	HEK293T	nuclear receptor	51.9
TOX21_ERb_BLA_Antagonist_ratio	ESR2	human	HEK293T	nuclear receptor	39.3
TOX21_ERR_Antagonist	ESRRA	human	ERR-HEK293T	nuclear receptor	58.0

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
ATG_PXRE_CIS_up	NR1I2	human	HepG2	nuclear receptor	35.3
NVS_NR_hCAR_Antagonist	NR1I3	human	NA	nuclear receptor	18.7
ATG_DR4_LXR_CIS_dn	NR1H3	human	HepG2	nuclear receptor	30.1
ATG_PPRE_CIS_up	PPARA	human	HepG2	nuclear receptor	30.6
ATG_PPARa_TRANS_up	PPARA	human	HepG2	nuclear receptor	21.8
TOX21_PPARd_BLA_agonist_ratio	PPARD	human	HepG2	nuclear receptor	253
TOX21_PPARd_BLA_antagonist_ratio	PPARD	human	HepG2	nuclear receptor	46.7
ATG_PPARg_TRANS_up	PPARG	human	HepG2	nuclear receptor	49.6
NVS_NR_hPPARg	PPARG	human	NA	nuclear receptor	23.3
TOX21_PPARg_BLA_antagonist_ratio	PPARG	human	HepG2	nuclear receptor	5.51
ATG_RORE_CIS_dn	RORA	human	HepG2	nuclear receptor	0.557
ATG_THRa1_TRANS_dn	THRA	human	HepG2	nuclear receptor	22.3
LTEA_HepaRG_ACOX1_up	ACOX1	human	HepaRG	oxidase	15.2
LTEA_HepaRG_FMO3_up	FMO3	human	HepaRG	oxidoreductase	3.24
LTEA_HepaRG_SDHB_up	SDHB	human	HepaRG	oxidoreductase	30.1
NVS_ENZ_hACP1	ACP1	human	NA	phosphatase	0.217
LTEA_HepaRG_ALPP_dn	ALPP	human	HepaRG	phosphatase	116
NVS_ENZ_hPPP1CA	PPP1CA	human	NA	phosphatase	0.474
NVS_ENZ_hPPP2CA	PPP2CA	human	NA	phosphatase	21.4
NVS_ENZ_hBACE	BACE1	human	NA	protease	3.90
BSK_BE3C_MMP1_up	MMP1	human	bronchial epithelial cells	protease	40.0
CLD_GSTA2_48hr	GSTA2	human	primary hepatocyte	transferase	0.266
LTEA_HepaRG_SULT2A1_up	SULT2A1	human	HepaRG	transferase	23.8
LTEA_HepaRG_UGT1A1_up	UGT1A1	human	HepaRG	transferase	2.93
LTEA_HepaRG_ABCC2_up	ABCC2	human	HepaRG	transporter	71.5
LTEA_HepaRG_ABCC3_up	ABCC3	human	HepaRG	transporter	68.6
LTEA_HepaRG_FABP1_up	FABP1	human	HepaRG	transporter	17.2

[1] Assays are alphabetically ordered by “intended target family”, and within each “intended target family” assays are ordered alphabetically by “gene symbol”. This table includes assays with curve-fitting “flags”, although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as ‘background measurement’ assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.

NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

Table A9.2. 58 Active ToxCast assays[1] for PFOA ammonium salt

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_CAT_up	CAT	human	HepaRG	catalase	9.11
LTEA_HepaRG_BCL2L11_up	BCL2L11	human	HepaRG	cell cycle	5.20
LTEA_HepaRG_CCND1_up	CCND1	human	HepaRG	cell cycle	44.6
ACEA_AR_agonist_AUC_viability	NA	human	22Rv1 prostate cell line	cell cycle	54.4
ACEA_AR_antagonist_AUC_viability	NA	human	22Rv1 prostate cell line	cell cycle	33.7
TOX21_HRE_BLA_Agonist_viability	NA	human	cervis ME-180	cell cycle	48.3
TOX21_p53_BLA_p4_viability	NA	human	HCT116 intestinal cell line	cell cycle	42.4
TOX21_ERb_BLA_Agonist_viability	NA	human	HEK293T	cell cycle	27.0
TOX21_ERb_BLA_Antagonist_viability	NA	human	HEK293T	cell cycle	35.6
APR_HepG2_MitoMembPot_72h_dn	NA	human	HepG2	cell morphology	111
LTEA_HepaRG_CYP1A1_up	CYP1A1	human	HepaRG	cyp	32.6
LTEA_HepaRG_CYP3A4_up	CYP3A4	human	HepaRG	cyp	14.3
LTEA_HepaRG_CYP3A7_up	CYP3A7	human	HepaRG	cyp	17.3
LTEA_HepaRG_CYP4A11_up	CYP4A11	human	HepaRG	cyp	9.87
LTEA_HepaRG_CYP4A22_up	CYP4A22	human	HepaRG	cyp	9.54
LTEA_HepaRG_CYP2B6_up	CYP2B6	human	HepaRG	cyp	5.64
LTEA_HepaRG_CYP2C19_up	CYP2C19	human	HepaRG	cyp	6.44
NVS_ADME_hCYP2C9	CYP2C9	human	NA	cyp	0.202
BSK_LPS_IL8_up	CXCL8	human	Umbilical vein endothelium and peripheral blood mononuclear cells	cytokine	40.0
BSK_BE3C_IP10_down	CXCL10	human	Bronchial epithelial cells	cytokine	40.0
LTEA_HepaRG_IL6_dn	IL6	human	HepaRG	cytokine	21.7
LTEA_HepaRG_DDIT3_up	DDIT3	human	HepaRG	dna binding	86.3
LTEA_HepaRG_EGR1_dn	EGFR1	human	HepaRG	dna binding	19.4
LTEA_HepaRG_FOXO1_up	FOXO1	human	HepaRG	dna binding	48.0
LTEA_HepaRG_HSPA1A_up	HSPA1A	human	HepaRG	dna binding	34.7
LTEA_HepaRG_NFE2L2_up	NFE2L2	human	HepaRG	dna binding	72.6
ATG_NRF2_ARE_CIS_up	NFE2L2	human	HepG2	dna binding	57.2
TOX21_ARE_BLA_agonist_ratio	NFE2L2	human	HepG2	dna binding	43.9

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_TP53_up	TP53	human	HepaRG	dna binding	40.4
LTEA_HepaRG_LPL_up	LPL	human	HepaRG	esterase	58.1
LTEA_HepaRG_KRT19_up	KRT19	human	HepaRG	filaments	32.9
NVS_GPCR_hTXA2	TBXA2R	human	NA	gpcr	11.2
NVS_GPCR_gLTB4	Ltb4r	guinea pig	NA	gpcr	21.9
LTEA_HepaRG_TGFA_up	TGFA	human	HepaRG	growth factor	18.7
LTEA_HepaRG_TGFB1_up	TGFB1	human	HepaRG	growth factor	26.7
LTEA_HepaRG_THRSP_dn	THRSP	human	HepaRG	growth factor	33.7
LTEA_HepaRG_PDK4_up	PDK4	human	HepaRG	kinase	22.4
LTEA_HepaRG_HMGCS2_up	HMGCS2	human	HepaRG	lyase	10.2
TOX21_AR_BLA_Antagonist_ratio	AR	human	HEK293T	nuclear receptor	50.3
ACEA_AR_antagonist_80hr	AR	human	22Rv1 prostate cell line	nuclear receptor	45.0
NVS_NR_rAR	Ar	rat	NA	nuclear receptor	19.3
ATG_ERE_CIS_up	ESR1	human	HepG2	nuclear receptor	22.7
ATG_ERa_TRANS_up	ESR1	human	HepG2	nuclear receptor	44.7
TOX21_ERa_BLA_Antagonist_ratio	ESR1	human	HEK293T	nuclear receptor	46.9
TOX21_ERb_BLA_Antagonist_ratio	ESR2	human	HEK293T	nuclear receptor	41.5
ATG_PXRE_CIS_up	NR1I2	human	HepG2	nuclear receptor	21.1
ATG_PPARa_TRANS_up	PPARA	human	HepG2	nuclear receptor	6.73
ATG_PPARg_TRANS_up	PPARG	human	HepG2	nuclear receptor	21.8
NVS_NR_hPPARg	PPARG	human	NA	nuclear receptor	26.4
TOX21_PPARd_BLA_antagonist_ratio	PPARD	human	HepG2	nuclear receptor	53.7
ATG_RARa_TRANS_dn	RARA	human	HepG2	nuclear receptor	128
ATG_RXRb_TRANS_up	RXRB	human	HepG2	nuclear receptor	37.7
TOX21_TR_LUC_GH3_Antagonist	Thrb	rat	Pituitary gland cell line	nuclear receptor	77.1
LTEA_HepaRG_ACOX1_up	ACOX1	human	HepaRG	oxidase	8.16
NVS_ENZ_hBACE	BACE1	human	NA	protease	1.25
LTEA_HepaRG_UGT1A1_up	UGT1A1	human	HepaRG	transferase	6.90
LTEA_HepaRG_FABP1_up	FABP1	human	HepaRG	transporter	8.77
LTEA_HepaRG_IGFBP1_up	IGFBP1	human	HepaRG	transporter	42.5

[1] Assays are alphabetically ordered by “intended target family”, and within each “intended target family” assays are ordered alphabetically by “gene symbol”. This table includes assays with curve-fitting “flags”, although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as ‘background measurement’ assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.
NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

Table A9.3. 260 Active ToxCast assays[1] for PFOS

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_CAT_dn	CAT	human	HepaRG	catalase	82.1
BSK_hDFCGF_CollagenIII_down	COL3A1	human	foreskin fibroblast	cell adhesion molecules	40.0
BSK_BE3C_HLADR_down	HLA-DRA	human	bronchial epithelial cells	cell adhesion molecules	10.0
BSK_hDFCGF_VCAM1_down	VCAM1	human	foreskin fibroblast	cell adhesion molecules	40.0
LTEA_HepaRG_BAX_up	BAX	human	HepaRG	cell cycle	33.4
LTEA_HepaRG_BCL2_up	BCL2	human	HepaRG	cell cycle	34.1
LTEA_HepaRG_CCND1_up	CCND1	human	HepaRG	cell cycle	21.3
APR_HepG2_OxidativeStress_24h_up	γH2AX	human	HepG2	cell cycle	108
APR_HepG2_OxidativeStress_72h_up	γH2AX	human	HepG2	cell cycle	7.98
APR_HepG2_MitoticArrest_24h_up	pH3	human	HepG2	cell cycle	109
APR_HepG2_MitoticArrest_72h_up	pH3	human	HepG2	cell cycle	8.10
APR_HepG2_CellLoss_24h_dn	NA	human	HepG2	cell cycle	111
APR_HepG2_CellLoss_72h_dn	NA	human	HepG2	cell cycle	111
BSK_3C_SRB_down	NA	human	umbilical vein endothelium	cell cycle	40.0
BSK_BE3C_SRB_down	NA	human	bronchial epithelial cells	cell cycle	40.0
BSK_hDFCGF_Proliferation_down	NA	human	foreskin fibroblast	cell cycle	10.0
BSK_hDFCGF_SRB_down	NA	human	foreskin fibroblast	cell cycle	40.0
TOX21_TR_LUC_GH3_Antagonist_viability	NA	rat	pituitary gland GH4	cell cycle	120
TOX21_FXR_BLA_antagonist_viability	NA	human	HEK293T	cell cycle	31.4
TOX21_PPARd_BLA_antagonist_viability	NA	human	HEK293T	cell cycle	71.0
TOX21_DT40	NA	chicken	lymphoblast	cell cycle	115
LTEA_HepaRG_LDH_cytotoxicity	NA	human	HepaRG	cell cycle	80.3
TOX21_ARE_BLA_agonist_viability	NA	human	HepG2	cell cycle	54.2
TOX21_p53_BLA_p1_viability	NA	human	intestinal cells HCT116	cell cycle	78.1
TOX21_FXR_BLA_agonist_viability	NA	human	HEK293T	cell cycle	30.1
TOX21_PPARd_BLA_Agonist_viability	NA	human	HEK293T	cell cycle	57.8
TOX21_p53_BLA_p3_viability	NA	human	intestinal cells HCT116	cell cycle	100

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
TOX21_p53_BLA_p4_viability	NA	human	intestinal cells HCT116	cell cycle	81.4
TOX21_VDR_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	37.3
NCCT_HEK293T_ CellTiterGLO	NA	human	HEK293T	cell cycle	49.9
TOX21_RXR_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	30.1
ACEA_AR_agonist_AUC_ viability	NA	human	prostate 22Rv1	cell cycle	80.0
ACEA_AR_antagonist_AUC_ viability	NA	human	prostate 22Rv1	cell cycle	33.7
TOX21_HRE_BLA_Agonist_ viability	NA	human	cervix ME-180	cell cycle	41.6
TOX21_RT_HEK293_FLO_ 08hr_viability	NA	human	HEK293T	cell cycle	30.7
TOX21_RT_HEK293_FLO_ 16hr_viability	NA	human	HEK293T	cell cycle	29.8
TOX21_RT_HEK293_FLO_ 24hr_viability	NA	human	HEK293T	cell cycle	28.1
TOX21_RT_HEK293_FLO_ 32hr_viability	NA	human	HEK293T	cell cycle	27.9
TOX21_RT_HEK293_FLO_ 40hr_viability	NA	human	HEK293T	cell cycle	27.7
TOX21_RT_HEK293_GLO_ 08hr_viability	NA	human	HEK293T	cell cycle	71.7
TOX21_RT_HEK293_GLO_ 16hr_viability	NA	human	HEK293T	cell cycle	70.5
TOX21_RT_HEK293_GLO_ 24hr_viability	NA	human	HEK293T	cell cycle	73.4
TOX21_RT_HEK293_GLO_ 32hr_viability	NA	human	HEK293T	cell cycle	66.4
TOX21_RT_HEK293_GLO_ 40hr_viability	NA	human	HEK293T	cell cycle	68.5
NIS_HEK293T_CTG_ Cytotoxicity	NA	human	HEK293T	cell cycle	0.100
TOX21_ERb_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	25.4
TOX21_ERb_BLA_Antagonist _viability	NA	human	HEK293T	cell cycle	30.3
TOX21_PR_BLA_Agonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	27.7
TOX21_PR_BLA_Antagonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	32.8
TOX21_DT40_657	NA	chicken	lymphoblast	cell cycle	67.0
TOX21_PXR_viability	NA	human	PXR-Luc HepG2 cells	cell cycle	99.4
APR_HepG2_MitoMass_24h_ dn	NA	human	HepG2	cell morphology	114
APR_HepG2_MitoMass_72h_ dn	NA	human	HepG2	cell morphology	17.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
APR_HepG2_NuclearSize_72h_dn	NA	human	HepG2	cell morphology	8.10
BSK_3C_Vis_down	NA	human	umbilical vein endothelium	cell morphology	40.0
TOX21_MMP_ratio_up	NA	human	HepG2	cell morphology	33.2
LTEA_HepaRG_CYP1A2_dn	CYP1A2	human	HepaRG	cyp	20.8
LTEA_HepaRG_CYP3A4_up	CYP3A4	human	HepaRG	cyp	10.1
LTEA_HepaRG_CYP3A7_up	CYP3A7	human	HepaRG	cyp	10.2
LTEA_HepaRG_CYP4A11_dn	CYP4A11	human	HepaRG	cyp	82.5
LTEA_HepaRG_CYP4A11_up	CYP4A11	human	HepaRG	cyp	10.8
LTEA_HepaRG_CYP4A22_dn	CYP4A22	human	HepaRG	cyp	88.2
LTEA_HepaRG_CYP4A22_up	CYP4A22	human	HepaRG	cyp	8.70
LTEA_HepaRG_CYP7A1_dn	CYP7A1	human	HepaRG	cyp	84.1
NVS_ADME_hCYP19A1	CYP19A1	human	NA	cyp	4.14
LTEA_HepaRG_CYP2B6_dn	CYP2B6	human	HepaRG	cyp	90.7
LTEA_HepaRG_CYP2B6_up	CYP2B6	human	HepaRG	cyp	5.52
CLD_CYP2B6_24hr	CYP2B6	human	primary hepatocyte	cyp	34.5
LTEA_HepaRG_CYP2C8_up	CYP2C8	human	HepaRG	cyp	16.7
NVS_ADME_hCYP2C8	CYP2C8	human	NA	cyp	4.70
LTEA_HepaRG_CYP2C9_dn	CYP2C9	human	HepaRG	cyp	85.4
NVS_ADME_hCYP2C9	CYP2C9	human	NA	cyp	2.17e-2
NVS_ADME_rCYP2C11	Cyp2c11	rat	NA	cyp	9.27e-2
NVS_ADME_hCYP2C18	CYP2C18	human	NA	cyp	0.822
LTEA_HepaRG_CYP2C19_up	CYP2C19	human	HepaRG	cyp	11.8
NVS_ADME_hCYP2C19	CYP2C19	human	NA	cyp	4.91
LTEA_HepaRG_CYP2E1_dn	CYP2E1	human	HepaRG	cyp	30.9
NVS_ADME_hCYP4F12	CYP4F12	human	NA	cyp	1.53
BSK_SAg_CD40_down	CD40	human	umbilical vein endothelium and peripheral blood mononuclear cells	cytokine	40.0
BSK_LPS_IL8_up	CXCL8	human	umbilical vein endothelium and peripheral blood mononuclear cells	cytokine	10.0
BSK_BE3C_IP10_down	CXCL10	human	bronchial epithelial cells	cytokine	10.0
BSK_KF3CT_IP10_down	CXCL10	human	keratinocytes and foreskin fibroblasts	cytokine	10.0
BSK_hDFCGF_IP10_down	CXCL10	human	foreskin fibroblast	cytokine	40.0

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
BSK_BE3C_IL1a_down	IL1A	human	bronchial epithelial cells	cytokine	40.0
BSK_BE3C_PAI1_down	SERPINE1	human	bronchial epithelial cells	cytokine	40.0
LTEA_HepaRG_FAS_up	FAS	human	HepaRG	cytokine receptor	17.4
LTEA_HepaRG_IL6R_dn	IL6R	human	HepaRG	cytokine receptor	85.3
NHEERL_MED_hDIO2_dn	null	human	NA	deiodinase	93.2
NHEERL_MED_hDIO3_dn	null	human	NA	deiodinase	173
LTEA_HepaRG_DDIT3_up	DDIT3	human	HepaRG	dna binding	33.9
LTEA_HepaRG_EGR1_dn	EGR1	human	HepaRG	dna binding	10.4
ATG_AP_1_CIS_up	FOS	human	HepG2	dna binding	33.7
LTEA_HepaRG_FOXO1_up	FOXO1	human	HepaRG	dna binding	23.8
LTEA_HepaRG_FOXO3_up	FOXO3	human	HepaRG	dna binding	27.3
LTEA_HepaRG_HSPA1A_up	HSPA1A	human	HepaRG	dna binding	19.3
ATG_MRE_CIS_up	MTF1	human	HepG2	dna binding	42.0
LTEA_HepaRG_MYC_up	MYC	human	HepaRG	dna binding	32.3
ATG_NRF2_ARE_CIS_up	NFE2L2	human	HepG2	dna binding	5.82
LTEA_HepaRG_NFE2L2_up	NFE2L2	human	HepaRG	dna binding	14.1
TOX21_ARE_BLA_agonist_ratio	NFE2L2	human	HEK293T	dna binding	25.2
ATG_Pax6_CIS_up	PAX6	human	HepG2	dna binding	84.0
ATG_Sp1_CIS_up	SP1	human	HepG2	dna binding	34.0
APR_HepG2_p53Act_24h_up	TP53	human	HepG2	dna binding	109
APR_HepG2_p53Act_72h_up	TP53	human	HepG2	dna binding	5.43
LTEA_HepaRG_TP53_up	TP53	human	HepaRG	dna binding	302
ATG_p53_CIS_dn	TP53	human	HepG2	dna binding	69.1
TOX21_p53_BLA_p4_ratio	TP53	human	intestinal cells HCT116	dna binding	159
LTEA_HepaRG_LIPC_dn	LIPC	human	HepaRG	esterase	47.2
NVS_ENZ_hPDE4A1	PDE4A	human	NA	esterase	9.62
NVS_ENZ_hPDE5	PDE5A	human	NA	esterase	25.6
LTEA_HepaRG_KRT19_up	KRT19	human	HepaRG	filaments	30.9
NVS_GPCR_hAdoRA2a	ADORA2A	human	NA	gpcr	5.17
NVS_GPCR_hAdra2C	ADRA2C	human	NA	gpcr	16.8
NVS_GPCR_hAdrb1	ADRB1	human	NA	gpcr	31.2
NVS_GPCR_gLTD4	Cysltr1	guinea pig	NA	gpcr	27.2
NVS_GPCR_hDRD4.4	DRD4	human	NA	gpcr	19.3
NVS_GPCR_h5HT5A	HTR5A	human	NA	gpcr	16.7
NVS_GPCR_h5HT6	HTR6	human	NA	gpcr	24.0
NVS_GPCR_h5HT7	HTR7	human	NA	gpcr	7.26
NVS_GPCR_hLTB4_BLT1	LTB4R	human	NA	gpcr	24.3
NVS_GPCR_gLTB4	Ltb4r	guinea pig	NA	gpcr	21.8
NVS_GPCR_hNPY2	NPY2R	human	NA	gpcr	28.0
NVS_GPCR_hOpiate_D1	OPRD1	human	NA	gpcr	12.6
NVS_GPCR_hPY2	P2RY1	human	NA	gpcr	11.8

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NVS_GPCR_hTXA2	TBXA2R	human	NA	gpcr	16.1
LTEA_HepaRG_IGF1_dn	IGF1	human	HepaRG	growth factor	34.7
LTEA_HepaRG_TGFA_up	TGFA	human	HepaRG	growth factor	13.4
ATG_TGFb_CIS_up	TGFB1	human	HepG2	growth factor	70.7
BSK_BE3C_TGFb1_down	TGFB1	human	bronchial epithelial cells	growth factor	40.0
BSK_KF3CT_TGFb1_down	TGFB1	human	keratinocytes and foreskin fibroblasts	growth factor	40.0
LTEA_HepaRG_THRSP_dn	THRSP	human	HepaRG	growth factor	48.2
TOX21_SBE_BLA_Antagonist_ratio	null	human	SBE-bla HEK 293T cell line	growth factor receptor	71.6
NVS_ENZ_hHDAC3	HDAC3	human	NA	hydrolase	9.92
NVS_ENZ_hSIRT2	SIRT2	human	NA	hydrolase	7.58
NVS_ENZ_hSIRT3_Activator	SIRT3	human	NA	hydrolase	7.55
NVS_ENZ_hAKT1	AKT1	human	NA	kinase	38.3
NVS_ENZ_hAKT2	AKT2	human	NA	kinase	26.2
NVS_ENZ_hAurA	AURKA	human	NA	kinase	26.8
NVS_ENZ_hBTK	BTK	human	NA	kinase	21.9
NVS_ENZ_hCDK6	CDK6	human	NA	kinase	20.0
NVS_ENZ_hIKKa	CHUK	human	NA	kinase	30.4
NVS_ENZ_hCSF1R	CSF1R	human	NA	kinase	23.0
NVS_ENZ_hCSF1R_Activator	CSF1R	human	NA	kinase	0.406
NVS_ENZ_hCK1a	CSNK1A1	human	NA	kinase	24.2
NVS_ENZ_hCK1D	CSNK1D	human	NA	kinase	26.4
NVS_ENZ_hDYRK1a	DYRK1A	human	NA	kinase	20.7
NVS_ENZ_hEGFR	EGFR	human	NA	kinase	19.1
NVS_ENZ_hEphA2	EPHA2	human	NA	kinase	20.8
NVS_ENZ_hFGFR1	FGFR1	human	NA	kinase	20.8
NVS_ENZ_hFGFR3	FGFR3	human	NA	kinase	33.3
NVS_ENZ_hVEGFR1	FLT1	human	NA	kinase	8.92
NVS_ENZ_hVEGFR3	FLT4	human	NA	kinase	8.36
NVS_ENZ_hFyn	FYN	human	NA	kinase	41.0
NVS_ENZ_hGSK3b	GSK3B	human	NA	kinase	25.5
NVS_ENZ_hInsR	INSR	human	NA	kinase	12.5
NVS_ENZ_hInsR_Activator	INSR	human	NA	kinase	40.9
NVS_ENZ_hVEGFR2	KDR	human	NA	kinase	32.2
NVS_ENZ_hLck	LCK	human	NA	kinase	40.9
NVS_ENZ_hLynA_Activator	LYN	human	NA	kinase	27.8
NVS_ENZ_hMARK1	MARK1	human	NA	kinase	32.5
NVS_ENZ_hMAPK1	MAPK1	human	NA	kinase	30.4
NVS_ENZ_hMAPK3	MAPK3	human	NA	kinase	31.5
NVS_ENZ_hMAPKAPK5	MAPKAPK5	human	NA	kinase	36.4
NVS_ENZ_hMet	MET	human	NA	kinase	25.7
NVS_ENZ_hTrkA	NTRK1	human	NA	kinase	30.2
NVS_ENZ_hPAK4	PAK4	human	NA	kinase	26.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_PDK4_up	PDK4	human	HepaRG	kinase	24.2
NVS_ENZ_hPI3Ka	PIK3CA	human	NA	kinase	7.42
NVS_ENZ_hAMPKa1	PRKAA1	human	NA	kinase	5.47
NVS_ENZ_hPKA	PRKACA	human	NA	kinase	26.4
NVS_ENZ_hRAF1	RAF1	human	NA	kinase	24.4
NVS_ENZ_hROCK1	ROCK1	human	NA	kinase	33.9
NVS_ENZ_hMsk1	RPS6KA5	human	NA	kinase	29.7
NVS_ENZ_hSGK1	SGK1	human	NA	kinase	29.8
NVS_ENZ_hTie2	TEK	human	NA	kinase	8.69
NVS_ENZ_hZAP70	ZAP70	human	NA	kinase	13.5
LTEA_HepaRG_ACLY_dn	ACLY	human	HepaRG	lyase	32.2
LTEA_HepaRG_FASN_dn	FASN	human	HepaRG	lyase	45.0
LTEA_HepaRG_HMGCS2_dn	HMGCS2	human	HepaRG	lyase	83.6
Tanguay_ZF_120hpf_MORT_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	0.547
Tanguay_ZF_120hpf_YSE_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	8.65
Tanguay_ZF_120hpf_AXIS_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	2.39
Tanguay_ZF_120hpf_SNOU_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	26.0
Tanguay_ZF_120hpf_JAW_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	29.2
Tanguay_ZF_120hpf_PE_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	28.7
Tanguay_ZF_120hpf_TRUN_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	16.8
Tanguay_ZF_120hpf_TR_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	1.92
NHEERL_ZF_144hpf_TERATOSCORE_up	NA	zebrafish	zebrafish embryo	malformation	42.3
Tanguay_ZF_120hpf_ActivityScore	NA	zebrafish	dechorionated zebrafish embryo	malformation	3.45
LTEA_HepaRG_EZR_up	EZR	human	HepaRG	membrane protein	56.5
LTEA_HepaRG_MIR122_dn	MIR122	human	HepaRG	microrna	72.0
ACEA_AR_antagonist_80hr	AR	human	prostate 22Rv1	nuclear receptor	39.0
NVS_NR_hAR	AR	human	NA	nuclear receptor	12.6

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NVS_NR_cAR	AR	chimpanzee	NA	nuclear receptor	7.31
NVS_NR_rAR	AR	rat	NA	nuclear receptor	4.27
ATG_ERE_CIS_up	ESR1	human	HepG2	nuclear receptor	18.1
ATG_ERa_TRANS_up	ESR1	human	HepG2	nuclear receptor	38.9
NVS_NR_hER	ESR1	human	NA	nuclear receptor	27.2
TOX21_ERa_BLA_Antagonist_ratio	ESR1	human	HEK293T	nuclear receptor	86.5
OT_ER_ERaERb_0480	ESR2	human	HEK293T	nuclear receptor	87.4
TOX21_ERb_BLA_Antagonist_ratio	ESR2	human	HEK293T	nuclear receptor	62.2
ATG_PXRE_CIS_up	NR1I2	human	HepG2	nuclear receptor	9.42
ATG_PXR_TRANS_up	NR1I2	human	HepG2	nuclear receptor	19.7
NVS_NR_hPXR	NR1I2	human	NA	nuclear receptor	40.9
NVS_NR_hCAR_Antagonist	NR1I3	human	NA	nuclear receptor	17.6
NVS_NR_hGR	NR3C1	human	NA	nuclear receptor	2.27
ATG_DR4_LXR_CIS_dn	NR1H3	human	HepG2	nuclear receptor	23.8
TOX21_PR_BLA_Antagonist_ratio	PGR	human	PR-UAS-bla-HEK293T	nuclear receptor	35.5
NVS_NR_hPR	PGR	human	NA	nuclear receptor	22.6
NVS_NR_bPR	PGR	bovine	NA	nuclear receptor	22.2
ATG_PPRE_CIS_up	PPARA	human	HepG2	nuclear receptor	33.9
ATG_PPARa_TRANS_up	PPARA	human	HepG2	nuclear receptor	58.9
ATG_PPARg_TRANS_up	PPARG	human	HepG2	nuclear receptor	26.7
NVS_NR_hPPARg	PPARG	human	NA	nuclear receptor	5.94
NVS_NR_hRAR_Antagonist	RARA	human	NA	nuclear receptor	28.4
NVS_NR_hTRa_Antagonist	THRA	human	NA	nuclear receptor	14.6
TOX21_TR_LUC_GH3_Antagonist	Thrb	rat	pituitary gland GH3	nuclear receptor	86.5
LTEA_HepaRG_ACOX1_up	ACOX1	human	HepaRG	oxidase	14.7
LTEA_HepaRG_FMO3_dn	FMO3	human	HepaRG	oxidoreductase	86.3
NVS_ENZ_oCOX2	PTGS2	sheep	NA	oxidoreductase	11.9

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NVS_ENZ_hACP1	ACP1	human	NA	phosphatase	24.1
LTEA_HepaRG_ALPP_up	ALPP	human	HepaRG	phosphatase	40.4
NVS_ENZ_hDUSP3	DUSP3	human	NA	phosphatase	26.0
NVS_ENZ_hPPP1CA	PPP1CA	human	NA	phosphatase	16.1
NVS_ENZ_hPPP2CA	PPP2CA	human	NA	phosphatase	5.39
NVS_ENZ_hPTEN	PTEN	human	NA	phosphatase	7.66
NVS_ENZ_hPTPN1	PTPN1	human	NA	phosphatase	5.33e-2
NVS_ENZ_hPTPN2	PTPN2	human	NA	phosphatase	26.6
NVS_ENZ_hPTPN6	PTPN6	human	NA	phosphatase	23.3
NVS_ENZ_hPTPN11	PTPN11	human	NA	phosphatase	24.3
NVS_ENZ_hPTPN12	PTPN12	human	NA	phosphatase	21.8
NVS_ENZ_hPTPN13	PTPN13	human	NA	phosphatase	10.5
NVS_ENZ_hPTPRB	PTPRB	human	NA	phosphatase	25.4
NVS_ENZ_hPTPRC	PTPRC	human	NA	phosphatase	16.0
NVS_ENZ_hPTPRF	PTPRF	human	NA	phosphatase	11.1
NVS_ENZ_hPTPRM	PTPRM	human	NA	phosphatase	31.0
NVS_ENZ_hBACE	BACE1	human	NA	protease	0.471
LTEA_HepaRG_CASP3_up	CASP3	human	HepaRG	protease	21.1
NVS_ENZ_hCASP5	CASP5	human	NA	protease	13.6
LTEA_HepaRG_CASP8_up	CASP8	human	HepaRG	protease	18.5
NVS_ENZ_hMMP3	MMP3	human	NA	protease	18.8
NVS_ENZ_hMMP7	MMP7	human	NA	protease	1.73
NVS_ENZ_hMMP9	MMP9	human	NA	protease	18.4
BSK_KF3CT_MMP9_down	MMP9	human	keratinocytes and foreskin fibroblasts	protease	10.0
LTEA_HepaRG_MMP10_up	MMP10	human	HepaRG	protease	83.4
NVS_ENZ_hMMP13	MMP13	human	NA	protease	8.08
BSK_BE3C_tPA_down	PLAT	human	bronchial epithelial cells	protease	40.0
BSK_BE3C_uPA_down	PLAU	human	bronchial epithelial cells	protease	40.0
LTEA_HepaRG_GSTA2_dn	GSTA2	human	HepaRG	transferase	41.2
LTEA_HepaRG_UGT1A1_up	UGT1A1	human	HepaRG	transferase	15.3
LTEA_HepaRG_UGT1A6_dn	UGT1A6	human	HepaRG	transferase	56.1
LTEA_HepaRG_ABCB1_up	ABCB1	human	HepaRG	transporter	21.2
LTEA_HepaRG_ABCB11_dn	ABCB11	human	HepaRG	transporter	44.4
LTEA_HepaRG_ABCC2_up	ABCC2	human	HepaRG	transporter	11.6
LTEA_HepaRG_ABCG2_up	ABCG2	human	HepaRG	transporter	6.93
LTEA_HepaRG_FABP1_dn	FABP1	human	HepaRG	transporter	85.9
LTEA_HepaRG_IGFBP1_up	IGFBP1	human	HepaRG	transporter	57.5
NIS_RAIU_inhibition	SLC5A5	human	HEK293T	transporter	11.2
LTEA_HepaRG_SLC22A1_dn	SLC22A1	human	HepaRG	transporter	85.1
LTEA_HepaRG_SLCO1B1_dn	SLCO1B1	human	HepaRG	transporter	82.9

[1] Assays are alphabetically ordered by “intended target family”, and within each “intended target family” assays are ordered alphabetically by “gene symbol”. This table includes assays with curve-fitting “flags”,

although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as 'background measurement' assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.
NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

Table A9.4. 179 Active ToxCast assays[1] for PFOS potassium salt

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_CAT_dn	CAT	human	HepaRG	catalase	87.0
BSK_BE3C_HLADR_down	HLA-DRA	human	bronchial epithelial cells	cell adhesion molecules	10.0
BSK_LPS_Eselectin_up	SELE	human	umbilical vein endothelium and peripheral blood mononuclear cells	cell adhesion molecules	40.0
BSK_4H_Pselectin_up	SELP	human	umbilical vein endothelium	cell adhesion molecules	40.0
APR_HepG2_CellLoss_24h_dn	NA	human	HepG2	cell cycle	115
APR_HepG2_MitoticArrest_24h_up	pH3	human	HepG2	cell cycle	110
APR_HepG2_OxidativeStress 24h_up	γH2AX	human	HepG2	cell cycle	121
APR_HepG2_CellLoss_72h_dn	NA	human	HepG2	cell cycle	111
APR_HepG2_MitoticArrest_72h_up	pH3	human	HepG2	cell cycle	107
APR_HepG2_OxidativeStress_72h_up	γH2AX	human	HepG2	cell cycle	111
APR_HepG2_StressKinase_72h_up	NA	human	HepG2	cell cycle	112
BSK_KF3CT_SRB_down	NA	human	keratinocytes and foreskin fibroblasts	cell cycle	40.0
BSK_BE3C_SRB_down	NA	human	bronchial epithelial cells	cell cycle	40.0
TOX21_TR_LUC_GH3_Antagonist_viability	NA	rat	pituitary gland GH4	cell cycle	49.4
LTEA_HepaRG_BAX_up	BAX	human	HepaRG	cell cycle	53.6
LTEA_HepaRG_BCL2_up	BCL2	human	HepaRG	cell cycle	43.0
LTEA_HepaRG_CCND1_up	CCND1	human	HepaRG	cell cycle	11.4
TOX21_FXR_BLA_antagonist_viability	NA	human	HEK293T	cell cycle	31.9
TOX21_PPARd_BLA_antagonist_viability	NA	human	HEK293T	cell cycle	43.2
TOX21_DT40	NA	chicken	lymphoblast	cell cycle	44.9

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_LDH_ cytotoxicity	NA	human	HepaRG	cell cycle	83.4
TOX21_ARE_BLA_agonist_ viability	NA	human	HepG2	cell cycle	76.2
TOX21_FXR_BLA_agonist_ viability	NA	human	HEK293T	cell cycle	29.1
TOX21_PPARd_BLA_Agonist _viability	NA	human	HEK293T	cell cycle	32.4
TOX21_p53_BLA_p3_viability	NA	human	intestinal cells HCT116	cell cycle	54.1
TOX21_p53_BLA_p4_viability	NA	human	intestinal cells HCT116	cell cycle	45.4
TOX21_VDR_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	18.8
TOX21_AP1_BLA_Agonist_vi ability	NA	human	cervix cell line	cell cycle	50.2
ACEA_AR_agonist_AUC_ viability	NA	human	prostate 22Rv1	cell cycle	69.3
ACEA_AR_antagonist_AUC_ viability	NA	human	prostate 22Rv1	cell cycle	56.0
TOX21_ERR_viability	NA	human	ERR-HEK293T	cell cycle	38.3
TOX21_HRE_BLA_Agonist_ viability	NA	human	cervix ME-180	cell cycle	26.3
TOX21_RT_HEK293_FLO_ 08hr_viability	NA	human	HEK293T	cell cycle	28.5
TOX21_RT_HEK293_FLO_ 16hr_viability	NA	human	HEK293T	cell cycle	24.0
TOX21_RT_HEK293_FLO_ 24hr_viability	NA	human	HEK293T	cell cycle	29.2
TOX21_RT_HEK293_FLO_ 32hr_viability	NA	human	HEK293T	cell cycle	28.8
TOX21_RT_HEK293_FLO_ 40hr_viability	NA	human	HEK293T	cell cycle	29.1
TOX21_RT_HEPG2_FLO_16h r_ctrl_viability	NA	human	HEK293T	cell cycle	14.3
TOX21_RT_HEPG2_FLO_24h r_viability	NA	human	HEK293T	cell cycle	13.4
TOX21_RT_HEPG2_FLO_32h r_ctrl_viability	NA	human	HEK293T	cell cycle	15.7
TOX21_RT_HEPG2_FLO_40h r_ctrl_viability	NA	human	HEK293T	cell cycle	14.8
TOX21_RT_HEPG2_GLO_00 hr_ctrl_viability	NA	human	HEK293T	cell cycle	43.6
NIS_HEK293T_CTG_ Cytotoxicity	NA	human	HEK293T	cell cycle	10.3
TOX21_ERb_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	42.2
TOX21_ERb_BLA_Antagonist _viability	NA	human	HEK293T	cell cycle	42.5
TOX21_PR_BLA_Agonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	40.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
TOX21_PR_BLA_Antagonist_viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	55.9
TOX21_DT40_100	NA	chicken	lymphoblast	cell cycle	43.3
TOX21_DT40_657	NA	chicken	lymphoblast	cell cycle	65.3
TOX21_PXR_viability	NA	human	PXR-Luc HepG2 cells	cell cycle	88.4
APR_HepG2_MitoMass_24h_dn	NA	human	HepG2	cell morphology	120
APR_HepG2_MitoMass_72h_dn	NA	human	HepG2	cell morphology	113
TOX21_MMP_ratio_up	NA	human	HepG2	cell morphology	72.5
LTEA_HepaRG_CYP1A2_dn	CYP1A2	human	HepaRG	cyp	202
LTEA_HepaRG_CYP3A4_up	CYP3A4	human	HepaRG	cyp	8.63
LTEA_HepaRG_CYP3A5_dn	CYP3A5	human	HepaRG	cyp	90.0
LTEA_HepaRG_CYP3A7_up	CYP3A7	human	HepaRG	cyp	9.25
LTEA_HepaRG_CYP4A11_dn	CYP4A11	human	HepaRG	cyp	87.0
LTEA_HepaRG_CYP4A22_dn	CYP4A22	human	HepaRG	cyp	85.8
LTEA_HepaRG_CYP7A1_dn	CYP7A1	human	HepaRG	cyp	82.2
LTEA_HepaRG_CYP2B6_dn	CYP2B6	human	HepaRG	cyp	86.4
LTEA_HepaRG_CYP2B6_up	CYP2B6	human	HepaRG	cyp	3.31
LTEA_HepaRG_CYP2C8_dn	CYP2C8	human	HepaRG	cyp	89.2
LTEA_HepaRG_CYP2C8_up	CYP2C8	human	HepaRG	cyp	21.2
LTEA_HepaRG_CYP2C9_dn	CYP2C9	human	HepaRG	cyp	86.3
NVS_ADME_hCYP2C9	CYP2C9	human	NA	cyp	1.30e-2
NVS_ADME_hCYP2C19	CYP2C19	human	NA	cyp	6.09
LTEA_HepaRG_CYP2C19_dn	CYP2C19	human	HepaRG	cyp	81.9
LTEA_HepaRG_CYP2C19_up	CYP2C19	human	HepaRG	cyp	11.1
LTEA_HepaRG_CYP2E1_dn	CYP2E1	human	HepaRG	cyp	60.4
BSK_BE3C_MIG_down	CXCL9	human	bronchial epithelial cells	cytokine	40.0
BSK_BE3C_IP10_down	CXCL10	human	bronchial epithelial cells	cytokine	10.0
BSK_KF3CT_IP10_down	CXCL10	human	keratinocytes and foreskin fibroblasts	cytokine	40.0
BSK_BE3C_IL1a_down	IL1A	human	bronchial epithelial cells	cytokine	40.0
BSK_KF3CT_IL1a_down	IL1A	human	keratinocytes and foreskin fibroblasts	cytokine	40.0
BSK_BE3C_uPAR_down	PLAUR	human	bronchial epithelial cells	cytokine	40.0
BSK_BE3C_PAI1_down	SERPINE1	human	bronchial epithelial cells	cytokine	40.0
LTEA_HepaRG_IL6R_dn	IL6R	human	HepaRG	cytokine receptor	95.7
NHEERL_MED_hDIO1_dn	DIO1	human	NA	deiodinase	175
NHEERL_MED_hDIO2_dn	NA	human	NA	deiodinase	122

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NHEERL_MED_hDIO3_dn	NA	human	NA	deiodinase	109
LTEA_HepaRG_DDIT3_up	DDIT3	human	HepaRG	dna binding	8.61
LTEA_HepaRG_EGR1_dn	EGR1	human	HepaRG	dna binding	42.6
ATG_AP_1_CIS_up	FOS	human	HepG2	dna binding	37.7
LTEA_HepaRG_JUN_up	JUN	human	HepaRG	dna binding	53.6
ATG_MRE_CIS_up	MTF1	human	HepG2	dna binding	30.7
LTEA_HepaRG_MYC_up	MYC	human	HepaRG	dna binding	12.5
ATG_NRF2_ARE_CIS_up	NFE2L2	human	HepG2	dna binding	30.8
LTEA_HepaRG_NFE2L2_up	NFE2L2	human	HepaRG	dna binding	66.9
ATG_TCF_b_cat_CIS_dn	TCF7	human	HepG2	dna binding	111
APR_HepG2_p53Act_24h_up	TP53	human	HepG2	dna binding	111
APR_HepG2_p53Act_72h_up	TP53	human	HepG2	dna binding	23.7
ATG_p53_CIS_dn	TP53	human	HepG2	dna binding	49.0
LTEA_HepaRG_TP53_up	TP53	human	HepaRG	dna binding	82.6
TOX21_p53_BLA_p4_ratio	TP53	human	intestinal cells HCT116	dna binding	49.1
LTEA_HepaRG_XBP1_dn	XBP1	human	HepaRG	dna binding	73.6
LTEA_HepaRG_LIPC_dn	LIPC	human	HepaRG	esterase	34.2
LTEA_HepaRG_LPL_up	LPL	human	HepaRG	esterase	15.5
LTEA_HepaRG_KRT19_up	KRT19	human	HepaRG	filaments	35.5
NVS_GPCR_hAdoRA2a	ADORA2A	human	NA	gpcr	10.3
NVS_GPCR_hAdra2C	ADRA2C	human	NA	gpcr	17.6
NVS_GPCR_hAdrb1	ADRB1	human	NA	gpcr	31.5
NVS_GPCR_gLTD4	Cysltr1	guinea pig	NA	gpcr	30.9
NVS_GPCR_hDRD4.4	DRD4	human	NA	gpcr	18.8
NVS_GPCR_h5HT5A	HTR5A	human	NA	gpcr	32.7
NVS_GPCR_h5HT7	HTR7	human	NA	gpcr	30.2
NVS_GPCR_hLTB4_BLT1	LTB4R	human	NA	gpcr	25.9
NVS_GPCR_gLTB4	Ltb4r	guinea pig	NA	gpcr	12.1
NVS_GPCR_hTXA2	TBXA2R	human	NA	gpcr	16.4
LTEA_HepaRG_IGF1_dn	IGF1	human	HepaRG	growth factor	90.0
LTEA_HepaRG_TGFA_up	TGFA	human	HepaRG	growth factor	71.6
ATG_TGFb_CIS_up	TGFB1	human	HepG2	growth factor	26.2
LTEA_HepaRG_TGFB1_up	TGFB1	human	HepaRG	growth factor	54.0
BSK_BE3C_TGFb1_down	TGFB1	human	bronchial epithelial cells	growth factor	40.0
BSK_KF3CT_TGFb1_down	TGFB1	human	keratinocytes and foreskin fibroblasts	growth factor	40.0
LTEA_HepaRG_THRSP_dn	THRSP	human	HepaRG	growth factor	57.8
TOX21_SBE_BLA_Antagonist_ratio	null	human	SBE-bla HEK 293T cell line	growth factor receptor	34.6
LTEA_HepaRG_KCNK1_up	KCNK1	human	HepaRG	ion channel	34.0
LTEA_HepaRG_PDK4_up	PDK4	human	HepaRG	kinase	23.0
LTEA_HepaRG_FASN_dn	FASN	human	HepaRG	lyase	65.1
LTEA_HepaRG_HMGCS2_dn	HMGCS2	human	HepaRG	lyase	82.3

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
Tanguay_ZF_120hpf_MORT_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	19.8
Tanguay_ZF_120hpf_YSE_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	38.0
Tanguay_ZF_120hpf_AXIS_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	34.6
Tanguay_ZF_120hpf_TR_up	NA	zebrafish	dechorionated zebrafish embryo	malformation	12.5
NHEERL_ZF_144hpf_TERATOSCORE_up	NA	zebrafish	zebrafish embryo	malformation	9.62
Tanguay_ZF_120hpf_ActivityScore	NA	zebrafish	dechorionated zebrafish embryo	malformation	33.7
LTEA_HepaRG_EZR_up	EZR	human	HepaRG	membrane protein	24.7
LTEA_HepaRG_MIR122_dn	MIR122	human	HepaRG	microrna	49.7
LTEA_HepaRG_GADD45B_up	GADD45B	human	HepaRG	mutagenicity response	34.8
LTEA_HepaRG_GADD45G_dn	GADD45G	human	HepaRG	mutagenicity response	36.2
ACEA_AR_antagonist_80hr	AR	human	prostate 22Rv1	nuclear receptor	69.3
NVS_NR_hAR	AR	human	NA	nuclear receptor	20.9
NVS_NR_rAR	AR	rat	NA	nuclear receptor	12.0
ATG_ERE_CIS_up	ESR1	human	HepG2	nuclear receptor	32.6
ATG_ERa_TRANS_up	ESR1	human	HepG2	nuclear receptor	35.9
NVS_NR_bER	ESR1	bovine	NA	nuclear receptor	2.59e-2
ATG_LXRb_TRANS_dn	NR1H2	human	HepG2	nuclear receptor	13.4
ATG_DR4_LXR_CIS_dn	NR1H3	human	HepG2	nuclear receptor	32.2
ATG_PXRE_CIS_up	NR1I2	human	HepG2	nuclear receptor	9.76
ATG_PXR_TRANS_up	NR1I2	human	HepG2	nuclear receptor	14.0
ATG_CAR_TRANS_up	NR1I3	human	HepG2	nuclear receptor	39.5
NVS_NR_hGR	NR3C1	human	NA	nuclear receptor	20.7
TOX21_PR_BLA_Antagonist_ratio	PGR	human	PR-UAS-bla-HEK293T	nuclear receptor	63.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
NVS_NR_hPR	PGR	human	NA	nuclear receptor	25.4
NVS_NR_bPR	PGR	bovine	NA	nuclear receptor	29.6
ATG_PPARa_TRANS_up	PPARA	human	HepG2	nuclear receptor	17.1
NVS_NR_hPPARa	PPARA	human	NA	nuclear receptor	11.2
ATG_PPARg_TRANS_up	PPARG	human	HepG2	nuclear receptor	22.2
NVS_NR_hPPARg	PPARG	human	NA	nuclear receptor	20.3
NVS_NR_hRARa_Antagonist	RARA	human	NA	nuclear receptor	1.91
TOX21_TR_LUC_GH3_ Antagonist	Thrb	rat	pituitary gland GH3	nuclear receptor	65.1
LTEA_HepaRG_FMO3_dn	FMO3	human	HepaRG	oxidoreductase	90.4
LTEA_HepaRG_ALPP_up	ALPP	human	HepaRG	phosphatase	57.1
NVS_ENZ_hPPP1CA	PPP1CA	human	NA	phosphatase	41.0
LTEA_HepaRG_PPP2R4_up	PPP2R4	human	HepaRG	phosphatase	55.9
NVS_ENZ_hPTPRC	PTPRC	human	NA	phosphatase	18.9
NVS_ENZ_hBACE	BACE1	human	NA	protease	8.72
LTEA_HepaRG_CASP3_up	CASP3	human	HepaRG	protease	22.5
NVS_ENZ_hCASP5	CASP5	human	NA	protease	27.8
NVS_ENZ_hMMP3	MMP3	human	NA	protease	29.4
BSK_KF3CT_MMP9_down	MMP9	human	keratinocytes and foreskin fibroblasts	protease	40.0
LTEA_HepaRG_MMP10_up	MMP10	human	HepaRG	protease	46.6
BSK_BE3C_tPA_down	PLAT	human	bronchial epithelial cells	protease	40.0
BSK_BE3C_uPA_down	PLAU	human	bronchial epithelial cells	protease	40.0
BSK_KF3CT_uPA_down	PLAU	human	keratinocytes and foreskin fibroblasts	protease	40.0
BSK_hDFCGF_TIMP1_up	TIMP1	human	foreskin fibroblast	protease inhibitor	10.0
BSK_KF3CT_TIMP2_down	TIMP2	human	keratinocytes and foreskin fibroblasts	protease inhibitor	40.0
LTEA_HepaRG_GSTA2_dn	GSTA2	human	HepaRG	transferase	71.1
LTEA_HepaRG_SULT2A1_dn	SULT2A1	human	HepaRG	transferase	88.4
LTEA_HepaRG_UGT1A1_dn	UGT1A1	human	HepaRG	transferase	85.5
LTEA_HepaRG_UGT1A1_up	UGT1A1	human	HepaRG	transferase	10.2
LTEA_HepaRG_UGT1A6_dn	UGT1A6	human	HepaRG	transferase	38.5
LTEA_HepaRG_ABCB11_dn	ABCB11	human	HepaRG	transporter	86.8
LTEA_HepaRG_FABP1_dn	FABP1	human	HepaRG	transporter	82.8
LTEA_HepaRG_IGFBP1_up	IGFBP1	human	HepaRG	transporter	73.5

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
LTEA_HepaRG_SLC22A1_dn	SLC22A1	human	HepaRG	transporter	78.6
LTEA_HepaRG_SLCO1B1_dn	SLCO1B1	human	HepaRG	transporter	86.2
NIS_RAIU_inhibition	SLC5A5	human	HEK293T	transporter	22.8

[1] Assays are alphabetically ordered by "intended target family", and within each "intended target family" assays are ordered alphabetically by "gene symbol". This table includes assays with curve-fitting "flags", although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as 'background measurement' assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.
NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

Table A9.5. 26 Active ToxCast assays[1] for PFOS lithium salt

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
TOX21_MMP_viability	NA	human	HepG2	cell cycle	67.4
TOX21_TR_LUC_GH3_ Antagonist_viability	NA	rat	pituitary gland GH4	cell cycle	52.3
TOX21_ARE_BLA_agonist_ viability	NA	human	HepG2	cell cycle	76.2
TOX21_FXR_BLA_antagonist _viability	NA	human	HEK293T	cell cycle	253
TOX21_PPARd_BLA_ Agonist_viability	NA	human	HEK293T	cell cycle	31.4
TOX21_p53_BLA_p2_viability	NA	human	intestinal cells HCT116	cell cycle	70.0
TOX21_p53_BLA_p3_viability	NA	human	intestinal cells HCT116	cell cycle	49.0
TOX21_p53_BLA_p4_viability	NA	human	intestinal cells HCT116	cell cycle	65.8
TOX21_AP1_BLA_Agonist_vi ability	NA	human	cervix ME-180	cell cycle	38.7
TOX21_H2AX_HTRF_CHO_vi ability	NA	Chinese hamster	CHO-K1	cell cycle	158
TOX21_HRE_BLA_Agonist_ viability	NA	human	cervix ME-180	cell cycle	40.5
TOX21_RT_HEK293_FLO_ 16hr_viability	NA	human	HEK293T	cell cycle	7.40e-2
TOX21_RT_HEK293_FLO_ 24hr_viability	NA	human	HEK293T	cell cycle	8.21e-2
TOX21_RT_HEK293_FLO_ 40hr_viability	NA	human	HEK293T	cell cycle	0.289
TOX21_RT_HEPG2_GLO_00 hr_ctrl_viability	NA	human	HEK293T	cell cycle	45.2
TOX21_ERb_BLA_Agonist_ viability	NA	human	HEK293T	cell cycle	43.4
TOX21_ERb_BLA_Antagonist _viability	NA	human	HEK293T	cell cycle	95.9
TOX21_PR_BLA_Agonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	50.0
TOX21_PR_BLA_Antagonist_ viability	NA	human	PR-UAS-bla-HEK293T	cell cycle	84.3
TOX21_MMP_ratio_up	NA	human	HepG2	cell morphology	40.5
TOX21_ARE_BLA_agonist_ ratio	NFE2L2	human	HEK293T	dna binding	85.2
TOX21_p53_BLA_p4_ratio	TP53	human	intestinal cells HCT116	dna binding	70.8
TOX21_SBE_BLA_Antagonist _ratio	NA	human	SBE-bla HEK 293T cell line	growth factor receptor	45.3
NVS_NR_bER	ESR1	bovine	NA	nuclear receptor	15.1
TOX21_PR_BLA_Antagonist_ ratio	PGR	human	PR-UAS-bla-HEK293T	nuclear receptor	98.6

Assay Name	Gene Symbol	Organism	Cells/Cell Lines	Intended Target Family	AC50 (µM)
TOX21_TR_LUC_GH3_ Antagonist	Thrb	rat	pituitary gland GH3	nuclear receptor	63.5

[1] Assays are alphabetically ordered by "intended target family", and within each "intended target family" assays are ordered alphabetically by "gene symbol". This table includes assays with curve-fitting "flags", although the flags are not shown here. The table does not include assays classified by the US EPA Comptox Chemicals Dashboard as 'background measurement' assays (e.g., artifact fluorescence, baseline controls, and internal markers).
AC50: the concentration that induces a half-maximal assay response.
NA, not applicable. This notation is used when no specific target genes are reported by the US EPA Comptox Chemicals Dashboard, and used for cell-free assays such as cell-free systems utilizing enzymes or receptors extracted from tissues or cells of various organisms.

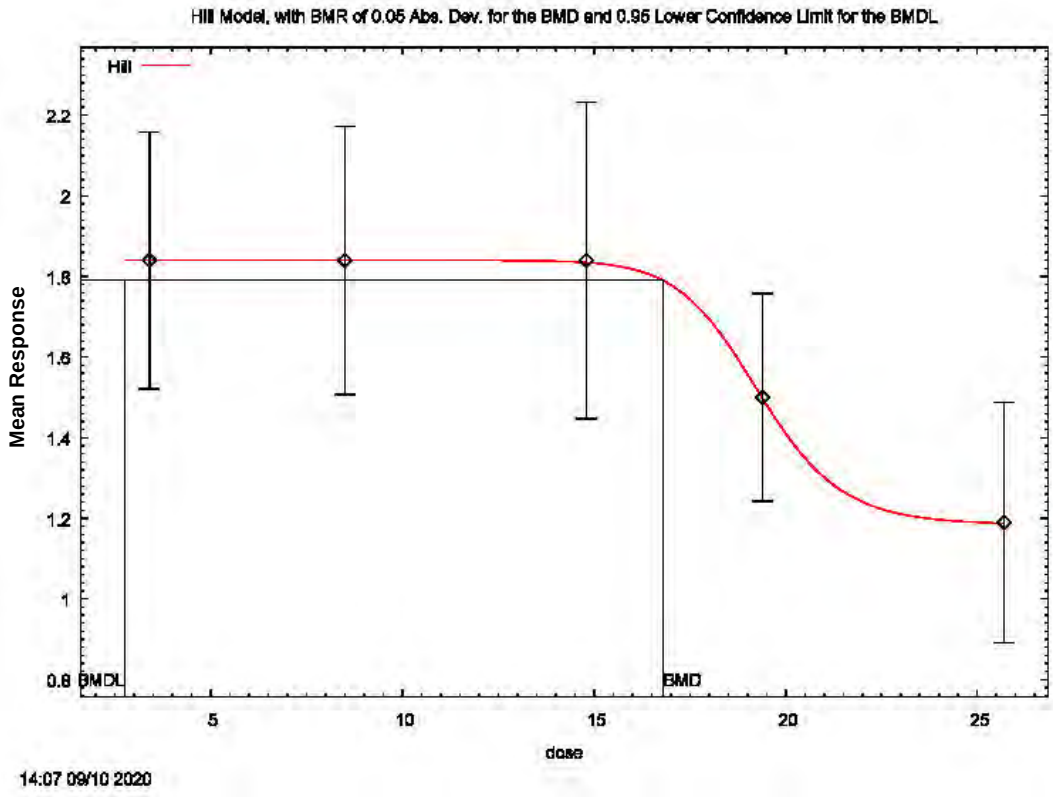
APPENDIX 10. BENCHMARK DOSE MODELING

This appendix provides the BMD modeling outputs for toxicity data that were amenable to dose-response modeling. All models are run with default parameters and a benchmark response of 5% for dichotomous data and one standard deviation from the control mean for continuous data. When appropriate, some models for continuous data are run with modeled variance instead of the default constant variance. Model selection criteria when comparing outputs of different models for the same endpoint/dataset are: scaled residual ≤ the absolute value of two, goodness of fit p-value ≥ 0.05,[28] the Akaike's information criterion (AIC), and visual inspection of the dose-response curve. The lower limit of the 95% confidence interval of the BMD resulting in the benchmark response, the BMDL, is selected as the POD. The model selected for each study to derive a POD is presented below.

Benchmark Dose Analysis Results for Noncancer Endpoints

Perfluorooctanoic Acid

Figure A10.1. BMD modeling of decreased influenza (Hib) antibody levels in humans from Abraham et al. (2020)



[28] US EPA's Benchmark Dose Technical Guidance (2012) suggests using a goodness of fit p-value ≥ 0.1; however, models with less adequate fit (goodness of fit p-value ≥ 0.05) may be used when other criteria are taken into account, such as variability in the endpoint and visual fit.

Model run output for Figure A10.1: Hill Model for decreased influenza (Hib) antibody levels in humans from Abraham et al. (2020).
Note, the benchmark response factor (BMRF, or user defined benchmark response) was derived by OEHHA as follows:

Power(10, at dose Q1) = power(10, 1.84) = 69.2 mg/dl;

10% decrease = 69.2 mg/dl × 0.90 = 62.3 mg/dl;

Log_{10}(62.3 mg/dl) = 1.79.

So, a 10% decrease from Q1 corresponds to a log_{10} value of 1.79, or a difference of 1.84 – 1.79 = 0.05 on the log_{10} scale.

Thus, the BMRF is an absolute deviation of 0.05.

```
================================================================
Hill Model. (Version: 2.18;  Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham PFOA
Hib quintiles_Opt.(d)
Gnuplot Plotting File:  C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham
PFOA Hib quintiles_Opt.plt
Thu Sep 10 14:07:15 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha =  0.469219
rho =  0 Specified
intercept =  1.84
v =  -0.65
n =  8.78409
k =  19.1971

Asymptotic Correlation Matrix of Parameter Estimates
 ( *** The model parameter(s)  -rho    -n
```

have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	v	k
lalpha	1	-5.7e-008	2.1e-007	-3.9e-007
intercept	-5.7e-008	1	-0.47	-0.25
v	2.1e-007	-0.47	1	-0.31
k	-3.9e-007	-0.25	-0.31	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.445284	0.0636119	0.320606	0.569961
intercept	1.84178	0.0868517	1.67155	2.012
v	-0.655607	0.181528	-1.0114	-0.299819
n	18	NA		
k	19.3091	1.1682	17.0194	21.5987

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
3.4	20	1.84	1.84	0.68	0.667	-0.0119
8.5	20	1.84	1.84	0.71	0.667	-0.0119
14.8	20	1.84	1.84	0.84	0.667	0.0244
19.4	20	1.5	1.5	0.55	0.667	-0.000805
25.7	18	1.19	1.19	0.6	0.667	0.000231

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-9.356408	6	30.712816
A2	-7.232118	10	34.464235
A3	-9.356408	6	30.712816
fitted	-9.356848	4	26.713697
R	-16.086495	2	36.172990

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	17.7088	8	0.02352
Test 2	4.24858	4	0.3734
Test 3	4.24858	4	0.3734
Test 4	0.000880611	2	0.9996

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

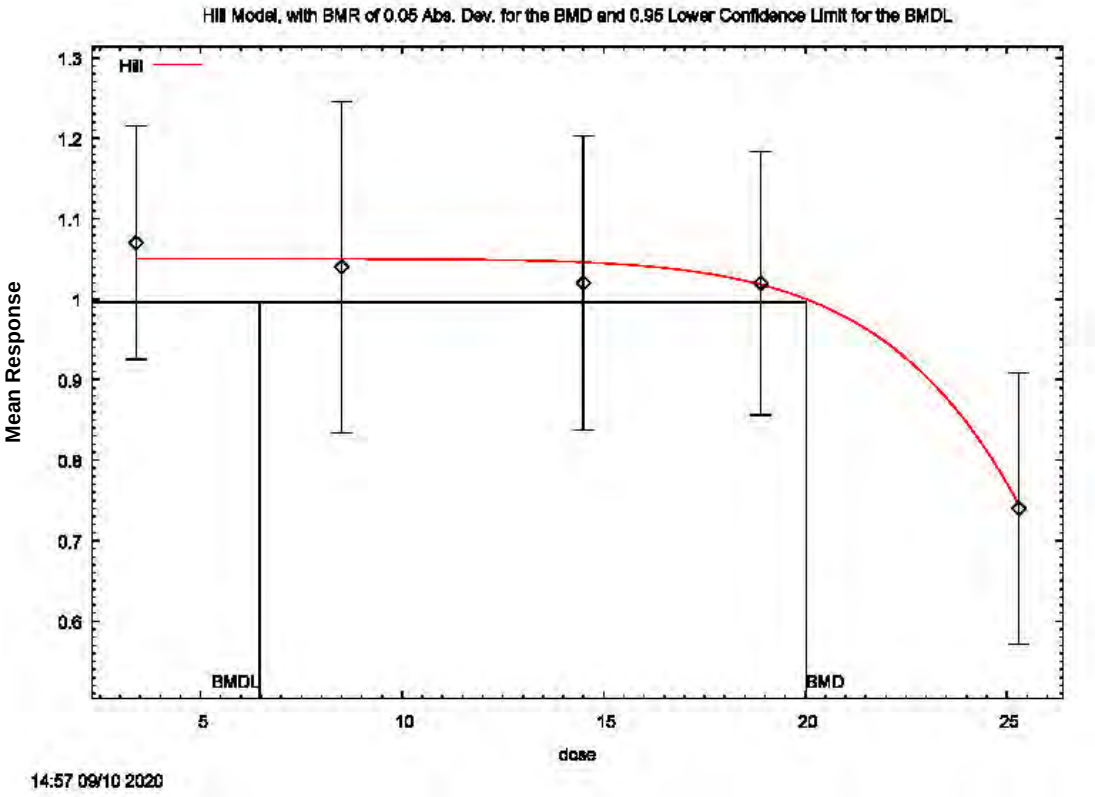
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Absolute deviation
Confidence level = 0.95
BMD = 16.8106
BMDL = 2.75177
BMDU = 19.2936

Figure A10.2. BMD modeling of decreased tetanus antibody levels in humans from Abraham et al. (2020)



Model run output for Figure A10.2: Hill Model for decreased tetanus antibody levels in humans from Abraham et al. (2020).
Note, the BMRF was derived by OEHHA as follows:

Power(10, at dose Q1) = power(10, 1.07) = 11.75 mg/L (mean antibody cocentration at dose Q1);

10% decrease = 11.75 mg/L × 0.90 = 10.57 mg/L;

Log_{10}(10.57 mg/L) = 1.02.
So, a 10% decrease from Q1 corresponds to a log_{10} value of 1.02, or a difference of 1.07 – 1.02 = 0.05 on the log_{10} scale.
Thus, the BMRF is an absolute deviation of 0.05.
==========
Hill Model. (Version: 2.18; Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham PFOA tetanus quintiles_Opt.(d)
Gnuplot Plotting File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham PFOA tetanus quintiles_Opt.plt
Thu Sep 10 14:57:53 2020
==========
BMDS Model Run
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The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 0.13878
rho = 0 Specified
intercept = 1.07
v = -0.33
n = 6.68878
k = 21.5286

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	n	v	k
lalpha	1	-1.6e-005	0.00062	8.6e-005	-0.00062
intercept	-1.6e-005	1	-0.025	-0.62	0.07
v	0.00062	-0.025	1	0.14	-1
n	8.6e-005	-0.62	0.14	1	-0.21
k	-0.00062	0.07	-1	-0.21	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.132066	0.018677	0.09546	0.168672
intercept	1.04647	0.0533738	0.941856	1.15108
v	-79.6551	8878.19	-17480.6	17321.3
n	7.75749	11.8916	-15.5497	31.0647
k	51.79	756.924	-1431.75	1535.33

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
3.4	20	1.07	1.05	0.31	0.363	0.29
8.5	20	1.04	1.05	0.44	0.363	-0.0788
14.5	20	1.02	1.04	0.39	0.363	-0.275

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
18.9	20	1.02	1.01	0.35	0.363	0.0679
25.3	20	0.74	0.74	0.36	0.363	-0.0034

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	51.307931	6	-90.615863
A2	52.669574	10	-85.339147
A3	51.307931	6	-90.615863
fitted	51.222612	5	-92.445225
R	46.092155	2	-88.184310

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	13.1548	8	0.1066
Test 2	2.72328	4	0.6051
Test 3	2.72328	4	0.6051
Test 4	0.170638	1	0.6795

The p-value for Test 1 is greater than .05. There may not be a
diffence between responses and/or variances among the dose levels
Modelling the data with a dose/response curve may not be appropriate

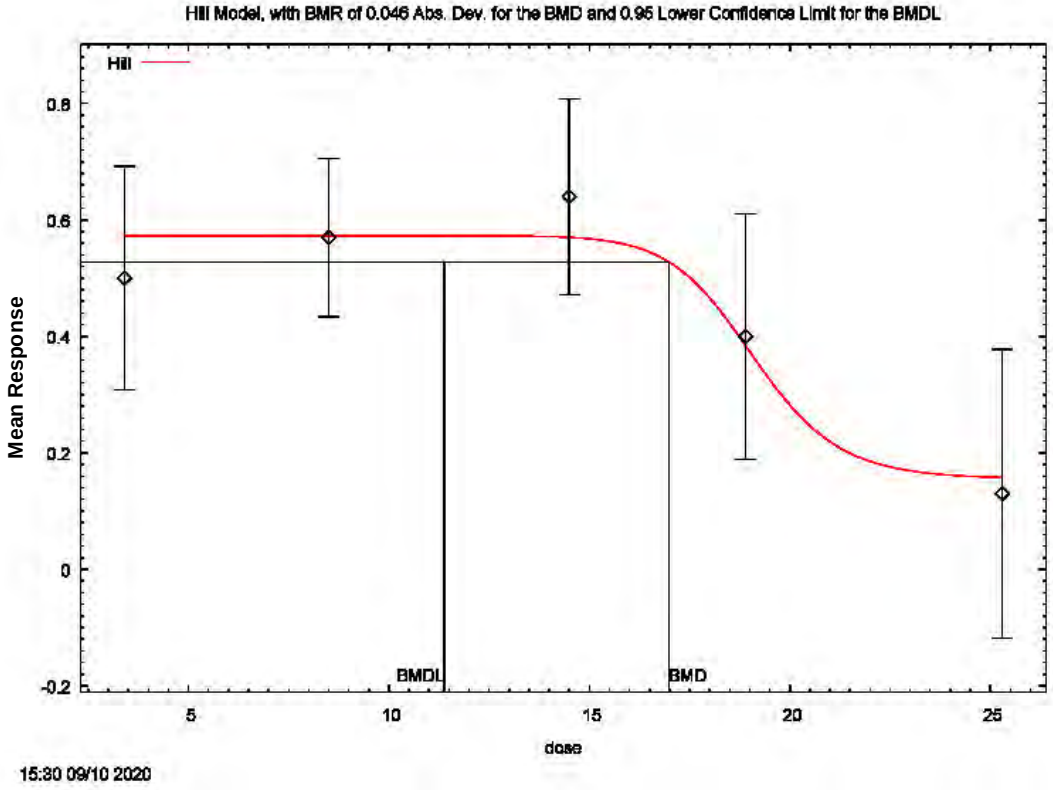
The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Absolute deviation
Confidence level = 0.95
BMD = 20.0211
BMDL = 6.4617
BMDU = 23.8054

Figure A10.3. BMD modeling of decreased diphtheria antibody levels in humans from Abraham et al. (2020)



Model run output for Figure A10.3: Hill Model for decreased diphtheria antibody levels in humans from Abraham et al. (2020).
Note, the BMRF was derived by OEHHA as follows:

Power(10, at dose Q1) = power(10, 0.50) = 3.16 IU/ml (mean antibody concentration at dose Q1);

10% decrease = 3.16 IU/ml × 0.90 = 2.846 IU/ml;

Log_{10}(2.846 IU/ml) = 0.454.

So, a 10% decrease from Q1 corresponds to a log_{10} value of 0.454, or a difference of 0.50 –

0.454 = 0.046 on the $\log_{10}$ scale.

Thus, the BMRF is an absolute deviation of 0.046.

==========

Hill Model. (Version: 2.18; Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham PFOA diph quintiles_Opt.(d)
Gnuplot Plotting File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Abraham PFOA diph quintiles_Opt.plt
Thu Sep 10 15:30:13 2020

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BMDS Model Run

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The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
Independent variable = Dose
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = exp(lalpha + rho * ln(mean(i)))
Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
lalpha = -1.75423
rho = 0
intercept = 0.5
v = -0.37
n = 9.80546
k = 20.9148

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	k
lalpha	1	0.92	0.17	-0.65	0.38
rho	0.92	1	0.093	-0.77	0.41
intercept	0.17	0.093	1	-0.39	-0.2
v	-0.65	-0.77	-0.39	1	-0.39
k	0.38	0.41	-0.2	-0.39	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	-2.3853	0.398083	-3.16553	-1.60507
~~~~~~~~~~

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
rho	-0.606513	0.480212	-1.54771	0.334686
intercept	0.572972	0.0462347	0.482353	0.66359
v	-0.417998	0.12579	-0.664542	-0.171455
n	18	NA		
k	19.0701	1.05478	17.0027	21.1374

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
3.4	20	0.5	0.573	0.41	0.359	-0.908
8.5	20	0.57	0.573	0.29	0.359	-0.037
14.5	20	0.64	0.57	0.36	0.36	0.87
18.9	20	0.4	0.381	0.45	0.407	0.211
25.3	20	0.13	0.158	0.53	0.531	-0.232

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	40.276290	6	-68.552579
A2	44.254859	10	-68.509717
A3	43.092126	7	-72.184252
fitted	42.034964	5	-74.069927
R	31.491216	2	-58.982431

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	25.5273	8	0.001265
Test 2	7.95714	4	0.09316
Test 3	2.32547	3	0.5077
Test 4	2.11432	2	0.3474

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

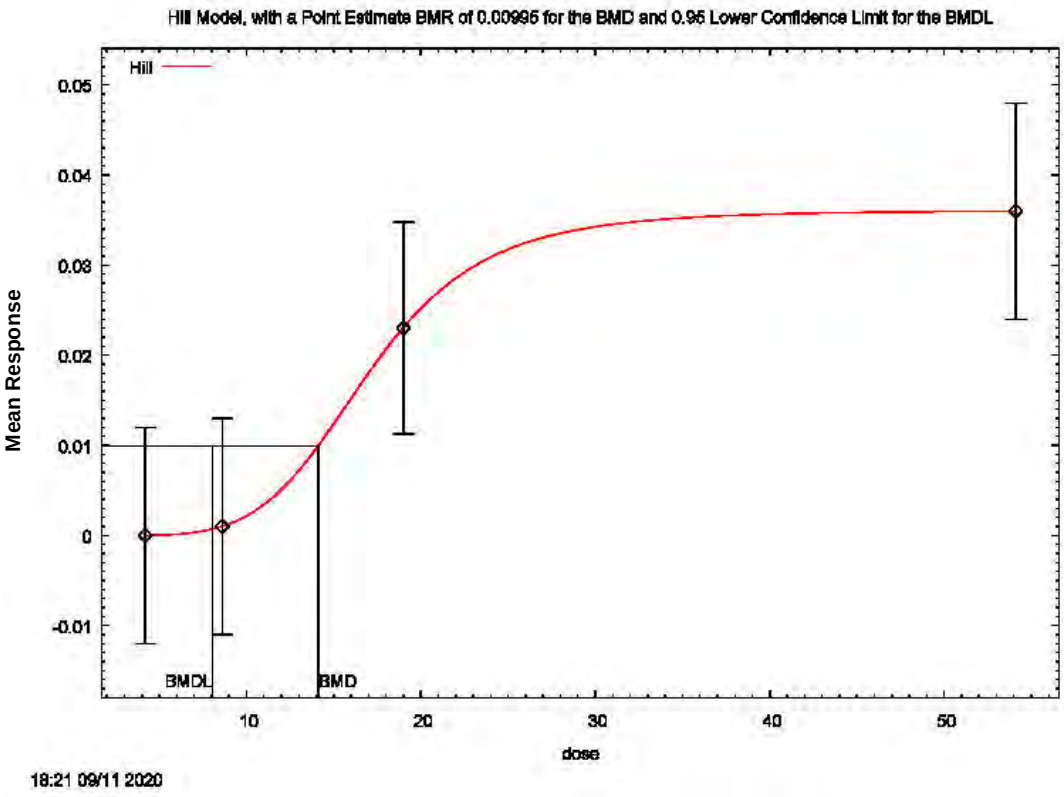
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.046
Risk Type = Absolute deviation
Confidence level = 0.95
BMD = 16.9793
BMDL = 11.3762
BMDU = 19.2778

Figure A10.4. BMD modeling of increased mean ALT concentrations in humans from Darrow et al. (2016)



Model run output for Figure A10.4: Hill Model for increased mean ALT levels in humans from Darrow et al. (2016).
Note, the BMRF was derived by OEHHA as follows:

A BMR of 1% would give a b of:

b = ln(1 + 0.01) = 0.00995. Thus, 0.00995 was used as the BMR of 1%.
Furthermore, the model did not converge with all quintiles, thus, quintile 5 was excluded.

```
================================================================
Hill Model. (Version: 2.18;  Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Darrow PFOA
ALT means b 4 levels_Opt.(d)
Gnuplot Plotting File:  C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Darrow
PFOA ALT means b 4 levels_Opt.plt
Fri Sep 11 18:21:52 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
```

Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 0.228025
rho = 0 Specified
intercept = 0
v = 0.036
n = 18
k = 21.3636

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	n	v	k
lalpha	1	-2.1e-008	4.9e-009	-1.1e-008	-4.1e-008
intercept	-2.1e-008	1	-0.75	0.71	0.7
v	4.9e-009	-0.75	1	-0.62	-0.38
n	-1.1e-008	0.71	-0.62	1	0.81
k	-4.1e-008	0.7	-0.38	0.81	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.227988	0.00205653	0.223957	0.232019
intercept	-2.59953e-005	0.00640626	-0.012582	0.01253
v	0.0361177	0.00950303	0.0174921	0.0547433
n	5.1685	11.1492	-16.6836	27.0206
k	17.0337	5.10574	7.02659	27.0407

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
4.2	6145	0	-1.41e-008	0.48	0.477	2.32e-006
8.6	6145	0.001	0.001	0.48	0.477	-2.46e-007
19	6145	0.023	0.023	0.47	0.477	-5.79e-006
54.1	6145	0.036	0.036	0.48	0.477	1.72e-006

Degrees of freedom for Test A3 vs. fitted <= 0

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	5880.307248	5	-11750.614495
A2	5882.335686	8	-11748.671371
A3	5880.307248	5	-11750.614495
fitted	5880.307248	5	-11750.614495
R	5867.834254	2	-11731.668509

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	29.0029	6	<.0001
Test 2	4.05688	3	0.2554
Test 3	4.05688	3	0.2554
Test 4	4.36557e-011	0	NA

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0. The Chi-Square
test for fit is not valid

Benchmark Dose Computation

Specified effect = 0.00995
Risk Type = Point estimate
Confidence level = 0.95
BMD = 14.1371
BMDL = 8.04229
BMDU = 18.5908

BMD modeling of increased ORs for high ALT in humans from Gallo et al. (2012)

Note: When all ten deciles are used, logprobit was the best fitting model (p=0.18; Figure A10.5A). Although p >0.10, this model resulted in a very steep dose-response curve at the lower doses (almost vertical) and an unusually low BMD (0.43 ng/ml), which did not correlate well with the observed data. Because of this, and because the fit was much improved with the logistic when the higher doses were removed, only deciles 1-5 were used in these BMD calculations (Figure A10.5B).

Figure A10.5A: BMD modeling of increased ORs for high ALT in humans from Gallo et al. (2012) (all ten deciles plotted)

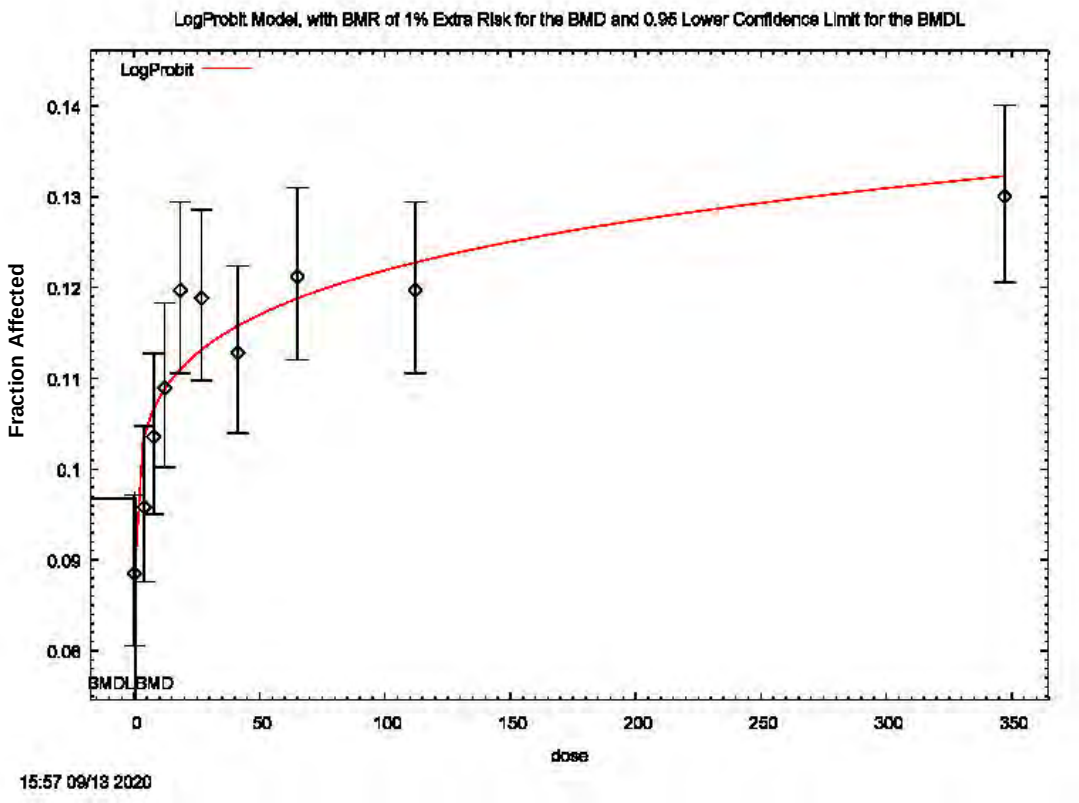
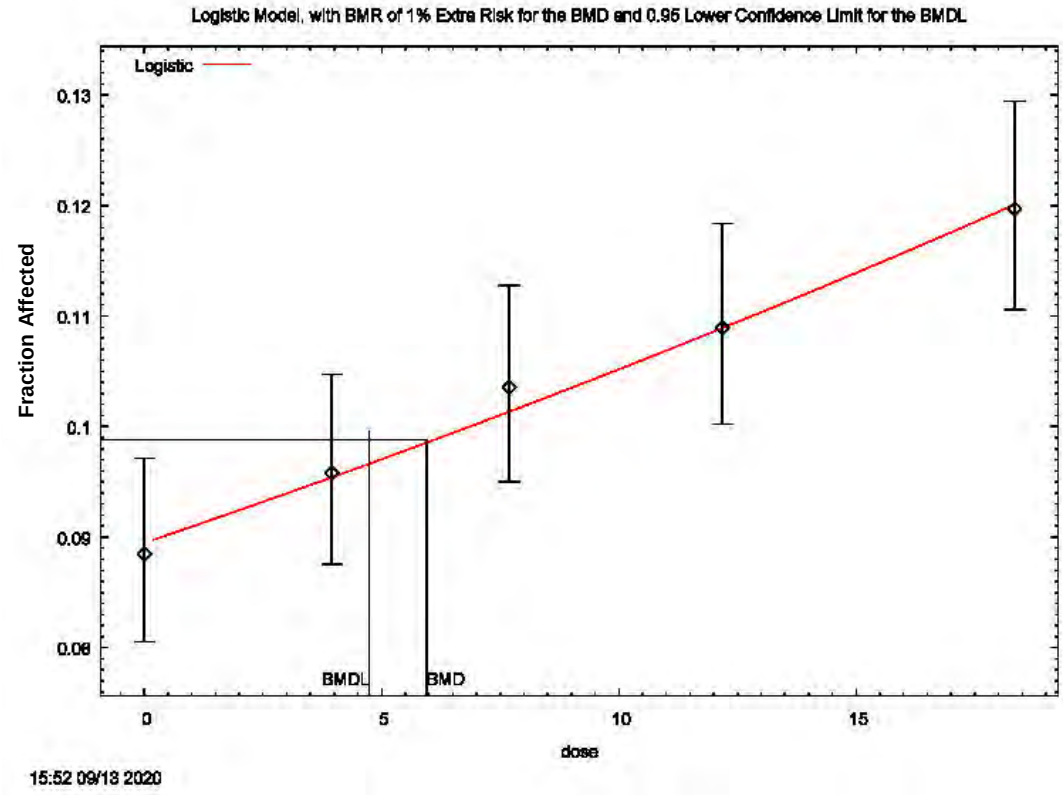


Figure A10.5B: BMD modeling of increased ORs for high ALT in humans from Gallo et al. (2012) (deciles 1-5 plotted)



Model run output for Figure A10.5B: Logistic Model for increased ORs for high ALT levels in humans from Gallo et al. (2012).

==========

Logistic Model. (Version: 2.15; Date: 3/20/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/log_Gallo PFOA ALT ORs 5_Opt.(d)
Gnuplot Plotting File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/log_Gallo PFOA ALT ORs 5_Opt.plt
Sun Sep 13 15:52:04 2020

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BMDS_Model_Run

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The form of the probability function is:
P[response] = 1/[1+EXP(-intercept-slope*dose)]

Dependent variable = case
Independent variable = dose0
Slope parameter is not restricted

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
~~~~~~~~~~

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
background = 0 Specified
intercept = -2.31763
slope = 0.018037

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.81
slope	-0.81	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
intercept	-2.3176	0.0371119	-2.39034	-2.24487
slope	0.0179348	0.0033493	0.0113703	0.0244993

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-7702.32	5			
Fitted model	-7702.47	2	0.302117	3	0.9596
Reduced model	-7716.75	1	28.8656	4	<.0001

AIC: 15408.9

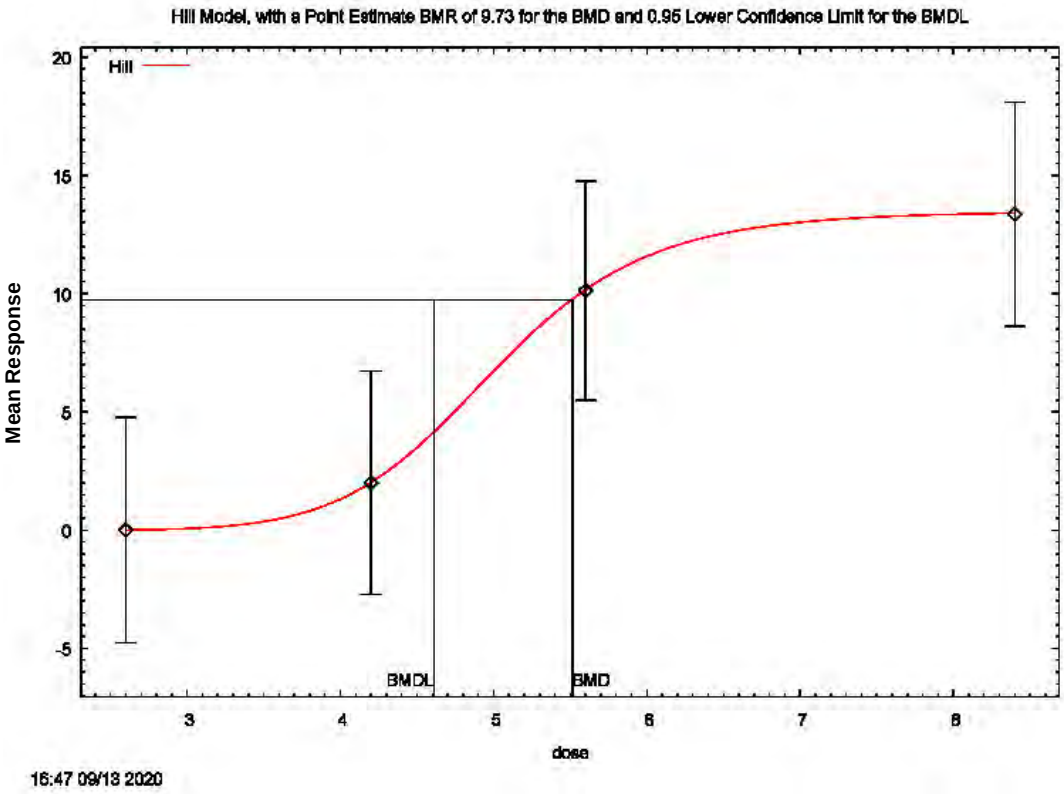
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0897	416.543	411.000	4645.000	-0.285
3.9500	0.0956	444.198	445.000	4645.000	0.040
7.6900	0.1016	471.884	481.000	4645.000	0.443
12.1800	0.1092	507.135	506.000	4645.000	-0.053
18.3400	0.1204	559.242	556.000	4645.000	-0.146

Chi^2 = 0.30 d.f. = 3 P-value = 0.9595

Benchmark Dose Computation
Specified effect = 0.01
Risk Type = Extra risk
Confidence level = 0.95
BMD = 5.9513
BMDL = 4.74341
BMDU = 8.24403

Figure A10.6. BMD modeling of increased mean serum total cholesterol in humans from Lin et al. (2019)



Model run output for Figure A10.6: Hill Model for increased mean serum total cholesterol levels in humans from Lin et al. (2019).

Note, the BMRF was derived by OEHHA as follows. The mean TC values in each quartile were not provided. However, the average TC in all subjects combined was provided. This overall average could be used, in combination with the mean differences, to estimate the mean TC in each quartile. These calculations are given in the "lipid reference level calculations" excel spreadsheet. Based on these calculations, the estimated mean TC in quartile 1 is 194.6. A 5% increase in this value is a mean difference of 194.6 × 5% = 9.73. Thus, 9.73 was used as the BMR of 5%.

==========

Hill Model. (Version: 2.18; Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Lin PFOA TC_Opt.(d)
Gnuplot Plotting File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Lin PFOA TC_Opt.plt
Sun Sep 13 16:47:22 2020

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BMDS Model Run

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The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
~~~~~~~~~~

Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 1286.34
rho = 0 Specified
intercept = 0
v = 13.36
n = 18
k = 6.1941

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	n	v	k
lalpha	1	-9.6e-009	3.6e-009	1.9e-009	3.1e-009
intercept	-9.6e-009	1	-0.72	0.5	0.53
v	3.6e-009	-0.72	1	-0.59	-0.045
n	1.9e-009	0.5	-0.59	1	0.2
k	3.1e-009	0.53	-0.045	0.2	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	1280.61	60.4356	1162.16	1399.06
intercept	-0.0203507	2.47126	-4.86394	4.82324
v	13.4584	3.62057	6.36216	20.5545
n	9.92358	7.34258	-4.46761	24.3148
k	5.00174	0.577992	3.8689	6.13459

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
2.6	221	0	-5.08e-007	35.9	35.8	2.11e-007
4.2	222	2	2	35.7	35.8	2.84e-007
5.6	227	10.1	10.1	35.5	35.8	1.69e-007
8.4	228	13.4	13.4	36.4	35.8	9.4e-008

Degrees of freedom for Test A3 vs. fitted <= 0
Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-3661.635617	5	7333.271234
A2	-3661.551213	8	7339.102426
A3	-3661.635617	5	7333.271234
fitted	-3661.635617	5	7333.271234
R	-3672.265777	2	7348.531554

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.4291	6	0.001536
Test 2	0.168809	3	0.9825
Test 3	0.168809	3	0.9825
Test 4	9.09495e-013	0	NA

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

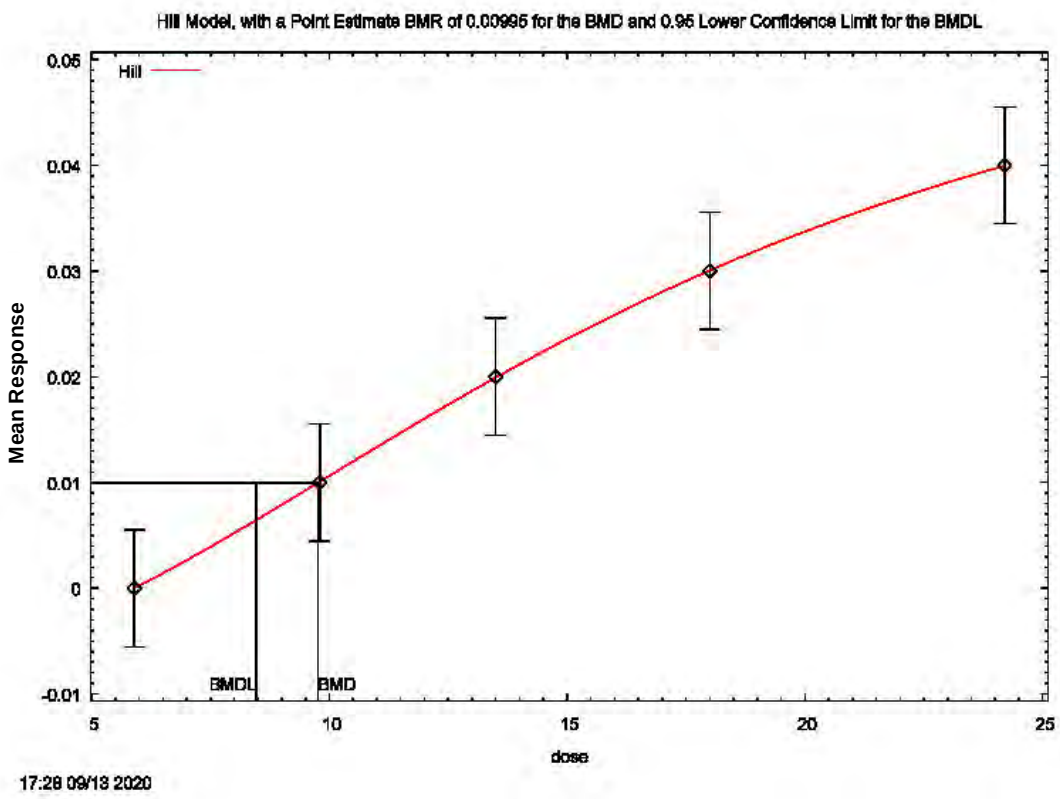
The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

NA - Degrees of freedom for Test 4 are less than or equal to 0. The Chi-Square
test for fit is not valid

Benchmark Dose Computation
Specified effect = 9.73
Risk Type = Point estimate
Confidence level = 0.95

BMD = 5.51357
BMDL = 4.61048
BMDU = 7.05482

Figure A10.7. BMD modeling of increased mean serum total cholesterol in humans from Steenland et al. (2009)



Model run output for Figure A10.7: Hill Model for increased mean serum total cholesterol levels from Steenland et al. (2009).

Note, the BMRF was derived by OEHHA as follows. A 5% increase was within the range of the observed effects but close to the upper limit (the percent increase in the highest decile was 5.1%). Because of this, a BMR of 1% was used.
Percent change in TC = [exp(b) - 1] x 100%
or, exp(b) = percent change + 1
or, exp(b) = 0.01 + 1 = 1.01
or, b = ln(1.01) = 0.00995.
Thus, b = 0.00995 was used as the BMR of 1%.

==========

Hill Model. (Version: 2.18; Date: 03/14/2017)
Input Data File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Steenland PFOA TC means 5_Opt.(d)
Gnuplot Plotting File: C:/Users/csteinmaus/Documents/BMDS/BMDS2704/Data/hil_Steenland PFOA TC means 5_Opt.plt
Sun Sep 13 17:28:05 2020

==========

BMDS Model Run
~~~~~~

The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 0.0370352
rho = 0 Specified
intercept = 0
v = 0.04
n = 18
k = 13.5

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	intercept	n	v	k
lalpha	1	2.1e-008	-1.3e-008	2.1e-008	-6.5e-009
intercept	2.1e-008	1	-0.93	0.95	-0.75
v	-1.3e-008	-0.93	1	-0.99	0.94
n	2.1e-008	0.95	-0.99	1	-0.9
k	-6.5e-009	-0.75	0.94	-0.9	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.0370272	0.000344197	0.0363526	0.0377018
intercept	-0.00722434	0.0160511	-0.038684	0.0242353
v	0.0688417	0.0656045	-0.0597409	0.197424
n	2.07285	2.18476	-2.2092	6.3549
k	16.6084	9.65775	-2.32041	35.5373

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
5.9	4629	0	-1.18e-005	0.192	0.192	0.00416
9.8	4629	0.01	0.0101	0.192	0.192	-0.0185
13.5	4629	0.02	0.0199	0.192	0.192	0.0298
~~~~~~

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
18	4629	0.03	0.0301	0.192	0.192	-0.0213
24.2	4629	0.04	0.04	0.192	0.192	0.00581

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	26571.646401	6	-53131.292802
A2	26571.646401	10	-53123.292802
A3	26571.646401	6	-53123.292802
fitted	26571.645532	5	-53133.291064
R	26509.306509	2	-53014.613018

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	124.68	8	<.0001
Test 2	0	4	1
Test 3	0	4	1
Test 4	0.00173865	1	0.9667

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

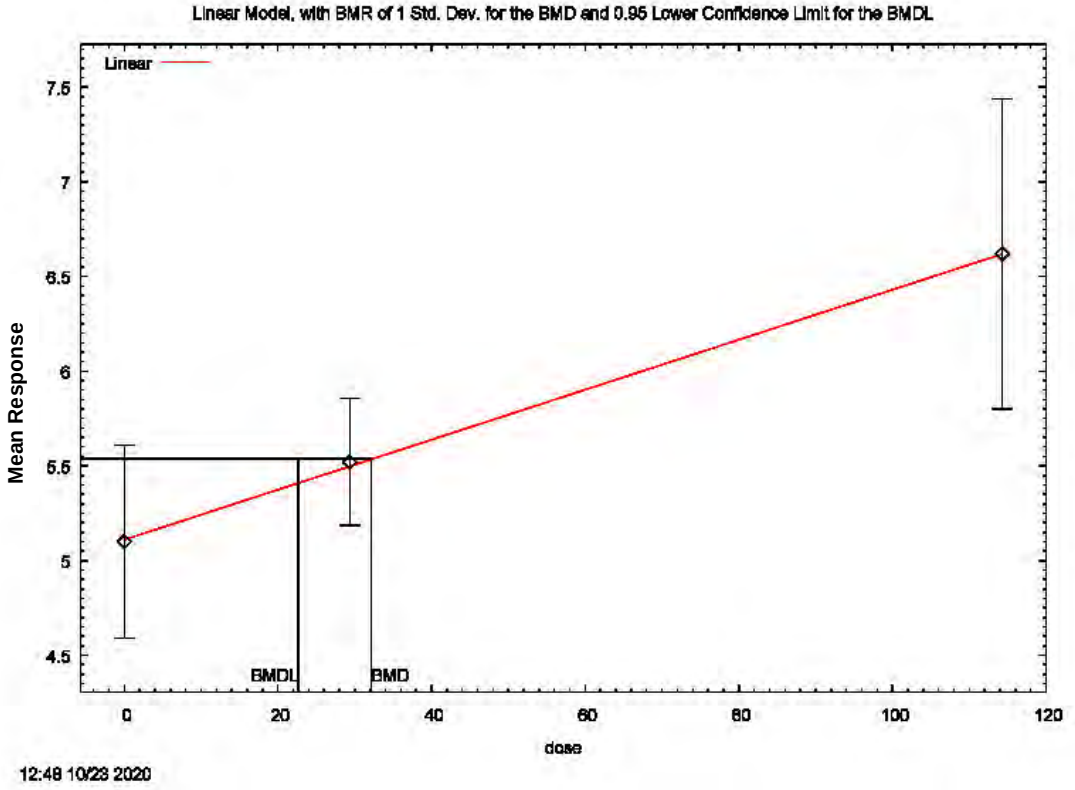
The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.00995
Risk Type = Point estimate
Confidence level = 0.95
BMD = 9.76261
BMDL = 8.46747
BMDU = 11.1839

Figure A10.8. BMD modeling of increased relative liver weight weight in male BALB/c mice from Yu et al. (2016)



Model run output for Figure A10.8: BMD modeling of increased relative liver weight in male BALB/c mice from Yu et al. (2016).

```
================================================================================
Polynomial Model. (Version: 2.21;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/lin_Yu 2016 rel liv weight_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOA/lin_Yu 2016 rel liv weight_Opt.plt
Fri Oct 23 12:48:38 2020
 ================================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
```

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit
Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
Default Initial Parameter Values
alpha = 0.225533
rho = 0 Specified
beta_0 = 5.1136
beta_1 = 0.0132208
Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	1.3e-009	1.1e-009
beta_0	1.3e-009	1	-0.7
beta_1	1.1e-009	-0.7	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.180611	0.0659499	0.0513518	0.309871
beta_0	5.1136	0.154253	4.81127	5.41593
beta_1	0.0132208	0.00226408	0.00878333	0.0176584

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.011	5	5.1	5.11	0.41	0.425	-0.0723
29.34	5	5.52	5.5	0.27	0.425	0.0973
114.3	5	6.62	6.62	0.66	0.425	-0.025

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	5.343231	4	-2.686463
A2	7.255811	6	-2.511622
A3	5.343231	4	-2.686463
fitted	5.335563	3	-4.671127
R	-3.557768	2	11.115535

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	21.6272	4	0.0002377
Test 2	3.82516	2	0.1477
Test 3	3.82516	2	0.1477
Test 4	0.0153362	1	0.9014

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

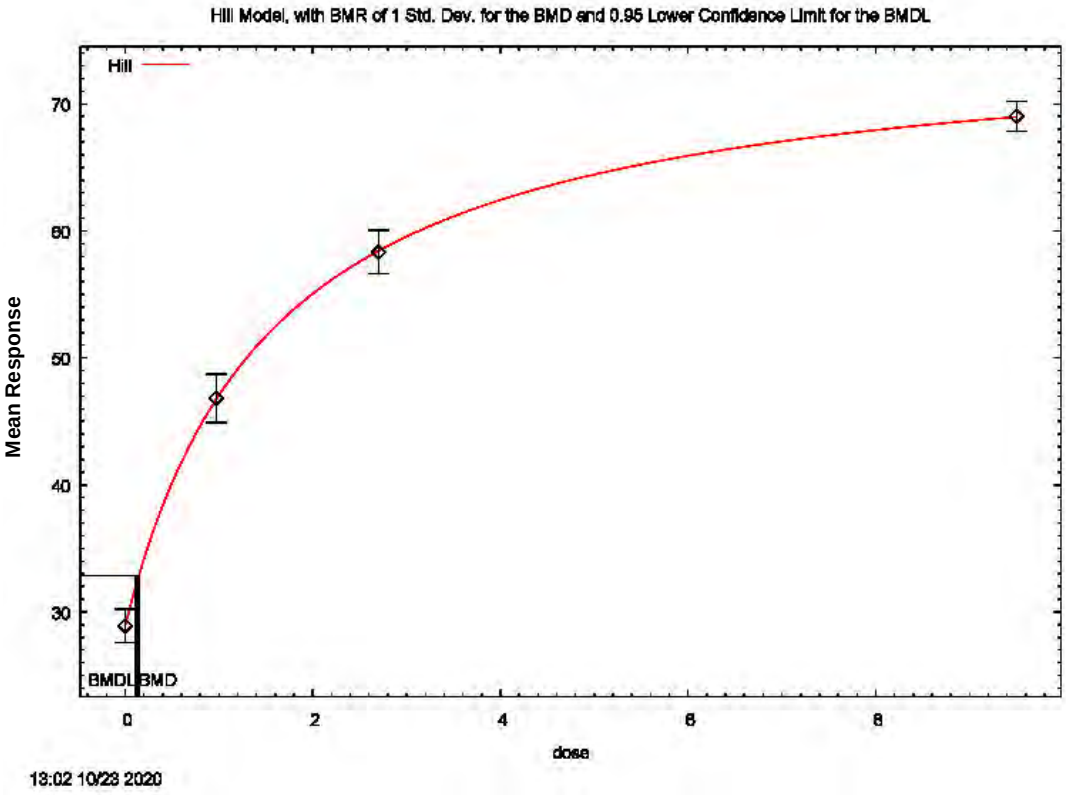
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 32.145
BMDL = 22.6469
BMDU = 53.8869

Figure A10.9. BMD modeling of increased p53 levels in male BALB/c mice Li et al. (2017)



Model run output for Figure A10.9: BMD modeling of increased p53 levels in male BALB/c mice Li et al. (2017).

```
================================================================================
Hill Model. (Version: 2.18;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/hil_Li female p53_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOA/hil_Li female p53_Opt.plt
Fri Oct 23 13:02:53 2020
================================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)
Dependent variable = Mean
Independent variable = Dose
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))
Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
lalpha = 2.84693
```

rho = 0
intercept = 28.8963
v = 40.1338
n = 0.0741803
k = 4.10814

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	k
lalpha	1	-1	0.041	0.045	0.088
rho	-1	1	-0.042	-0.045	-0.088
intercept	0.041	-0.042	1	-0.38	0.44
v	0.045	-0.045	-0.38	1	0.55
k	0.088	-0.088	0.44	0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	2.41404	1.80205	-1.11792	5.946
rho	0.102867	0.463534	-0.805643	1.01138
intercept	28.9066	0.721242	27.493	30.3203
v	46.6915	1.39224	43.9628	49.4203
n	1	NA		
k	1.56858	0.153601	1.26753	1.86963

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	30	28.9	28.9	3.48	3.98	-0.0142
0.97	30	46.8	46.7	5.08	4.07	0.101
2.7	30	58.3	58.4	4.55	4.12	-0.15
9.5	30	69	69	3.21	4.16	0.0643

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-228.781417	5	467.562835
A2	-224.581124	8	465.162248
A3	-228.756484	6	469.512968
fitted	228.775516	-5	467.551033
R	-388.287724	2	780.575449

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	327.413	6	<.0001
Test 2	8.40059	3	0.03842
Test 3	8.35072	2	0.01537
Test 4	0.038065	1	0.8453

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

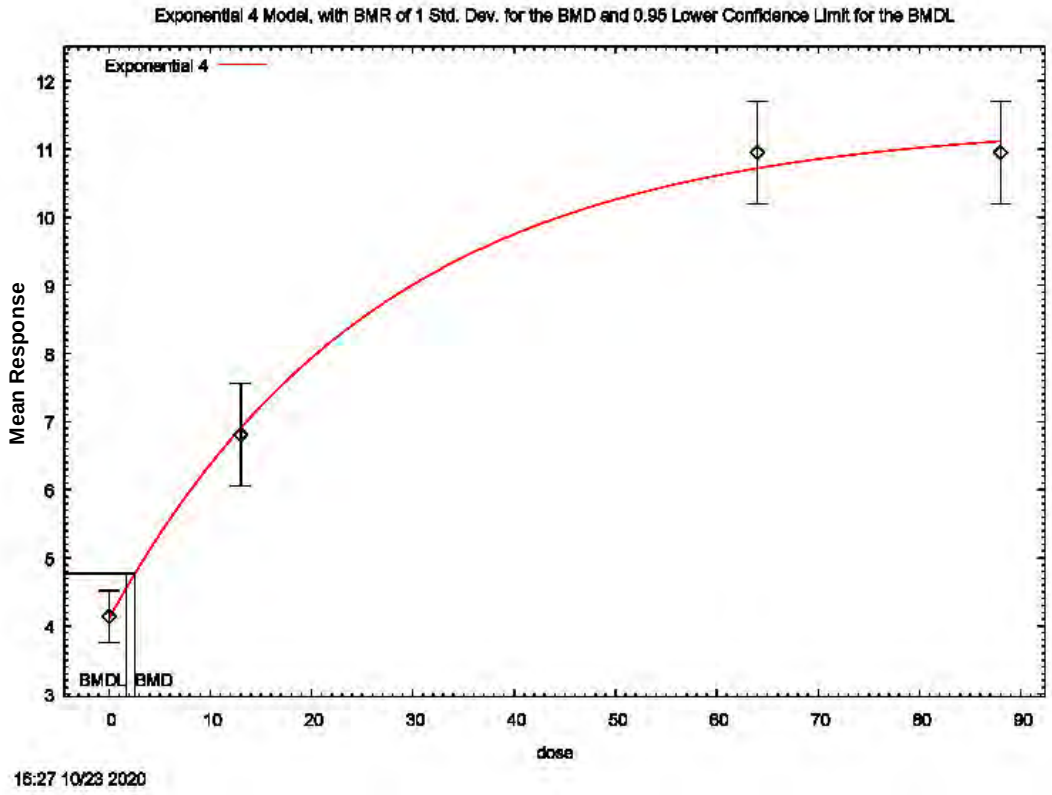
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a different variance model

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 0.145968
BMDL = 0.112334
BMDU = 0.2355

Figure A10.10. BMD modeling of increased relative liver weight in male BALB/c mice from Guo et al. (2019)



Model run output for Figure A10.10: BMD modeling of increased relative liver weight in male BALB/c mice from Guo et al. (2019).

```
==========================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/exp_Guo 2019 rel liv weight_Opt.(d)
Gnuplot Plotting File:
Fri Oct 23 16:27:42 2020
==========================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function by Model:
Model 2:     Y[dose] = a * exp{sign * b * dose}
Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]
Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-2.6178	-2.6178	-2.6178	-2.6178
rho	1.29136	1.29136	1.29136	1.29136
a	4.98718	4.98718	3.933	3.933
b	0.0102914	0.0102914	0.0337541	0.0337541
c	0 *	0 *	2.92334	2.92334
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-0.178462	-0.178462	-2.65372	-2.57698
rho	0.483039	0.483039	1.29459	1.25001
a	5.35374	5.35374	4.10528	4.12238
b	0.00909555	0.00909555	0.0373018	0.0547297
c	--	--	2.77402	2.65328
d	--	1	--	1.96892

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	1.5504	1.5504	1.17786	1.18104
rho	0.750434	0.750434	0.570423	0.572084
a	0.316622	0.316622	0.189448	0.193895
b	0.000907091	0.000907091	0.00650846	3.96733
c	NA	NA	0.149678	0.153129
d	NA	NA	NA	419.199

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.02	12	4.14	0.6
13	12	6.81	1.19

Dose	N	Obs Mean	Obs Std Dev
64	12	10.95	1.19
88	12	10.95	1.19

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.02	5.355	1.372	-3.068
	13	6.026	1.411	1.925
	64	9.582	1.579	3.001
	88	11.92	1.664	-2.019
3	0.02	5.355	1.372	-3.068
	13	6.026	1.411	1.925
	64	9.582	1.579	3.001
	88	11.92	1.664	-2.019
4	0.02	4.111	0.6624	0.1531
	13	6.904	0.9266	-0.3506
	64	10.72	1.232	0.6495
	88	11.11	1.261	-0.4527
5	0.02	4.122	0.6682	0.09129
	13	6.852	0.9179	-0.1574
	64	10.94	1.23	0.03446
	88	10.94	1.23	0.03432

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-25.30928	5	60.61856
A2	-22.04414	8	60.08828
A3	-23.09363	6	58.18726
R	-77.88171	2	159.7634
2	-43.5176	4	95.0352
3	-43.5176	4	95.0352
4	-23.42929	5	56.85859
5	-23.09363	6	58.18726

Additive constant for all log-likelihoods = -44.11. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.
Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)

Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)
Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)
Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	111.7	6	< 0.0001
Test 2	6.53	3	0.08848
Test 3	2.099	2	0.3501
Test 4	40.85	2	< 0.0001
Test 5a	40.85	2	< 0.0001
Test 5b	-1.421e-014	0	N/A
Test 6a	0.6713	1	0.4126
Test 6b	40.18	1	< 0.0001
Test 7a	1.048e-008	0	N/A
Test 7b	40.85	2	< 0.0001
Test 7c	0.6713	1	0.4126

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0.
The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

Degrees of freedom for Test 7a are less than or equal to 0.
The Chi-Square test for fit is not valid.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

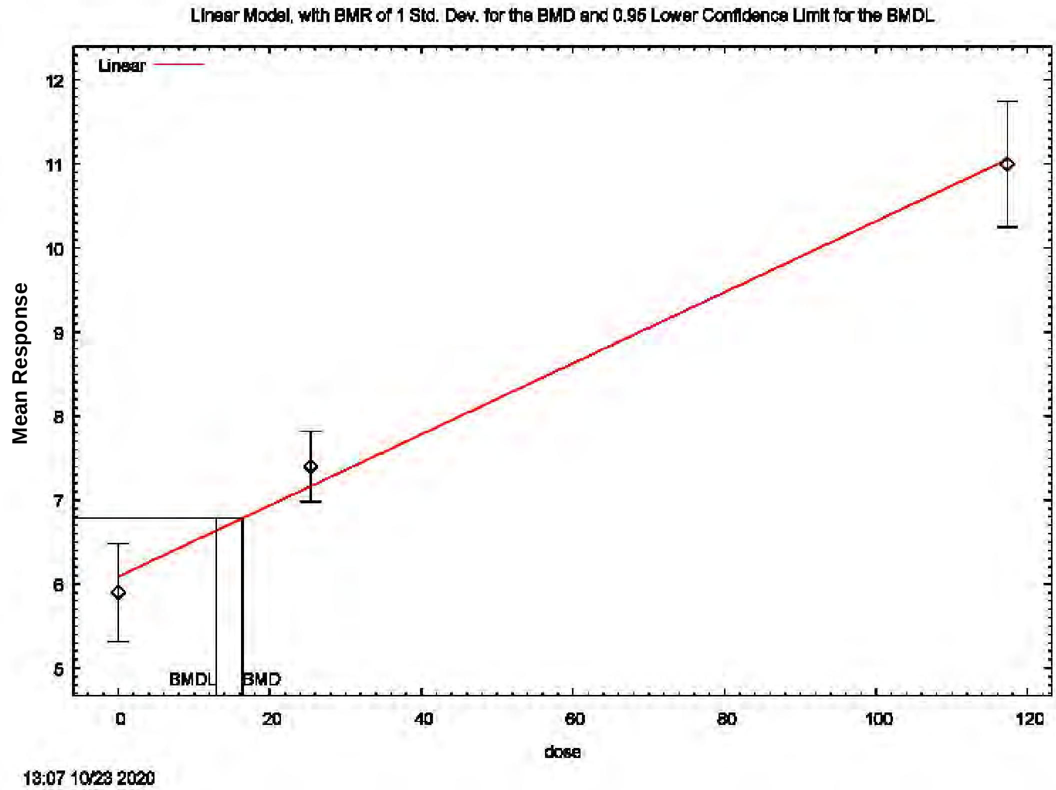
The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	25.077	18.7265	35.2831
3	25.077	18.7265	35.2831
4	2.55421	1.70954	4.13933
5	5.76493	1.79716	12.2396

Figure A10.11. BMD modeling of increased relative liver weight in pregnant CD-1 mice at ED 11.5 from Blake et al. (2020)



Model run output for Figure A10.11: BMD modeling of increased relative liver weight in pregnant CD-1 mice at ED 11.5 from Blake et al. (2020).

==
Polynomial Model. (Version: 2.21; Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/lin_Blake 2020 rel liv ED11_Opt.(d)
Gnuplot Plotting File: K:/BMD saved files/Chemicals/PFOA/lin_Blake 2020 rel liv ED11_Opt.plt
Fri Oct 23 13:07:29 2020
==
BMDS Model Run
~~~~~~
The form of the response function is:
Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...

Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
~~~~~~

Parameter Convergence has been set to: 1e-008
Default Initial Parameter Values
alpha = 0.516667
rho = 0 Specified
beta_0 = 6.08666
beta_1 = 0.0423268

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	alpha	beta_0	beta_1
alpha	1	3.1e-009	4e-008
beta_0	3.1e-009	1	-0.69
beta_1	4e-008	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	0.483504	0.139576	0.209941	0.757067
beta_0	6.08666	0.195191	5.70409	6.46922
beta_1	0.0423268	0.0028169	0.0368058	0.0478478

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	5.9	6.09	0.7	0.695	-0.759
25.4	8	7.4	7.16	0.5	0.695	0.969
117.3	8	11	11.1	0.9	0.695	-0.21

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-2.473335	4	12.946670
A2	-1.156162	6	14.312324
A3	-2.473335	4	12.946670
fitted	-3.279650	3	12.559300
R	-31.390009	2	66.780018

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	60.4677	4	<.0001
Test 2	2.63435	2	0.2679
Test 3	2.63435	2	0.2679
Test 4	1.61263	1	0.2041

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

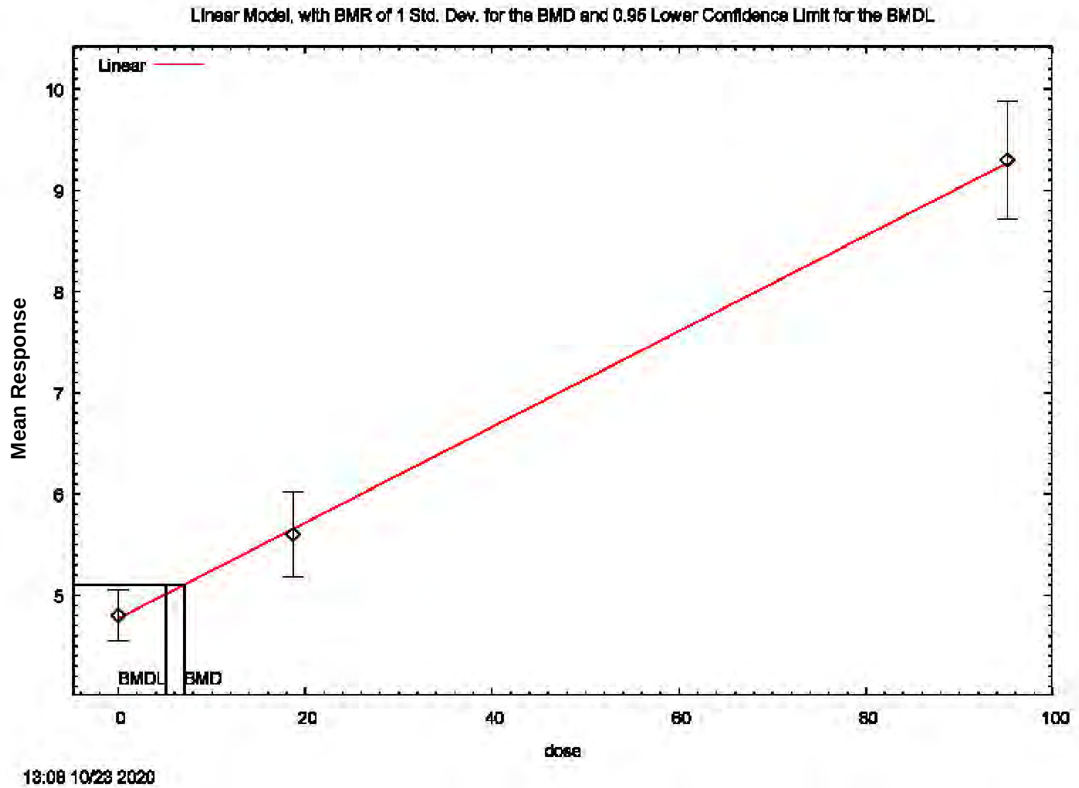
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 16.428
BMDL = 12.9484
BMDU = 21.9988

Figure A10.12. BMD modeling of increased relative liver weight in pregnant CD-1 mice at ED 17.5 from Blake et al. (2020)



Model run output for Figure A10.12: BMD modeling of increased relative liver weight in pregnant CD-1 mice at ED 17.5 from Blake et al. (2020).

==
Polynomial Model. (Version: 2.21; Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/lin_Blake 2020 rel liv ED17_Opt.(d)
Gnuplot Plotting File: K:/BMD saved files/Chemicals/PFOA/lin_Blake 2020 rel liv ED17_Opt.plt
Fri Oct 23 13:08:54 2020
==
BMDS Model Run
~~~~~~

The form of the response function is:
Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
Dependent variable = Mean
Independent variable = Dose
Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
~~~~~~

Default Initial Parameter Values
lalpha = -1.28494
rho = 0
beta_0 = 4.75952
beta_1 = 0.0476401

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.99	-0.063	0.095
rho	-0.99	1	0.063	-0.096
beta_0	-0.063	0.063	1	-0.52
beta_1	0.095	-0.096	-0.52	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	-5.62452	2.05483	-9.65192	-1.59712
rho	2.19217	1.10534	0.0257544	4.35859
beta_0	4.76933	0.0996775	4.57397	4.96469
beta_1	0.0473241	0.00285874	0.0417211	0.0529271

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	8	4.8	4.77	0.3	0.333	0.261
18.7	8	5.6	5.65	0.5	0.401	-0.383
95.1	8	9.3	9.27	0.7	0.69	0.124

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	5.021679	4	-2.043358
A2	7.632736	6	-3.265472
A3	7.148940	5	-4.297880
fitted	7.078860	4	-6.157719
R	-28.885821	2	61.771641

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?

(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	73.0371	4	<.0001
Test 2	5.22211	2	0.07346
Test 3	0.967592	1	0.3253
Test 4	0.140161	1	0.7081

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

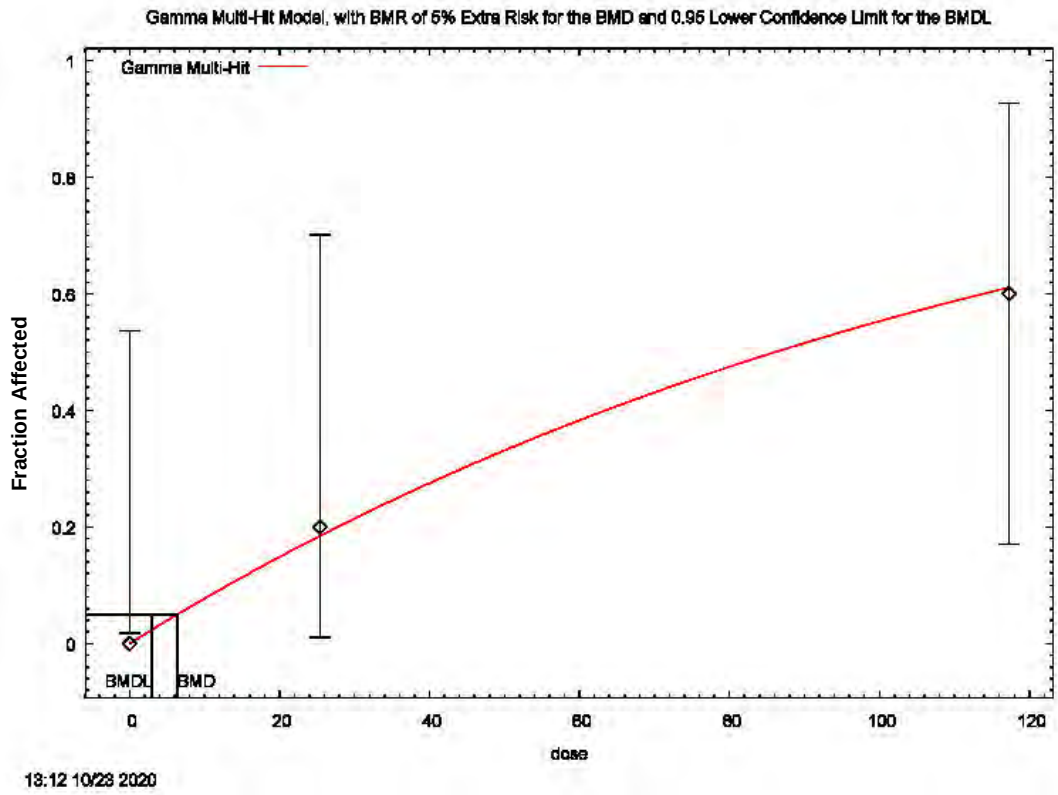
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 7.03424
BMDL = 5.06239
BMDU = 10.3874

Figure A10.13. BMD modeling of increased liver cell death in pregnant CD-1 mice at ED 11.5 from Blake et al. (2020)



Model run output for Figure A10.13: BMD modeling of increased liver cell death in pregnant CD-1 mice at ED 11.5 from Blake et al. (2020).

```
================================================================
Gamma Model. (Version: 2.17;  Date: 6/22/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOA/gam_Blake 2020 liv cell death
ed11_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOA/gam_Blake 2020 liv cell death
ed11_Opt.plt
Fri Oct 23 13:12:01 2020
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response]= background+(1-background)*CumGamma[slope*dose,power],
where CumGamma(.) is the cummulative Gamma distribution function
Dependent variable = Effect
Independent variable = Dose
Power parameter is restricted as power >=1
Total number of observations = 3
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
```

Default Initial (and Specified) Parameter Values
Background = 0.142857
Slope = 0.00977874
Power = 1.3

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -Background -Power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Slope
Slope	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	0.00804597	0.00413652	-6.14598e-005	0.0161534
Power	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-5.86707	3			
Fitted model	-5.87204	1	0.00994311	2	0.995
Reduced model	-8.69873	1	5.66331	2	0.05892

AIC: 13.7441

Goodness of Fit

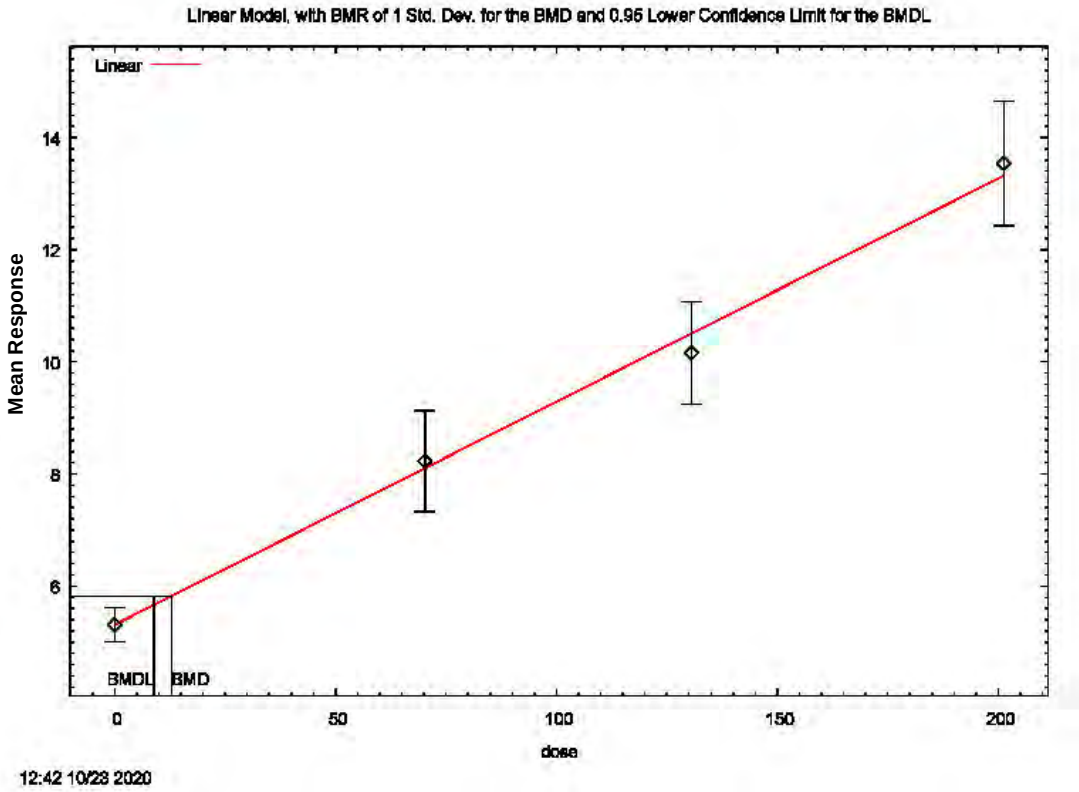
Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	5.000	0.000
25.4000	0.1848	0.924	1.000	5.000	0.087
117.3000	0.6109	3.054	3.000	5.000	-0.050

Chi^2 = 0.01 d.f. = 2 P-value = 0.9950

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 6.37503
BMDL = 3.00033
BMDU = 89.7605

Perfluorooctane Sulfonic Acid

Figure A10.14. BMD modeling of increased relative liver weight in male C57BL/6 mice from Xing et al. (2016)



Model run output for Figure A10.14: BMD modeling of increased relative liver weight in male C57BL/6 mice from Xing et al. (2016).

```
================================================================================
Polynomial Model. (Version: 2.21;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/lin_Xing 2016 liv weight_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/lin_Xing 2016 liv weight_Opt.plt
Fri Oct 23 12:42:38 2020
================================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
Dependent variable = Mean
Independent variable = Dose
Signs of the polynomial coefficients are not restricted
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
```

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
lalpha = 0.377751
rho = 0
beta_0 = 5.27261
beta_1 = 0.040168

Asymptotic Correlation Matrix of Parameter Estimates

	lalpha	rho	beta_0	beta_1
lalpha	1	-0.99	-0.078	0.083
rho	-0.99	1	0.076	-0.081
beta_0	-0.078	0.076	1	-0.54
beta_1	0.083	-0.081	-0.54	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	-5.91848	1.75177	-9.35188	-2.48508
rho	2.73789	0.798373	1.17311	4.30267
beta_0	5.31108	0.154427	5.00841	5.61375
beta_1	0.0397629	0.00222688	0.0353983	0.0441275

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.02	10	5.31	5.31	0.42	0.51	-0.0116
70.23	10	8.23	8.1	1.26	0.909	0.439
130.6	10	10.2	10.5	1.28	1.3	-0.835
201.2	10	13.5	13.3	1.56	1.79	0.4

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-25.447815	5	60.895630
A2	-18.444360	8	52.888720
A3	-20.036279	6	52.072559
fitted	-20.764402	4	49.528805
R	-66.562230	2	137.124460

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	96.2357	6	<.0001
Test 2	14.0069	3	0.002896
Test 3	3.18384	2	0.2035
Test 4	1.45625	2	0.4828

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

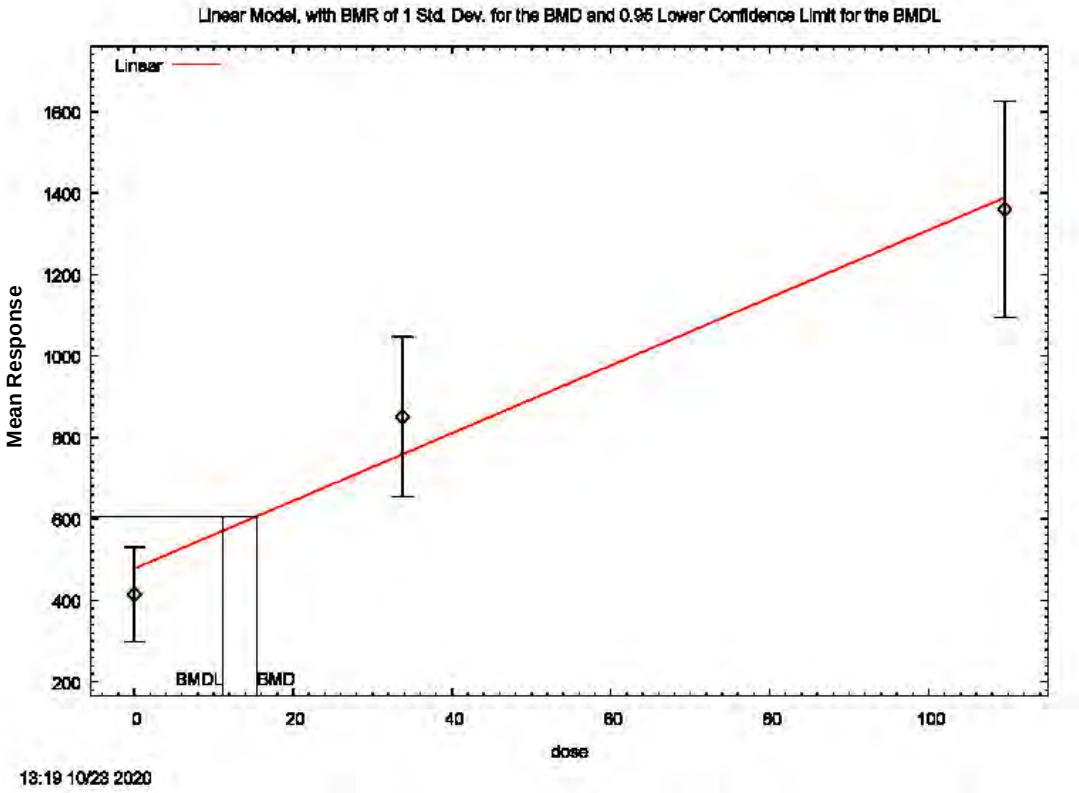
The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 12.8255
BMDL = 8.93086
BMDU = 19.588

Figure A10.15. BMD modeling of increased liver triglycerides in female CD-1 mice from Lai et al. (2018)



Model run output for Figure A10.15: BMD modeling of increased liver triglycerides in female CD-1 mice from Lai et al. (2018).

```
================================================================
Polynomial Model. (Version: 2.21;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/lin_Lai 2018 liv tg_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/lin_Lai 2018 liv tg_Opt.plt
Fri Oct 23 13:19:17 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function is:
Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...
Dependent variable = Mean
Independent variable = Dose
rho is set to 0
Signs of the polynomial coefficients are not restricted
A constant variance model is fit

Total number of dose groups = 3
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values
alpha = 1
rho = 0 Specified
beta_0 = 477.882
beta_1 = 8.30982

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -rho
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	beta_0	beta_1
lalpha	1	6.1e-010	1.9e-010
beta_0	6.1e-010	1	-0.72
beta_1	1.9e-010	-0.72	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
alpha	16567.9	6763.71	3311.3	29824.6
beta_0	477.882	53.6998	372.633	583.132
beta_1	8.30982	0.811248	6.7198	9.89983

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.0239	4	415	478	72.7	129	-0.988
33.78	4	851	759	124	129	1.43
109.6	4	1.36e+003	1.39e+003	167	129	-0.44

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-62.424521	4	132.849042
A2	-61.166630	6	134.333259
A3	-62.424521	4	132.849042
fitted	-64.291449	3	134.582898
R	-77.951075	2	159.902150

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	33.5689	4	<.0001
Test 2	2.51578	2	0.2843
Test 3	2.51578	2	0.2843
Test 4	3.73386	1	0.05332

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

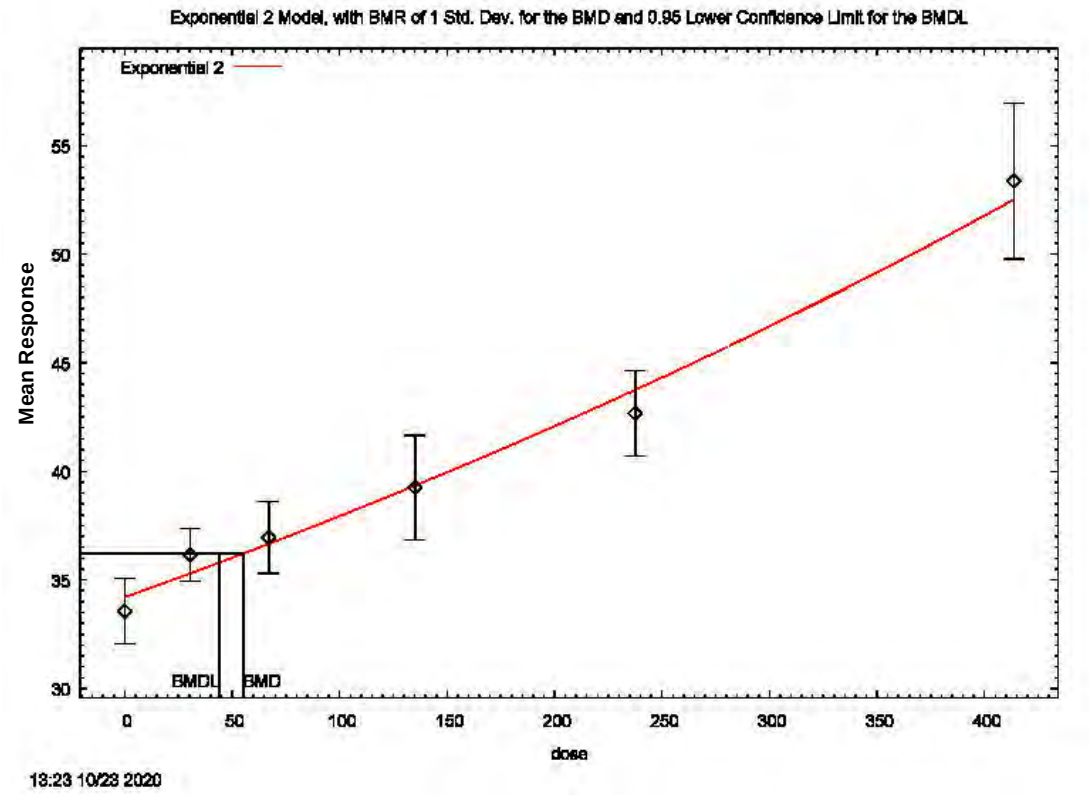
The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation
Specified effect = 1
Risk Type = Estimated standard deviations from the control mean
Confidence level = 0.95
BMD = 15.4897
BMDL = 11.1741
BMDU = 24.04

Figure A10.16. BMD modeling of increased relative liver weight in female Sprague Dawley rats from NTP (2019b)



Model run output for Figure A10.16: BMD modeling of increased relative liver weight in female Sprague Dawley rats from NTP (2019b).

==========

Exponential Model. (Version: 1.11; Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 female liv weight_Opt.(d)
Gnuplot Plotting File:
Fri Oct 23 13:24:29 2020

==========

BMDS Model Run

~~~~~~~~~~

The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
~~~~~~~~~~

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-12.1843	-12.1843	-12.1843	-12.1843
rho	3.83765	3.83765	3.83765	3.83765
a	34.1558	34.1558	31.882	31.882
b	0.00104589	0.00104589	0.000785948	0.000785948
c	0 *	0 *	3.34797	3.34797
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-11.8604	-11.8604	-13.7212	-12.3537
rho	3.75336	3.75336	4.26605	3.89281
a	34.2029	34.2029	33.9708	34.2256
b	0.0010363	0.0010363	1.71187e-007	7.36298e-007
c	--	--	7316.82	3879.51
d	--	1	--	1.10059

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	4.9863	4.9863	5.26548	5.64019
rho	1.35453	1.35453	1.43135	1.53272
a	0.399311	0.399311	0.419397	0.649752
b	7.07734e-005	7.07734e-005	9.41746e-006	3.75474e-005
c	NA	NA	402450	217472
d	NA	NA	NA	0.202644

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.054	10	33.56	2.09
30.5	10	36.15	1.71

Dose	N	Obs Mean	Obs Std Dev
67	10	36.95	2.31
135.1	10	39.25	3.35
237.5	10	42.67	2.75
413.6	9	53.37	4.68

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.054	34.2	2.012	-1.014
	30.5	35.3	2.134	1.258
	67	36.66	2.291	0.3974
	135.1	39.34	2.616	-0.1122
	237.5	43.75	3.192	-1.067
	413.6	52.51	4.496	0.5766
3	0.054	34.2	2.012	-1.014
	30.5	35.3	2.134	1.258
	67	36.66	2.291	0.3974
	135.1	39.34	2.616	-0.1122
	237.5	43.75	3.192	-1.067
	413.6	52.51	4.496	0.5766
4	0.054	33.97	1.934	-0.6755
	30.5	35.27	2.095	1.331
	67	36.82	2.296	0.1773
	135.1	39.72	2.699	-0.5489
	237.5	44.07	3.37	-1.318
	413.6	51.57	4.71	1.149
5	0.054	34.23	2.013	-1.047
	30.5	35.24	2.131	1.348
	67	36.64	2.299	0.4255
	135.1	39.45	2.655	-0.2396
	237.5	43.95	3.275	-1.234
	413.6	52.13	4.566	0.816

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-89.95135	7	193.9027
A2	-83.54031	12	191.0806
A3	-84.99057	8	185.9811
R	-143.3548	2	290.7096
2	-87.11333	4	182.2267

Model	Log(likelihood)	DF	AIC
3	-87.11333	4	182.2267
4	-87.82322	5	185.6464
5	-87.68515	6	187.3703

Additive constant for all log-likelihoods = -54.22. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	119.6	10	< 0.0001
Test 2	12.82	5	0.0251
Test 3	2.901	4	0.5746
Test 4	4.246	4	0.3738
Test 5a	4.246	4	0.3738
Test 5b	2.842e-014	0	N/A
Test 6a	5.665	3	0.1291
Test 6b	-1.42	1	N/A
Test 7a	5.389	2	0.06757
Test 7b	-1.144	2	N/A
Test 7c	0.2762	1	0.5992

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is greater than .1. Model 2 seems to adequately describe the data.

The p-value for Test 5a is greater than .1. Model 3 seems to adequately describe the data.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is less than .1. Model 5 may not adequately describe the data; you may want to consider another model.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

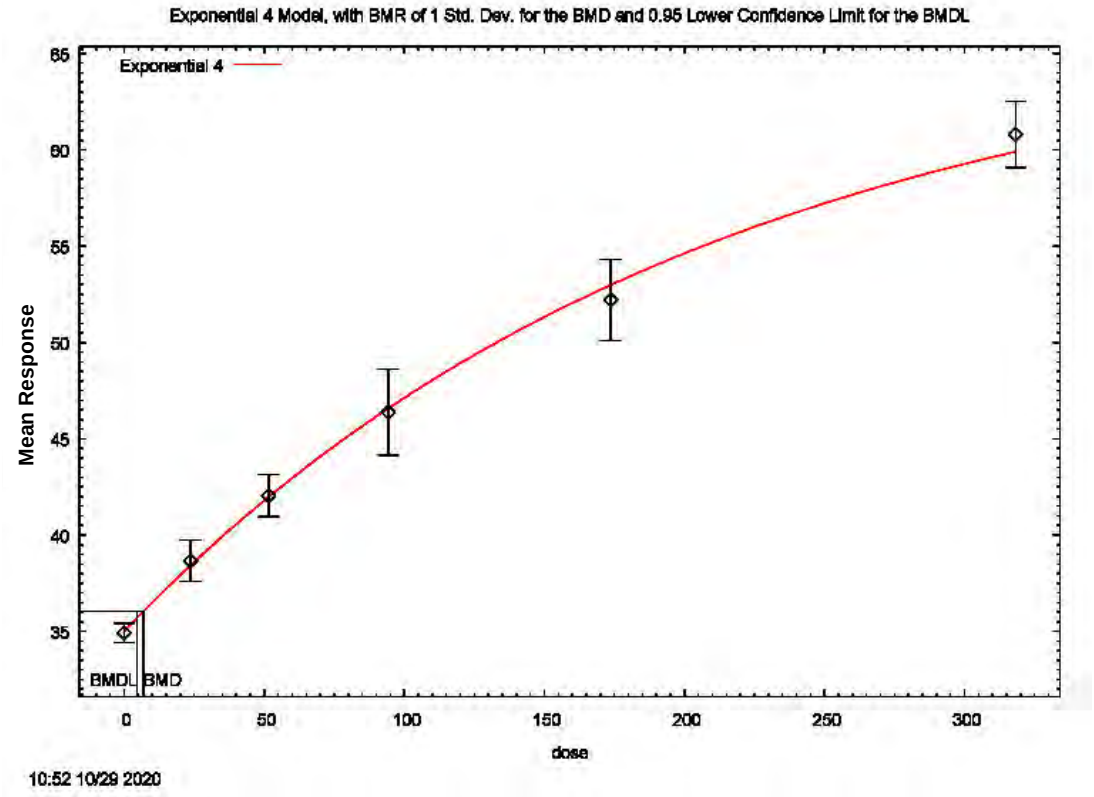
The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	55.1412	44.119	70.0943
3	55.1412	44.119	96.7343
4	45.4488	35.7996	58.8881
5	56.7917	36.2354	104.724

Figure A10.17. BMD modeling of increased relative liver weight in male Sprague Dawley rats from NTP (2019b)



Model run output for Figure A10.17: BMD modeling of increased relative liver weight in male Sprague Dawley rats from NTP (2019b).

```
==========================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 male liv weight_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 10:53:03 2020
==========================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact
Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-15.8946	-15.8946	-15.8946	-15.8946
rho	4.48618	4.48618	4.48618	4.48618
a	37.5477	37.5477	33.174	33.174
b	0.00165161	0.00165161	0.00682224	0.00682224
c	0 *	0 *	1.9244	1.9244
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-6.32147	-6.32147	-17.333	-17.333
rho	2.2163	2.2163	4.89051	4.89051
a	37.5374	37.5374	35.0088	35.0088
b	0.00166409	0.00166409	0.00478913	0.00478913
c	--	--	1.90908	1.90908
d	--	1	--	1

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	7.76758	7.76759	5.26047	5.26047
rho	2.0385	2.0385	1.38161	1.38161
a	0.681746	0.681747	0.300973	0.300973
b	0.000152463	0.000152463	0.000865858	0.000865859
c	NA	NA	0.0999593	0.0999594
d	NA	NA	NA	NA

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	34.92	0.696
23.7	10	38.66	1.49

Dose	N	Obs Mean	Obs Std Dev
51.6	10	42.04	1.52
94.3	10	46.38	3.13
173.7	10	52.21	2.94
318.2	10	60.8	2.43

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0	37.54	2.355	-3.514
	23.7	39.05	2.461	-0.4979
	51.6	40.9	2.59	1.388
	94.3	43.92	2.803	2.781
	173.7	50.12	3.245	2.038
	318.2	63.74	4.235	-2.197
3	0	37.54	2.355	-3.514
	23.7	39.05	2.461	-0.4979
	51.6	40.9	2.59	1.388
	94.3	43.92	2.803	2.781
	173.7	50.12	3.245	2.038
	318.2	63.74	4.235	-2.197
4	0	35.01	1.028	-0.273
	23.7	38.42	1.291	0.5791
	51.6	41.98	1.603	0.1242
	94.3	46.57	2.066	-0.2973
	173.7	52.98	2.832	-0.8629
	318.2	59.9	3.823	0.7436
5	0	35.01	1.028	-0.273
	23.7	38.42	1.291	0.5791
	51.6	41.98	1.603	0.1242
	94.3	46.57	2.066	-0.2973
	173.7	52.98	2.832	-0.8629
	318.2	59.9	3.823	0.7436

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-74.47572	7	162.9514
A2	-62.46333	12	148.9267
A3	-67.87277	8	151.7455
R	-161.2307	2	326.4615
2	-93.59988	4	195.1998

Model	Log(likelihood)	DF	AIC
3	-93.59988	4	195.1998
4	-68.62996	5	147.2599
5	-68.62996	5	147.2599

Additive constant for all log-likelihoods = -55.14. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	197.5	10	< 0.0001
Test 2	24.02	5	0.0002147
Test 3	10.82	4	0.02868
Test 4	51.45	4	< 0.0001
Test 5a	51.45	4	< 0.0001
Test 5b	-5.116e-013	0	N/A
Test 6a	1.514	3	0.679
Test 6b	49.94	1	< 0.0001
Test 7a	1.514	3	0.679
Test 7b	49.94	1	< 0.0001
Test 7c	-5.684e-013	0	N/A

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 4 is less than .1. Model 2 may not adequately

describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0.
The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

Degrees of freedom for Test 7c are less than or equal to 0.
The Chi-Square test for fit is not valid.

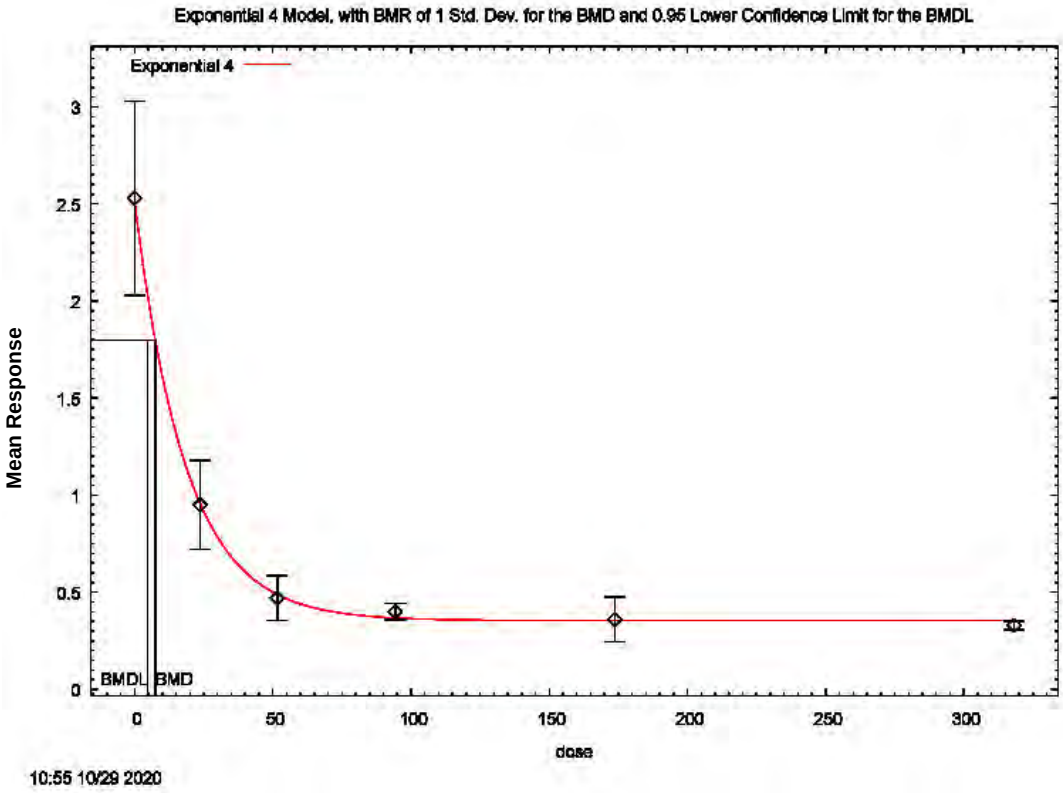
Benchmark Dose Computations:

Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	36.5705	20.6092	54.6849
3	36.5705	20.6092	54.6849
4	6.85744	4.70931	10.4181
5	6.85744	4.70931	10.5731

Figure A10.18. BMD modeling of decreased free T4 in male Sprague Dawley rats from NTP (2019b)



Model run output for Figure A10.18: BMD modeling of decreased free T4 in male Sprague Dawley rats from NTP (2019b).

==========

Exponential Model. (Version: 1.11; Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 male freeT4_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 10:55:42 2020

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BMDS Model Run

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The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
~~~~~~~~~~

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-2.62382	-2.62382	-2.62382	-2.62382
rho	2.46849	2.46849	2.46849	2.46849
a	0.364387	0.662731	2.6565	2.6565
b	0.0047355	1.3676e-005	0.01922	0.01922
c	0 *	0 *	0.118308	0.118308
d	1 *	2	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.69663	-6.02037	-2.50112	-2.50102
rho	0.0740169	-32.5821	1.98513	1.98559
a	2.46523	0.84	2.51089	2.5072
b	0.0329466	1.56641e-005	0.0538167	0.0533173
c	--	--	0.14101	0.141483
d	--	4.21197	--	1.01627

NC = No Convergence
-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.220405	NA	0.241422	0.241551
rho	0.0465958	NA	0.288022	0.288401
a	0.133022	NA	0.217577	0.223448
b	0.0032016	NA	0.00557817	0.00880357
c	NA	NA	0.0139465	0.0154707
d	NA	NA	NA	0.228883

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	2.53	0.7
23.7	10	0.95	0.32
51.6	10	0.47	0.16
94.3	10	0.4	0.06
173.7	10	0.36	0.16
318.2	10	0.33	0.03

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0	2.465	0.4427	0.4627
	23.7	1.129	0.4301	-1.317
	51.6	0.4503	0.4157	0.1496
	94.3	0.1103	0.3946	2.322
	173.7	0.008062	0.3582	3.107
	318.2	6.901e-005	0.3003	3.474
3	0	0.84	0.8438	6.333
	23.7	0.84	0.8438	0.4122
	51.6	0.84	0.8438	-1.387
	94.3	0.84	0.8438	-1.649
	173.7	0.84	0.8438	-1.799
	318.2	0.84	0.8438	-1.911
4	0	2.511	0.7141	0.08462
	23.7	0.9565	0.274	-0.07469
	51.6	0.4883	0.1406	-0.4112
	94.3	0.3675	0.106	0.968
	173.7	0.3542	0.1022	0.1779
	318.2	0.3541	0.1022	-0.7447
5	0	2.507	0.7132	0.1011
	23.7	0.9602	0.275	-0.1168
	51.6	0.486	0.1399	-0.3624
	94.3	0.3671	0.1059	0.9837
	173.7	0.3549	0.1024	0.1584
	318.2	0.3547	0.1023	-0.7641

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	39.92491	7	-65.84982
A2	87.97322	12	-151.9464
A3	76.18026	8	-136.3605

Model	Log(likelihood)	DF	AIC
R	-19.81312	2	43.62624
2	26.96114	4	-45.92229
3	-19.81312	5	49.62624
4	73.99365	5	-137.9873
5	73.9962	6	-135.9924

Additive constant for all log-likelihoods = -55.14. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	215.6	10	< 0.0001
Test 2	96.1	5	< 0.0001
Test 3	23.59	4	< 0.0001
Test 4	98.44	4	< 0.0001
Test 5a	192	3	< 0.0001
Test 5b	-93.55	1	N/A
Test 6a	4.373	3	0.2239
Test 6b	94.07	1	< 0.0001
Test 7a	4.368	2	0.1126
Test 7b	187.6	1	< 0.0001
Test 7c	0.005103	1	0.943

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to

consider a different variance model.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

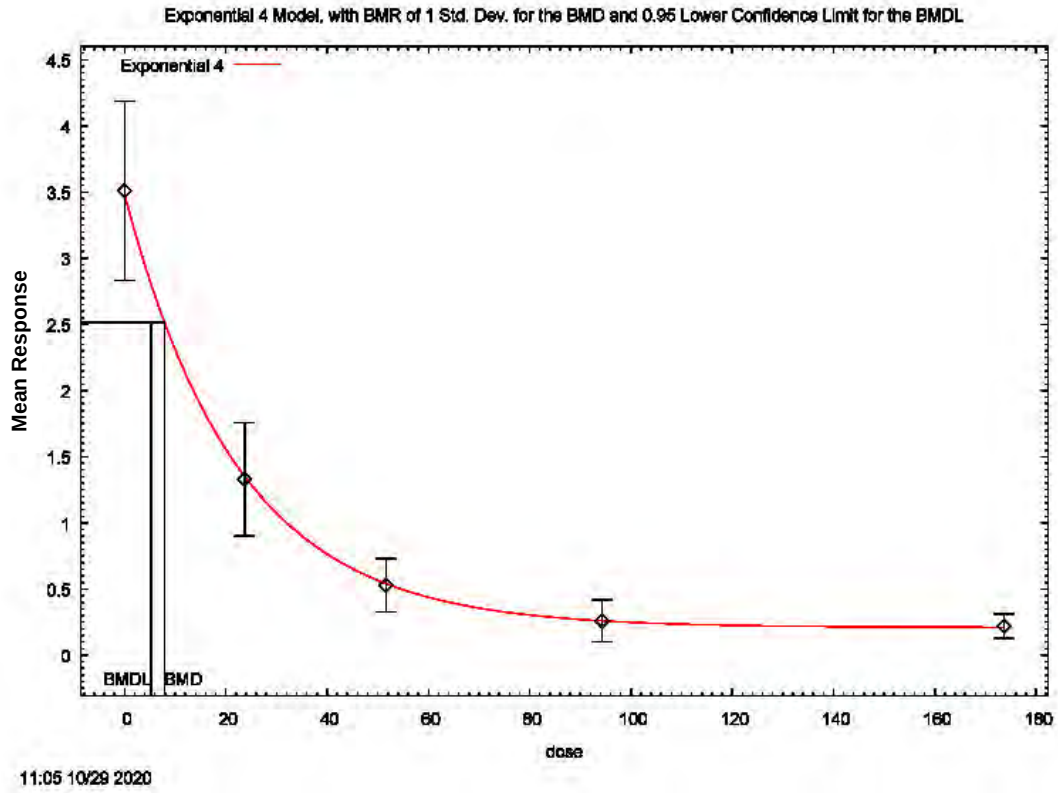
Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	6.00736	4.19822	9.4973
3	-0	-0	Not computed
4	7.47141	4.78086	14.083
5	7.65985	4.78261	15.7676

Figure A10.19. BMD modeling of decreased total T4 in male Sprague Dawley rats from NTP (2019b) (high dose excluded)



Model run output for Figure A10.19: BMD modeling of decreased total T4 in male Sprague Dawley rats from NTP (2019b) (high dose excluded).

```
==========================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 male totT4 high
dose_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 11:05:50 2020
==========================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
```

Model 4 is nested within Model 5.

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.63191	-1.63191	-1.63191	-1.63191
rho	1.33961	1.33961	1.33961	1.33961
a	0.240579	0.608535	3.6855	3.6855
b	0.0150546	7.02825e-005	0.0368227	0.0368227
c	0 *	0 *	0.0568509	0.0568509
d	1 *	2	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.22909	2.95289	-1.70796	-1.70796
rho	0.40255	-15.1302	1.3087	1.3087
a	3.42992	1.17	3.48038	3.48038
b	0.0352566	0.00159613	0.0443829	0.0443829
c	--	--	0.0603702	0.0603702
d	--	17.7628	--	1

NC = No Convergence
-- Indicates that this parameter does not appear in model
Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.222157	NA	0.230198	0.230198
rho	0.0896499	NA	0.218597	0.218597
a	0.20411	NA	0.29379	0.29379
b	0.00295564	NA	0.00502326	0.00502326
c	NA	NA	0.0119747	0.0119747
d	NA	NA	NA	NA

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	3.51	0.95
23.7	10	1.33	0.6
51.6	10	0.53	0.28
94.3	10	0.26	0.22
173.7	10	0.22	0.13

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0	3.43	0.6932	0.3653
	23.7	1.487	0.5859	-0.849
	51.6	0.5562	0.4806	-0.1721
	94.3	0.1234	0.355	1.217
	173.7	0.00751	0.2021	3.325
3	0	1.17	1.335	5.544
	23.7	1.17	1.335	0.3791
	51.6	1.17	1.335	-1.516
	94.3	1.17	1.335	-2.156
	173.7	1.17	1.335	-2.251
4	0	3.48	0.9628	0.09728
	23.7	1.352	0.5187	-0.1363
	51.6	0.5412	0.2849	-0.1246
	94.3	0.2599	0.1763	0.002205
	173.7	0.2116	0.1541	0.1728
5	0	3.48	0.9628	0.09728
	23.7	1.352	0.5187	-0.1363
	51.6	0.5412	0.2849	-0.1246
	94.3	0.2599	0.1763	0.002205
	173.7	0.2116	0.1541	0.1728

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	9.347685	6	-6.69537
A2	31.52834	10	-43.05669
A3	30.74894	7	-47.49788
R	-39.43493	2	82.86987
2	17.68492	4	-27.36985
3	-39.43493	5	88.86987
4	30.5611	5	-51.1222
5	30.5611	5	-51.1222

Additive constant for all log-likelihoods = -45.95. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	141.9	8	< 0.0001
Test 2	44.36	4	< 0.0001
Test 3	1.559	3	0.6688
Test 4	26.13	3	< 0.0001
Test 5a	140.4	2	< 0.0001
Test 5b	-114.2	1	N/A
Test 6a	0.3757	2	0.8287
Test 6b	25.75	1	< 0.0001
Test 7a	0.3757	2	0.8287
Test 7b	140	0	N/A
Test 7c	-4.619e-013	0	N/A

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

Degrees of freedom for Test 7b are less than or equal to 0. The Chi-Square test for fit is not valid.

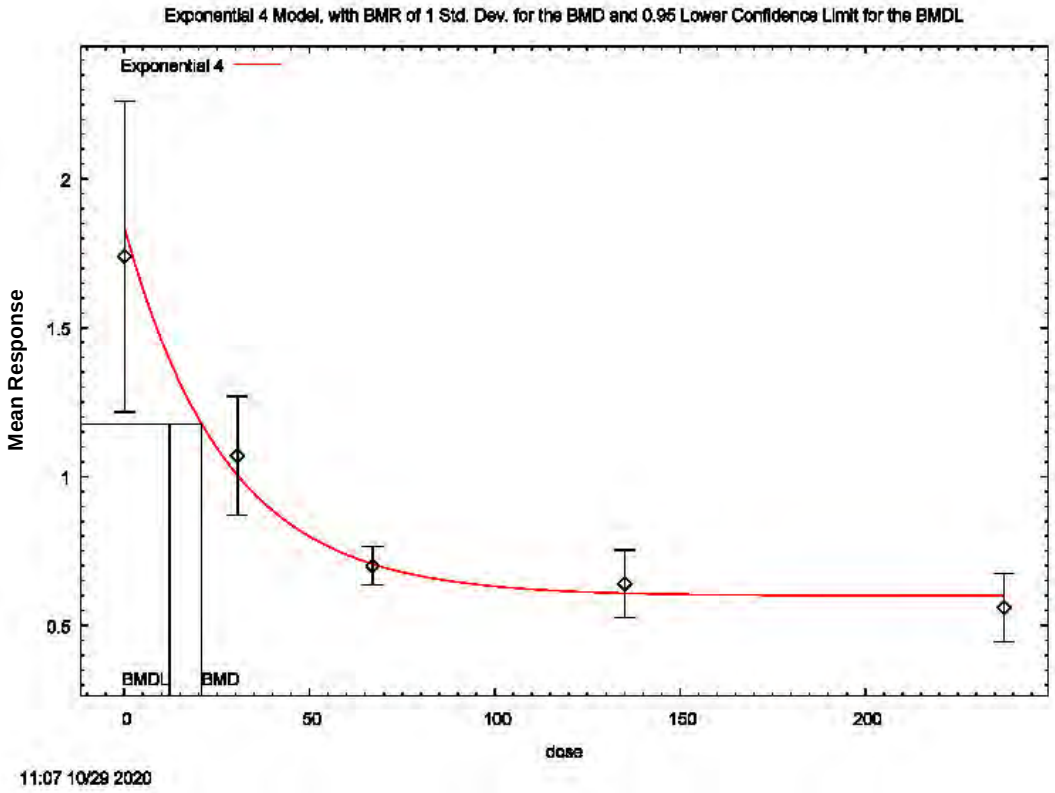
Degrees of freedom for Test 7c are less than or equal to 0. The Chi-Square test for fit is not valid.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	6.40359	4.41377	10.5654
3	-0	-0	Not computed
4	7.8571	5.19419	13.6877
5	7.8571	5.19419	14.725

Figure A10.20. BMD modeling of decreased free T4 in female Sprague Dawley rats from NTP (2019b) (high dose excluded)



Model run output for Figure A10.20: BMD modeling of decreased free T4 in female Sprague Dawley rats from NTP (2019b) (high dose excluded).

```
==========================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 female freeT4 high
dose_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 11:07:17 2020
==========================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
```

Model 4 is nested within Model 5.

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-2.59855	-2.59855	-2.59855	-2.59855
rho	3.05927	3.05927	3.05927	3.05927
a	0.582269	0.826107	1.827	1.827
b	0.00413243	1.41578e-005	0.017773	0.017773
c	0 *	0 *	0.291918	0.291918
d	1 *	2	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.744	-1.7883	-2.56371	-2.56769
rho	3.60746	-10.3545	2.84981	2.92667
a	1.21261	0.942	1.83708	1.79019
b	0.00355905	1.18396e-005	0.0365556	0.0328328
c	--	--	0.326198	0.343342
d	--	4.33687	--	1.43342

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.337133	NA	0.244905	0.249298
rho	0.919305	NA	0.505485	0.534869
a	0.10609	NA	0.195972	0.196265
b	0.00051845	NA	0.00643411	0.005464
c	NA	NA	0.0374588	0.0424576
d	NA	NA	NA	0.479716

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.054	10	1.74	0.73
30.5	10	1.07	0.28
67	10	0.7	0.09
135.1	10	0.64	0.16
237.5	10	0.56	0.16

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.054	1.212	0.5918	2.82
	30.5	1.088	0.4867	-0.1161
	67	0.9553	0.385	-2.097
	135.1	0.7497	0.2487	-1.395
	237.5	0.5207	0.1289	0.9633
3	0.054	0.942	0.5572	4.529
	30.5	0.942	0.5572	0.7264
	67	0.942	0.5572	-1.373
	135.1	0.942	0.5572	-1.714
	237.5	0.942	0.5572	-2.168
4	0.054	1.835	0.6589	-0.4542
	30.5	1.005	0.2796	0.7332
	67	0.7062	0.169	-0.1151
	135.1	0.6081	0.1366	0.7379
	237.5	0.5995	0.1339	-0.9323
5	0.054	1.79	0.6493	-0.2438
	30.5	1.046	0.2959	0.2539
	67	0.6678	0.1534	0.6632
	135.1	0.6149	0.136	0.5839
	237.5	0.6146	0.1359	-1.272

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	27.85952	6	-43.71904
A2	54.24186	10	-88.48372
A3	50.81643	7	-87.63287
R	4.240573	2	-4.481145
2	31.39641	4	-54.79282
3	4.240573	5	1.518855
4	49.70844	5	-89.41688
5	50.15681	6	-88.31362

Additive constant for all log-likelihoods = -45.95. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	100	8	< 0.0001
Test 2	52.76	4	< 0.0001
Test 3	6.851	3	0.07681
Test 4	38.84	3	< 0.0001
Test 5a	93.15	2	< 0.0001
Test 5b	-54.31	1	N/A
Test 6a	2.216	2	0.3302
Test 6b	36.62	1	< 0.0001
Test 7a	1.319	1	0.2507
Test 7b	91.83	1	< 0.0001
Test 7c	0.8967	1	0.3437

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is less than .1. You may want to consider a different variance model.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

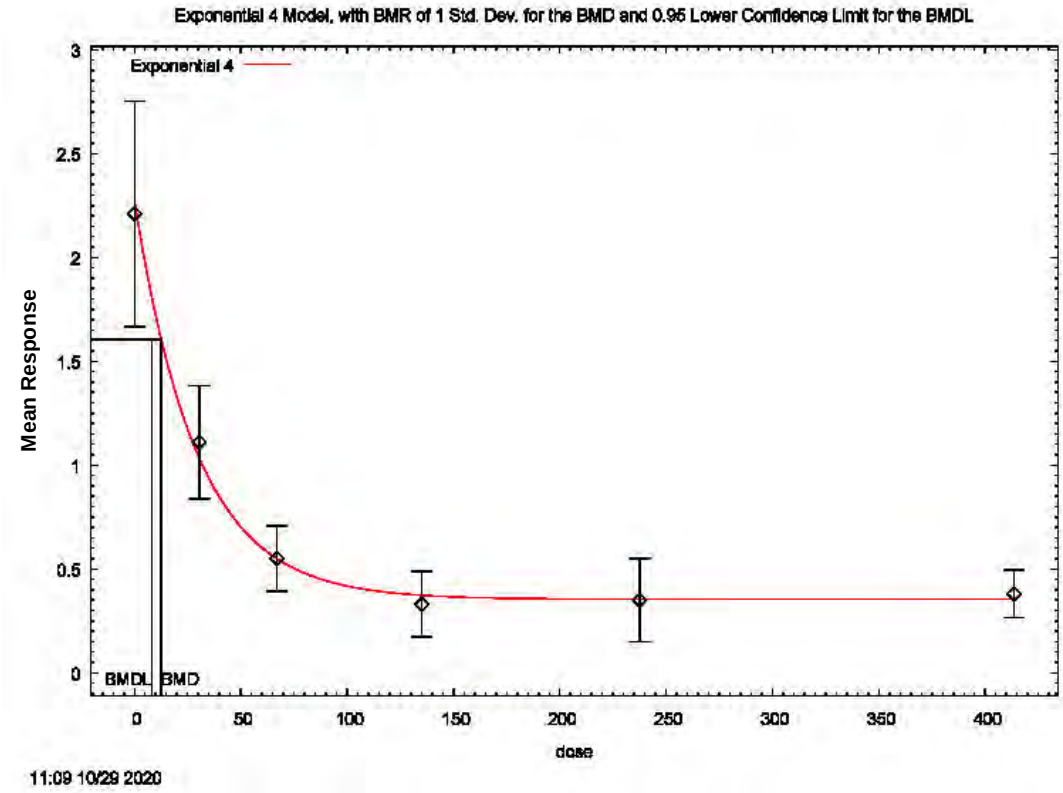
The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	188.193	100.614	445.578
3	82335.7	185.908	2.375e+006
4	20.8485	12.0925	52.8862
5	26.1553	13.1887	53.4664

Figure A10.21. BMD modeling of decreased total T4 in female Sprague Dawley rats from NTP (2019b)



Model run output for Figure A10.21: BMD modeling of decreased total T4 in female Sprague Dawley rats from NTP (2019b).

```
================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_NTP 2019 female totT4_Opt.(d)
Gnuplot Plotting File:
Thu Oct 29 11:09:42 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.85945	-1.85945	-1.85945	-1.85945
rho	1.31752	1.31752	1.31752	1.31752
a	0.378064	0.677915	2.3205	2.3205
b	0.00357207	7.7376e-006	0.0127321	0.0127321
c	0 *	0 *	0.135439	0.135439
d	1 *	2	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-1.55384	-3.5949	-1.86964	-1.87846
rho	0.0787933	-16.4755	1.29137	1.29137
a	2.14448	0.829153	2.27293	2.23463
b	0.0184725	6.02767e-006	0.034085	0.0312502
c	--	--	0.155619	0.161448
d	--	4.26099	--	1.23067

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	0.217422	NA	0.229921	0.229373
rho	0.0635918	NA	0.269636	0.268228
a	0.137424	NA	0.203019	0.205509
b	0.00215864	NA	0.00504129	0.00517153
c	NA	NA	0.0212704	0.0228504
d	NA	NA	NA	0.327459

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.054	10	2.21	0.76

Dose	N	Obs Mean	Obs Std Dev
30.5	10	1.11	0.38
67	10	0.55	0.22
135.1	10	0.33	0.22
237.5	10	0.35	0.28
413.6	9	0.38	0.15

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.054	2.142	0.4738	0.4516
	30.5	1.221	0.4634	-0.7558
	67	0.622	0.4513	-0.5047
	135.1	0.1768	0.4295	1.128
	237.5	0.02667	0.3986	2.565
	413.6	0.001031	0.3507	3.242
3	0.054	0.8292	0.7756	5.63
	30.5	0.8292	0.7756	1.145
	67	0.8292	0.7756	-1.138
	135.1	0.8292	0.7756	-2.035
	237.5	0.8292	0.7756	-1.954
	413.6	0.8292	0.7756	-1.737
4	0.054	2.269	0.6665	-0.2818
	30.5	1.032	0.4008	0.6127
	67	0.5493	0.2667	0.008358
	135.1	0.3729	0.2077	-0.6534
	237.5	0.3543	0.2009	-0.06764
	413.6	0.3537	0.2007	0.3929
5	0.054	2.234	0.6569	-0.1151
	30.5	1.091	0.4135	0.1466
	67	0.5172	0.2554	0.4055
	135.1	0.366	0.2043	-0.557
	237.5	0.3608	0.2024	-0.1686
	413.6			0.2849

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	28.51891	12	-43.03781
A2	46.17054	12	-68.34107
A3	43.71268	8	-71.42537
R	-14.50826	2	33.01651

Model	Log(likelihood)	DF	AIC
2	20.69547	4	-33.39095
3	-14.50826	5	39.01651
4	43.13407	5	-76.26814
5	43.4172	6	-76.26814

Additive constant for all log-likelihoods = -54.22. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	121.4	10	< 0.0001
Test 2	35.3	5	< 0.0001
Test 3	4.916	4	0.2961
Test 4	46.03	4	< 0.0001
Test 5a	116.4	3	< 0.0001
Test 5b	-70.41	1	N/A
Test 6a	1.157	3	0.7633
Test 6b	44.88	1	< 0.0001
Test 7a	0.591	2	0.7442
Test 7b	115.9	1	< 0.0001
Test 7c	0.5663	1	0.4517

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5b is less than .05. Model 3 appears to fit the data better than Model 2.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

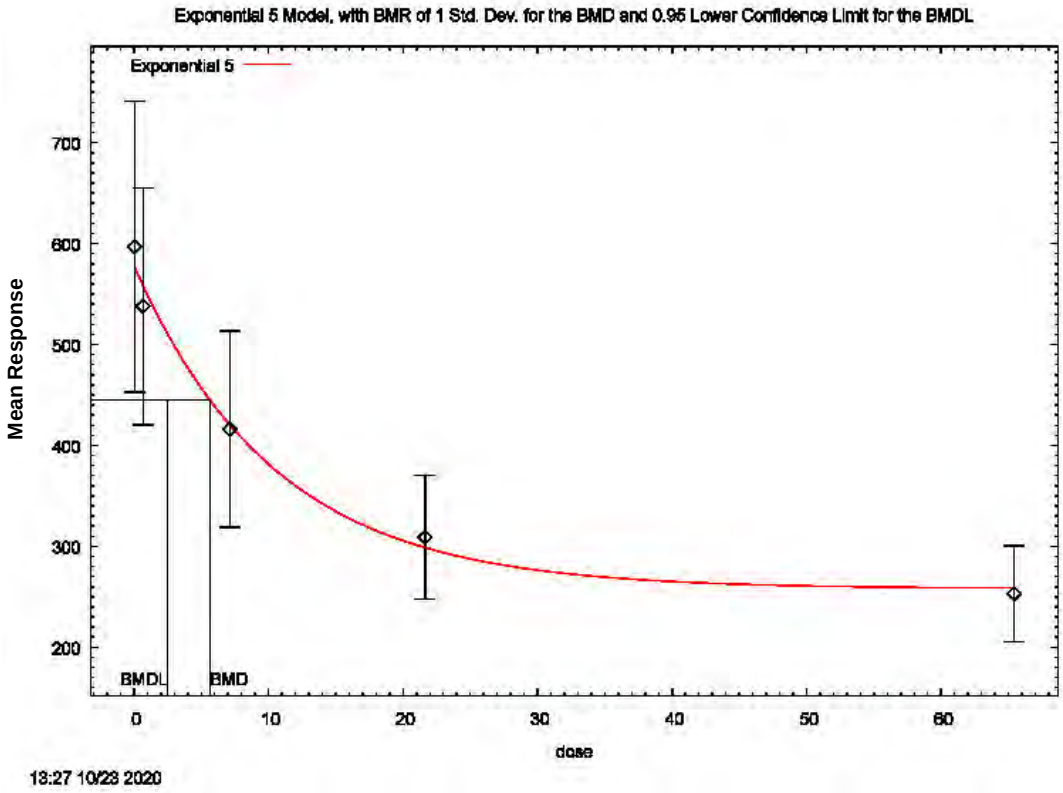
The p-value for Test 7c is greater than .05. Model 5 does not seem to fit the data better than Model 4.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	13.5173	8.90055	23.9262
3	210178	308.041	4.136e+006
4	12.5322	8.19881	22.3109
5	16.1711	8.55386	29.0349

Figure A10.22. BMD modeling of decreased plaque forming cell response in male c57/BL6 mice from Dong et al. (2009) (BMR = 1 standard deviation)



Model run output for Figure A10.22: BMD modeling of decreased plaque forming cell response in male c57/BL6 mice from Dong et al. (2009).

```
================================================================
Exponential Model. (Version: 1.11;  Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/exp_Dong 2009 pfc no high dose_Opt.(d)
Gnuplot Plotting File:
Fri Oct 23 13:28:02 2020
================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the response function by Model:
Model 2: Y[dose] = a * exp{sign * b * dose}
Model 3: Y[dose] = a * exp{sign * (b * dose)^d}
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Model 5: Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

Note: Y[dose] is the median response for exposure = dose;
sign = +1 for increasing trend in data;
sign = -1 for decreasing trend.

Model 2 is nested within Models 3 and 4.
Model 3 is nested within Model 5.
Model 4 is nested within Model 5.
```

Dependent variable = Mean
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

MLE solution provided: Exact

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-5.5662	-5.5662	-5.5662	-5.5662
rho	2.52721	2.52721	2.52721	2.52721
a	320.885	320.885	626.85	626.85
b	0.0118183	0.0118183	0.0562866	0.0562866
c	0 *	0 *	0.384386	0.384386
d	1 *	1	1 *	1

* Indicates that this parameter has been specified

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-7.40745	-7.40747	-5.92711	-5.92711
rho	2.857	2.85701	2.57226	2.57226
a	501.266	501.266	572.107	572.107
b	0.0112284	0.0112284	0.084283	0.084283
c	--	--	0.441593	0.441593
d	--	1	--	1

-- Indicates that this parameter does not appear in model

Std. Err. Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	5.64667	5.85396	4.26621	4.26621
rho	0.943073	0.978063	0.712088	0.712088
a	30.8634	32.0567	39.9535	39.9535
b	0.00164939	0.00178318	0.0245768	0.0245768
c	NA	NA	0.0454404	0.0454405
d	NA	NA	NA	NA

NA - Indicates that this parameter was specified (by the user or because of the model form) or has hit a bound implied by some inequality constraint and thus has no standard error.

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0.048	10	597	202

Dose	N	Obs Mean	Obs Std Dev
0.674	10	538	164
7.132	10	416	136
21.64	10	309	85.4
65.43	10	253	66.4

Estimated Values of Interest

Model	Dose	Est Mean	Est Std Dev	Scaled Res.
2	0.048	501	177.1	1.714
	0.674	497.5	175.3	0.7307
	7.132	462.7	158.1	-0.934
	21.64	393.1	125.3	-2.124
	65.43	240.4	62.06	0.6396
3	0.048	501	177.1	1.714
	0.674	497.5	175.3	0.7307
	7.132	462.7	158.1	-0.934
	21.64	393.1	125.3	-2.124
	65.43	240.4	62.06	0.6396
4	0.048	570.8	181.2	0.4569
	0.674	554.5	174.6	-0.2983
	7.132	427.8	125	-0.2977
	21.64	304.2	80.65	0.1879
	65.43	253.9	63.93	-0.04578
5	0.048	570.8	181.2	0.4569
	0.674	554.5	174.6	-0.2983
	7.132	427.8	125	-0.2977
	21.64	304.2	80.65	0.1879
	65.43	253.9	63.93	-0.04578

Other models for which likelihoods are calculated:
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)
Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-269.4221	6	550.8442
A2	-262.0043	10	544.0086
A3	-262.0841	7	538.1682
R	-286.3701	2	576.7402
2	-268.6484	4	545.2969
3	-268.6484	4	545.2969
4	-262.3839	5	534.7679
5	-262.3839	5	534.7679

Additive constant for all log-likelihoods = -45.95. This constant added to the

above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests
Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs. 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs. 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs. 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	48.73	8	< 0.0001
Test 2	14.84	4	0.005055
Test 3	0.1596	3	0.9838
Test 4	13.13	3	0.004367
Test 5a	13.13	3	0.004367
Test 5b	-6.071e-011	0	N/A
Test 6a	0.5996	2	0.741
Test 6b	12.53	1	0.0004007
Test 7a	0.5996	2	0.741
Test 7b	12.53	1	0.0004007
Test 7c	0	0	N/A

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0.

The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

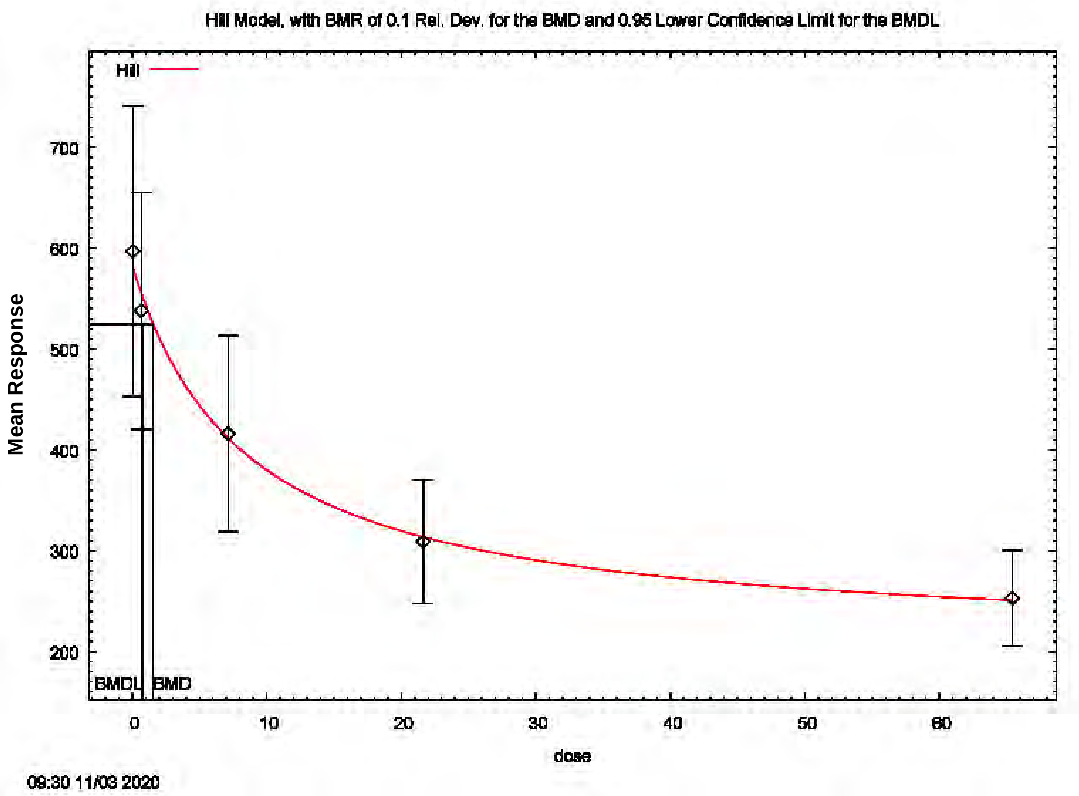
Degrees of freedom for Test 7c are less than or equal to 0.
The Chi-Square test for fit is not valid.

Benchmark Dose Computations:
Specified Effect = 1.000000
Risk Type = Estimated standard deviations from control
Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL	BMDU
2	38.856	24.4058	67.3771
3	38.856	24.4058	67.3771
4	9.98166	5.08531	23.9077
5	9.98166	5.08531	23.9077

Figure A10.23. BMD modeling of decreased plaque forming cell response in male c57/BL6 mice from Dong et al. (2009) (BMR = 0.1 relative deviation)



Model run output for Figure A10.23: BMD modeling of decreased plaque forming cell response in male c57/BL6 mice from Dong et al. (2009).

==
Hill Model. (Version: 2.18; Date: 03/14/2017)
Input Data File: K:/BMD saved files/Chemicals/PFOS/hil_Dong 2009 pfc no high dose_Opt.(d)
Gnuplot Plotting File: K:/BMD saved files/Chemicals/PFOS/hil_Dong 2009 pfc no high dose_Opt.plt
Tue Nov 03 09:30:20 2020
==
BMDS Model Run
~~~~~~

The form of the response function is:
Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Independent variable = Dose
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = exp(lalpha + rho * ln(mean(i)))

Total number of dose groups = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
~~~~~~

Default Initial Parameter Values
lalpha = 9.88224
rho = 0
intercept = 597
v = -344
n = 1.19729
k = 6.65559

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	lalpha	rho	intercept	v	k
lalpha	1	-1	0.34	-0.41	-0.12
rho	-1	1	-0.35	0.42	0.12
intercept	0.34	-0.35	1	-0.76	-0.54
v	-0.41	0.42	-0.76	1	-0.028
k	-0.12	0.12	-0.54	-0.028	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
lalpha	-5.75113	4.23737	-14.0562	2.55397
rho	2.54262	0.707137	1.15666	3.92858
intercept	582.785	43.8176	496.904	668.666
v	-374.609	50.0391	-472.684	-276.534
n	1	NA		
k	8.47933	4.00913	0.621581	16.3371

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0.048	10	597	581	202	184	0.28
0.674	10	538	555	164	174	-0.313
7.132	10	416	412	136	119	0.116
21.64	10	309	314	85.4	84.1	-0.175
65.43	10	253	251	66.4	63.4	0.092

Model Descriptions for likelihoods calculated
Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2
Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))

Model A3 uses any fixed variance parameters that
were specified by the user
Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-269.422105	6	550.844210
A2	-262.004309	10	544.008618
A3	-262.084125	7	538.168249
fitted	-262.335552	5	534.671105
R	-286.370083	2	576.740166

Explanation of Tests
Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs. A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	48.7315	8	<.0001
Test 2	14.8356	4	0.005055
Test 3	0.159631	3	0.9838
Test 4	0.502856	2	0.7777

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

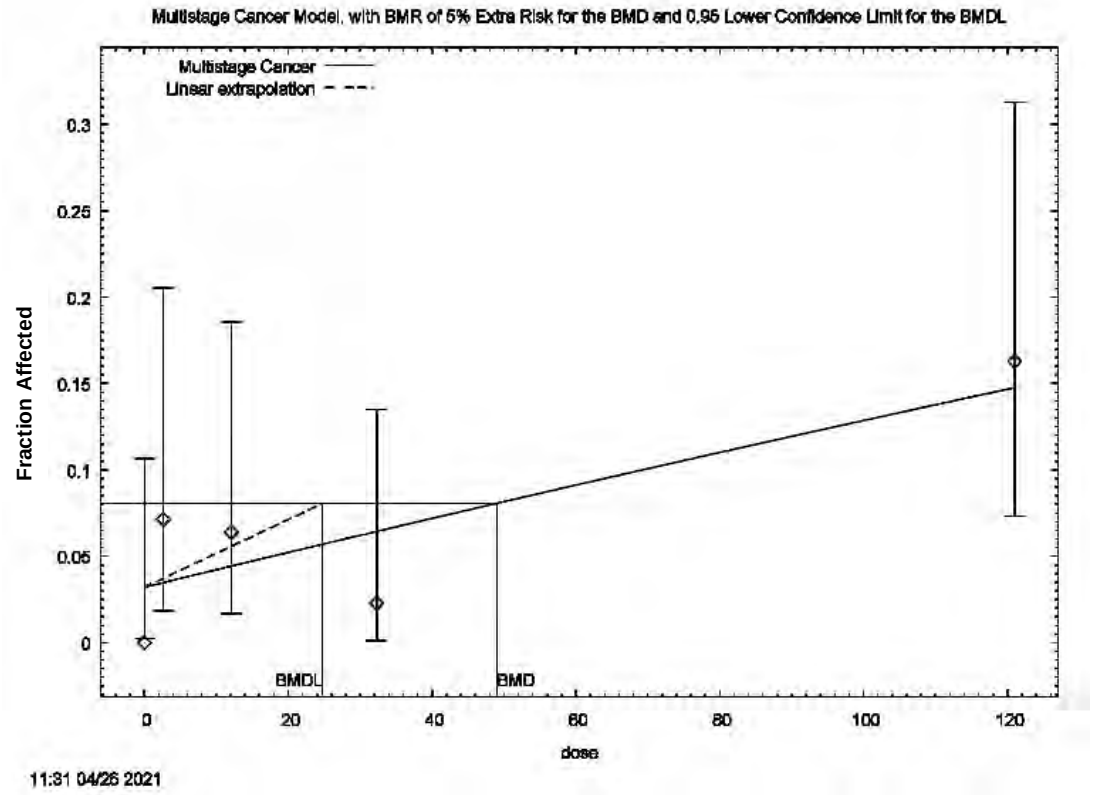
The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Relative deviation
Confidence level = 0.95
BMD = 1.56217
BMDL = 0.746895
BMDU = 6.44306

Benchmark Dose Analysis Results for Cancer Endpoints

Perfluorooctane Sulfonic Acid

Figure A10.24. BMD modeling of hepatocellular tumor incidence in male Sprague Dawley rats from Butenhoff et al. (2012b)



Model Run Output for Figure A10.24: BMD modeling of hepatocellular tumor incidence in male Sprague Dawley rats from Butenhoff et al. (2012b).

```
 ================================================================
Multistage Model. (Version: 3.4;  Date: 05/02/2014)
Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos male serum
hep_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos male serum
hep_Opt.plt
Mon Apr 26 11:30:59 2021
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]

The parameter betas are restricted to be positive
```

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0288539
Beta(1) = 0.00115644

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.46
Beta(1)	-0.46	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0321432	0.0165415	-0.000277513	0.0645638
Beta(1)	0.00104566	0.000531353	4.23e-006	0.00208709

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-45.8404	5			
Fitted model	-48.8849	2	6.08895	3	0.1074
Reduced model	-51.9101	1	12.1394	4	0.01634

AIC: 101.77

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0140	0.0322	1.318	0.000	41.000	-1.167
2.6400	0.0348	1.462	3.000	42.000	1.295
12.1000	0.0443	2.083	3.000	47.000	0.650
32.3000	0.0643	2.829	1.000	44.000	-1.124
121.0000	0.1472	6.328	7.000	43.000	0.289

Chi^2 = 4.81 d.f. = 3 P-value = 0.1864

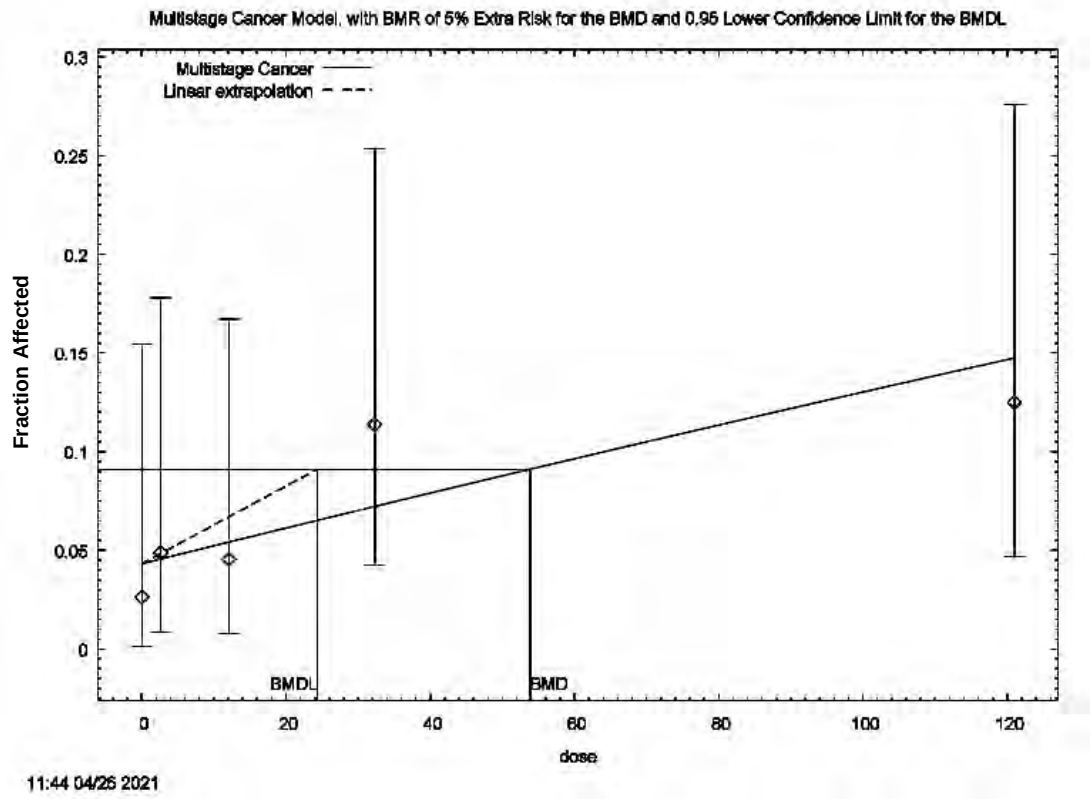
Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 49.0534
BMDL = 24.7835

BMDU = 171.534

Taken together, (24.7835, 171.534) is a 90 % two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00201747

Figure A10.25. BMD modeling of pancreatic islet cell carcinoma incidence in male Sprague Dawley rats from Butenhoff et al. (2012b)



Model Run Output for Figure A10.25: BMD modeling of pancreatic islet cell carcinoma incidence in male Sprague Dawley rats from Butenhoff et al. (2012b).

```
================================================================
Multistage Model. (Version: 3.4;  Date: 05/02/2014)
Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos male serum
panc_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos male serum
panc_Opt.plt
Mon Apr 26 11:44:10 2021
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
```

-beta1*dose^1)]

The parameter betas are restricted to be positive
Dependent variable = Effect
Independent variable = Dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0476913
Beta(1) = 0.000791577

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.56
Beta(1)	-0.56	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0432278	0.0206316	0.00279061	0.083665
Beta(1)	0.000952916	0.000613586	-0.00024969	0.00215552

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-51.4005	5			
Fitted model	-52.1608	2	1.52043	3	0.6776
Reduced model	-53.8129	1	4.82475	4	0.3058

AIC: 108.322

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0140	0.0432	1.643	1.000	38.000	-0.513
2.6400	0.0456	1.871	2.000	41.000	0.097
12.1000	0.0542	2.385	2.000	44.000	-0.256
32.3000	0.0722	3.178	5.000	44.000	1.061
121.0000	0.1474	5.897	5.000	40.000	-0.400

Chi^2 = 1.62 d.f. = 3 P-value = 0.6540

Benchmark Dose Computation

Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 53.8277
BMDL = 24.3624
BMDU = 657.141

Taken together, (24.3624, 657.141) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 0.00205235

Model Run Output for BMD modeling of multisite tumors: hepatocellular adenoma and pancreatic islet cell carcinoma in male Sprague Dawley rats from Butenhoff et al. (2012b).

```
================================================================
MS_COMBO. (Version: 1.10;  Date: 01/29/2017)
Input Data File: K:\BMD saved files\Chemicals\PFOS\butenhoff 2012 pfos multi.(d)
Gnuplot Plotting File:  K:\BMD saved files\Chemicals\PFOS\butenhoff 2012 pfos multi.plt
Mon Apr 26 11:48:44 2021
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose
Data file name = Butenhoffpfosmaleserumhep.dax

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0288539
Beta(1) = 0.00115644

Asymptotic Correlation Matrix of Parameter Estimates
                Background   Beta(1)
 Background     1            -0.57
```

	Background	Beta(1)
Beta(1)	-0.57	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0321432	*	*	*
Beta(1)	0.00104566	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-45.8404	5			
Fitted model	-48.8849	2	6.08895	3	0.1074
Reduced model	-51.9101	1	12.1394	4	0.01634

AIC: 101.77

Log-likelihood Constant 40.11446893476365

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0140	0.0322	1.318	0.000	41.000	-1.167
2.6400	0.0348	1.462	3.000	42.000	1.295
12.1000	0.0443	2.083	3.000	47.000	0.650
32.3000	0.0643	2.829	1.000	44.000	-1.124
121.0000	0.1472	6.328	7.000	43.000	0.289

Chi^2 = 4.81 d.f. = 3 P-value = 0.1864

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 49.0534
BMDL = 24.7835
BMDU = 171.534

Taken together, (24.7835, 171.534) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00201747

==========

MS_COMBO. (Version: 1.10; Date: 01/29/2017)
Input Data File: K:\BMD saved files\Chemicals\PFOS\butenhoff 2012 pfos multi.(d)
Gnuplot Plotting File: K:\BMD saved files\Chemicals\PFOS\butenhoff 2012 pfos multi.plt
Mon Apr 26 11:48:44 2021

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BMDS_Model_Run

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The form of the probability function is:
~~~~~~~~~~

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose
Data file name = Butenhoffpfosmaleserumpanc.dax

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0476913
Beta(1) = 0.000791577

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.63
Beta(1)	-0.63	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0432278	*	*	*
Beta(1)	0.000952916	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-51.4005	5			
Fitted model	-52.1608	2	1.52043	3	0.6776
Reduced model	-53.8129	1	4.82475	4	0.3058

AIC: 108.322

Log-likelihood Constant 44.494124583256585

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0140	0.0432	1.643	1.000	38.000	-0.513

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
2.6400	0.0456	1.871	2.000	41.000	0.097
12.1000	0.0542	2.385	2.000	44.000	-0.256
32.3000	0.0722	3.178	5.000	44.000	1.061
121.0000	0.1474	5.897	5.000	40.000	-0.400

Chi^2 = 1.62 d.f. = 3 P-value = 0.6540

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 53.8277
BMDL = 24.3624
BMDU = 3.61147e+276

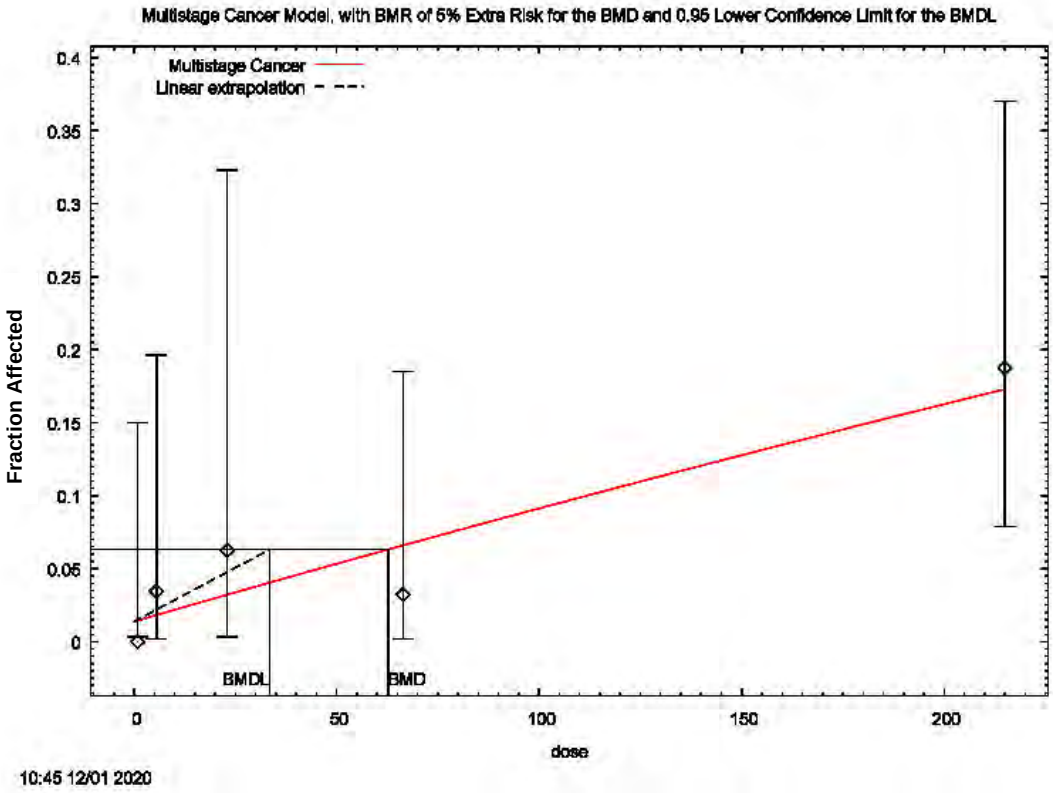
Taken together, (24.3624, 3.61147e+276) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00205235
**** Start of combined BMD and BMDL Calculations.****
Combined Log-Likelihood 101.04566952341376
Combined Log-likelihood Constant 84.608593518020228

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 25.6649
BMDL = 14.7461
BMDU = 64.2098

Multistage Cancer Slope Factor = 0.00339073

Figure A10.26. BMD modeling of hepatocellular tumor incidence in female Sprague Dawley rats from Butenhoff et al. (2012b)



Model Run Output for Figure A10.26: BMD modeling of hepatocellular tumor incidence in female Sprague Dawley rats from Butenhoff et al. (2012b).

```
================================================================
Multistage Model. (Version: 3.4;  Date: 05/02/2014)
Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos female serum
hep_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff pfos female serum
hep_Opt.plt
Tue Dec 01 10:45:30 2020
================================================================
BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]
The parameter betas are restricted to be positive
Dependent variable = Effect
Independent variable = Dose
Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1
```

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0145684
Beta(1) = 0.000858241

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.44
Beta(1)	-0.44	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval	
			Lower Conf. Limit	Upper Conf. Limit
Background	0.0137622	0.0167901	-0.0191458	0.0466703
Beta(1)	0.000818058	0.000367259	9.82424e-005	0.00153787

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-27.9507	5			
Fitted model	-29.0869	2	2.27239	3	0.5178
Reduced model	-33.1343	1	10.3672	4	0.03468

AIC: 62.1738

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.8400	0.0144	0.404	0.000	28.000	-0.640
5.4900	0.0182	0.527	1.000	29.000	0.657
23.0000	0.0321	0.514	1.000	16.000	0.688
66.4000	0.0659	2.043	1.000	31.000	-0.755
215.0000	0.1728	5.530	6.000	32.000	0.220

Chi^2 = 1.93 d.f. = 3 P-value = 0.5862

Benchmark Dose Computation
Specified effect = 0.05
Risk Type = Extra risk
Confidence level = 0.95
BMD = 62.7013
BMDL = 33.4769
BMDU = 168.724

Taken together, (33.4769, 168.724) is a 90% two-sided confidence interval for the BMD

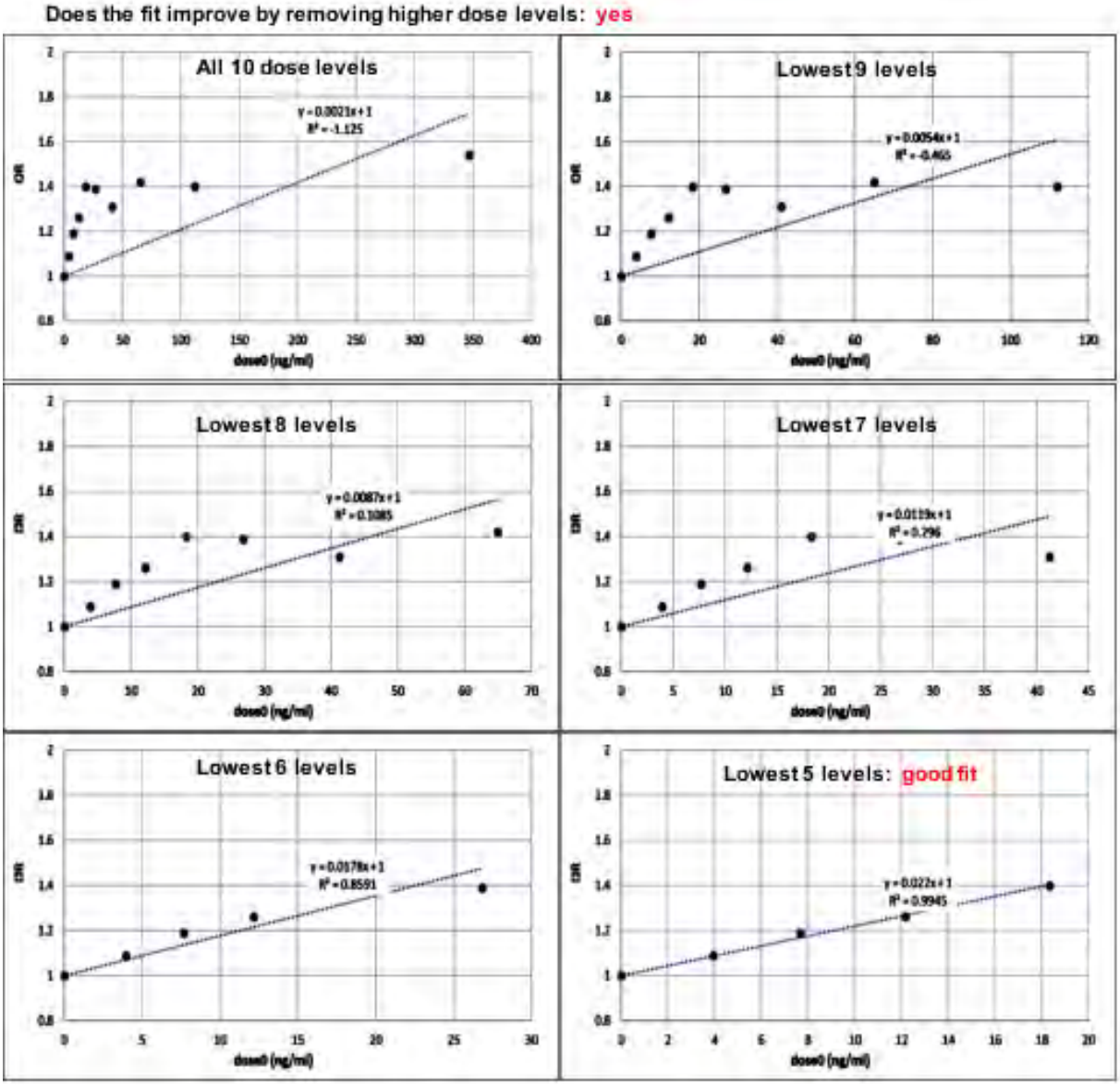
Cancer Slope Factor = 0.00149357

APPENDIX 11. GENERALIZED LEAST SQUARES FOR TREND IN STATA

Stata output of ORs for high ALT from Gallo et al. (2012).

"No intercept model" used: lowest dose subtracted out
Poor model fit when all ten dose levels are used. Fit improved substantially when only the lowest five doses were used.

Figure A11.1. Dose-response graphs plotting all ten dose levels in comparison with plotting only the lowest nine, eight, seven, six or five doses.



Results:
The regression model between PFOA and lnOR using GLST in Stata

Generalized least- squares regression — Number of obs = 4
Goodness-of-fit chi2(3) = 0.29 — Model ch2(1) = 25.71
Prob > chi2 = 0.9621 — Prob >chi2 = 0.0000

logrr |	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
dose0 |	.017	0.00354	5.07	0.000	.0110127	0.248891

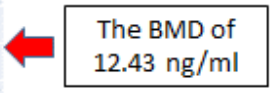
This regression coefficient was then used to calculate the BMD01 and BMDL01 using the following equations:

$OR = \exp^{(dose \times slope)}$ (eq 1)
lnOR = dose x slope (eq 2)
dose = lnOR / slope (eq 3)
BMD01* = 0.1181 / 0.01795 = 6.58 ng/ml
BMDL01* = 0.1181 / 0.024889 = 4.74 ng/ml

*Since the lowest dose (5.85 ng/ml) was subtracted out (i.e. no intercept model), this dose was added back in:

BMD = 5.85 + 6.58 = 12.43 ng/ml
BMDL = 5.85 + 4.74 = 10.59 ng/ml

Decile	Dose (ng/ml)	Log10 dose	N	OR	CIL	CIU	Logrr	Percent with high ALT	Percent over baseline
1	5.853917	0.767447	4645	1			0	8.85%	0.0%
2	9.807348	0.991552	4645	1.09	0.94	1.26	0.086178	9.6%	0.7%
3	13.53996	1.131617	4645	1.19	1.03	1.37	0.173953	10.4%	1.5%
4	18.03516	1.25612	4645	1.26	1.09	1.45	0.231112	10.9%	2.1%
5	24.19555	1.383735	4645	1.4	1.22	1.62	0.336472	12.0%	3.1%
6	32.69365	1.514463	4645	1.39	1.21	1.6	0.329304	11.9%	3.0%
7	47.11991	1.673204	4645	1.31	1.14	1.52	0.270027	11.3%	2.4%
8	70.89587	1.850621	4645	1.42	1.23	1.64	0.350657	12.1%	3.3%
9	117.927	2.071613	4645	1.4	1.21	1.62	0.336472	12.0%	3.1%
10	353.0504	2.547837	4645	1.54	1.33	1.78	0.431782	13.0%	4.2%



APPENDIX 12. ALTERNATIVE METHODS FOR CALCULATING THE CANCER SLOPE FACTOR AND CANCER HEALTH-PROTECTIVE CONCENTRATION

Generalized least-squares for trend (glst): A potential disadvantage of the method described in the main PHG document to calculate the CSFs is that it assumes that the relative risk (RR) estimates for the different exposure categories are independent. However, they are not independent because for each study they rely on a common reference group. This lack of independence can lead to an underestimate of the variance of the dose-response slope derived from these data (Greenland and Longnecker, 1992). That is, it can lead to an artificially narrow CI around b. It could also lead to a less efficient estimator of b. The glst method is a way of correcting for this lack of independence. However, a potential weakness of the glst model is that it assumes a linear relationship between the exposure and the logarithm of the RR, a relationship that may or may not fit the data.

The effect of this lack of independence was evaluated by running dose-response analyses of the Shearer et al. (2021) and Vieira et al. (2013) data with and without the glst correction. The glst command in Stata/IC (version 15.1) was used to provide dose-response and variance estimates corrected for the lack of independence, and the variance-weighted least squares (vwls) command (also in Stata/IC) was used to provide uncorrected estimates of these variables. The vwls method is similar to the glst method in that it also assumes a linear-log relationship between exposure and RR, however it does not correct for lack of independence in the data. Printouts from these analyses are shown in Figure A12.1. As seen, the correction for lack of independence had only a small effect on the dose-response slopes (labeled “Coef.” in the printouts) or their upper 95% CI. As such, these findings suggest that the lack of independence in the Shearer et al. (2021) and Vieira et al. (2013) results likely had only small effects on the CSF calculations.

Figure A12.1. Regression coefficients and their 95% CIs between the log of the RCC or kidney cancer ORs and serum PFOA concentrations using data from Shearer et al. (2021) and Vieira et al. (2013): adjusted (glst) and unadjusted (vwls) for OR dependence

Shearer et al. (2021):

Adjusted:

```
. glst logrr d0, se(se) cov(n case) cc

Generalized least-squares regression                Number of obs   =       3
Goodness-of-fit chi2(2)    =    0.84                Model chi2(1)   =    8.39
Prob > chi2                =  0.6568                Prob > chi2     =  0.0038
------------------------------------------------------------------------------
       logrr |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          d0 |   .0582026   .0200985     2.90   0.004     .0188103    .0975949
------------------------------------------------------------------------------
```

Unadjusted:

```
. vwls logrr d0 if logrr != 0, sd(se) nocons

Variance-weighted least-squares regression      Number of obs     =          3
Goodness-of-fit chi2(2)    =    0.44            Model chi2(1)     =       9.06
Prob > chi2                =  0.8018            Prob > chi2       =     0.0026
------------------------------------------------------------------------------
       logrr |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          d0 |   .0646938   .0214912     3.01   0.003     .0225718    .1068157
------------------------------------------------------------------------------
```

Vieira et al. (2013):

Adjusted:

```
. glst logrr dose0, se(se) cov(n case) cc

Generalized least-squares regression                Number of obs   =       3
Goodness-of-fit chi2(2)    =    0.58                Model chi2(1)   =    9.53
Prob > chi2                =  0.7478                Prob > chi2     =  0.0020
------------------------------------------------------------------------------
       logrr |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       dose0 |   .0108576   .0035177     3.09   0.002     .0039631    .0177521
------------------------------------------------------------------------------
```

Unadjusted:

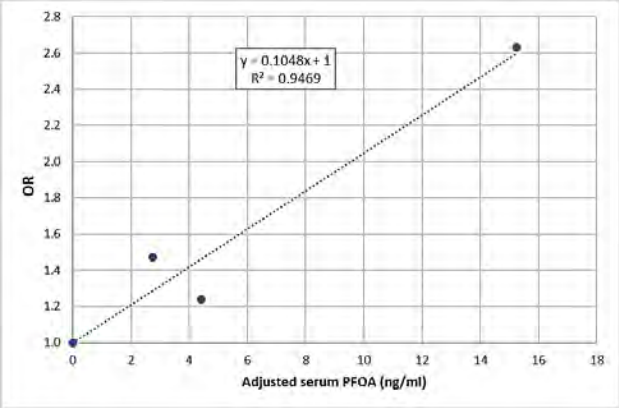
```
. vwls logrr dose0 if logrr != 0, sd(se) nocons

Variance-weighted least-squares regression      Number of obs     =          3
Goodness-of-fit chi2(2)    =    0.58            Model chi2(1)     =       9.42
Prob > chi2                =  0.7500            Prob > chi2       =     0.0021
------------------------------------------------------------------------------
       logrr |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       dose0 |   .0106432   .0034669     3.07   0.002     .0038482    .0174382
------------------------------------------------------------------------------
```

As noted above, another difference between the glst model and the linear model used for the final CSF calculations is that the former assumes a linear relationship between dose and the log of the RR estimates. Plotting both the linear-linear model (used in Equation 1) and the linear-log model (used in the glst method) shows that both provide a reasonably good fit (both visually and based on R^2 values), but with the linear model being slightly better for both studies (Figure A12.2). This suggests that the linear-linear model was a good choice for the CSF calculations.

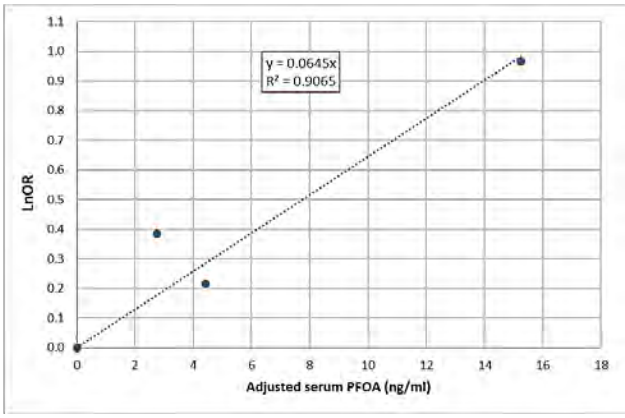
Figure A12.2. Plots, unweighted regression coefficients, and coefficients of determination (R^2) of RCC or kidney cancer ORs or natural log ORs (lnOR) and serum PFOA concentrations using data from Shearer et al. (2021) and Vieira et al. (2013)

Shearer et al. (2021):

OR (linear model, Equation 1)

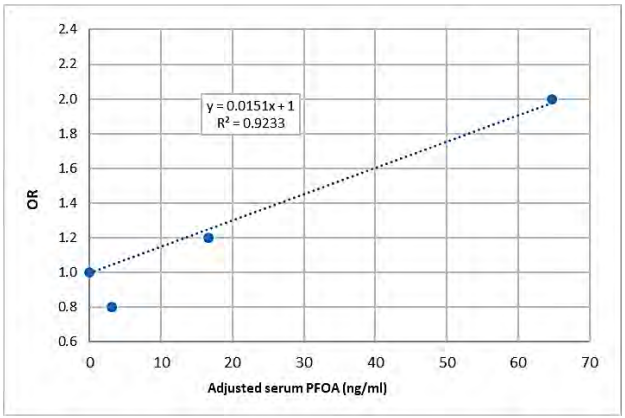


LnOR (glst model)

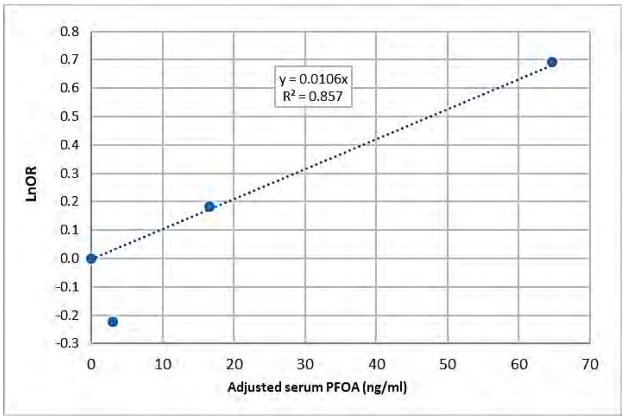


Vieira et al. (2013):

OR (linear model, Equation 1)



LnOR (glst model)



Lifetime, cumulative, or peak exposure: Exposure assessment in the Shearer et al. (2021) study was based on a single serum measurement collected in adulthood, while exposure assessment in the Vieira et al. (2013) study was based on modeled estimates of a single year of exposure, also in adulthood. Because of the long half-life of PFOA in serum, these measurements are likely a good indication of several years of exposure. Regardless, the use of a single measurement at a single point in time or for a relatively short period of time raises concerns about the importance of even longer-term cumulative exposure, and concerns about whether important time windows of exposure (e.g., childhood exposures) might have been missed.

Currently, little information is available on the relative cancer risks from longer-term lower PFOA exposures (e.g., cumulative exposure) versus shorter-term higher PFOA exposures (e.g., peak exposure). In addition, information from human studies on the most important time window (e.g., latency period) or life stage of exposure are not currently available. Despite this, several factors suggest that the exposure measurements in the Shearer et al. (2021) and Vieira et al. (2013) studies are good metrics for assessing the true cancer risks of PFOA. The first factor is that in animal studies, cancer risks from PFOA were not increased when exposure occurred perinatally compared to when it occurred later in life (NTP, 2020). This suggests that the Shearer et al. (2021) and Vieira et al. (2013) studies did not underestimate cancer risks by not including earlier life exposures. The second factor is that the Barry et al. (2013) study of PFOA and kidney cancer examined various exposure lag periods and found that the model using unlagged exposures provided a better fit than the models using lag periods of 10 to 20 years. This finding suggests that the cancer latency of PFOA is not several decades long, and that important latency effects were not missed in either study.

The third factor is that RR estimates for PFOA and kidney cancer in the two population-based studies in the C8 area, Barry et al. (2013) and Vieira et al. (2013), were similar regardless of whether the exposure metric was cumulative exposure or an indicator of lifetime peak exposure. Table A.X.1 shows the RR estimates for kidney cancer in these two studies. As shown, the RR estimates in the higher exposure categories of the C8 studies were all near 2.0, regardless of whether the exposure metric was cumulative exposure over a number of years or an estimate of the lifetime peak. The fact that the RRs for cumulative exposure are not markedly higher suggests that the peak exposure metrics OEHHA used in its CSF calculations are a good way to assess the true cancer risks of PFOA.

Table A12.1. Relative risk estimates for modeled serum PFOA and kidney cancer in the C8 population-based studies

Barry et al. (2013)			**Vieira et al. (2013)**					
Cumulative exposure Exposure window: 1952-2004			Cumulative exposure Exposure window: 1986-2005			Single year average Exposure window: 1986-1995		
Category	%	RR (95% CI)	Category	%	RR (95% CI)	Category	%	RR (95% CI)
Quartile 1	25%	1.00	Tertile 1	33%	0.8 (0.4-1.5)	Tertile 1	33%	0.8 (0.4-1.5)
Quartile 2	25%	1.34 (0.71-2.52)	Tertile 2	33%	1.2 (0.7-2.0)	Tertile 2	33%	1.2 (0.7-2.0)
Quartile 3	25%	1.95 (1.03-3.70)	Tertile 3*	23%	2.0 (1.3-3.2)	Tertile 3*	23%	2.0 (1.3-3.2)
Quartile 4	25%	2.04 (1.07-3.88)	>90th pct	10%	2.1 (1.1-4.2)	>90th pct	10%	2.0 (1.0-3.9)

Abbreviations: %, percentage of participants in each exposure category; pct, percentile; RR, relative risk estimate
* Tertile 3 excluding the upper 10th percentile

US EPA Benchmark Dose Software (BMDS): Categorical data from case-control studies cannot be used in the US EPA BMDS since these models are based on cancer risk, and the information needed to calculate risks is not available from the Shearer et al. (2021) or Vieira et al. (2013) publications. More specifically, although the number of RCC or kidney cancer cases in each exposure category is known, the exact number of people that generated these cases in each exposure category is not. These denominators could be estimated using the total number of participants in the underlying cohorts (e.g., the total number of people in the screening arm of the PLCO trial) and percentages of controls in each exposure category. However, entering

these estimates into the BMDS would lead to an artificially narrow 95% CI around the regression slope, and thus an artificially elevated BMDL.

One advantage of the BMDS is that the fit of a number of different dose-response models can be assessed (e.g., logistic, multi-stage, Weibull), allowing selection of the best fitting model. However, as discussed above, the linear model used in the above calculations provides a very good fit to the Shearer et al. (2021) and Vieira et al. (2013) data and it is unlikely that a different model will provide a fit that is substantially better.

APPENDIX 13. CANCER HEALTH-PROTECTIVE CONCENTRATIONS USING DIFFERENT VALUES FOR BASELINE RISK

Table A13.1. The effect on cancer health protective concentrations of using lower values of baseline risk (R_O)

Reduction in R_O	0%	5%	10%	15%	20%	25%
R_O Vieira	0.0202	0.0192	0.0182	0.0172	0.0162	0.0152
R_O Shearer	0.0182	0.0173	0.0164	0.0155	0.0146	0.0137
b Vieira	0.0146	0.0146	0.0146	0.0146	0.0146	0.0146
b Shearer	0.0980	0.0980	0.0980	0.0980	0.0980	0.0980
CSF_{serum} Vieira	0.00029	0.00028	0.00027	0.00025	0.00024	0.00022
CSF_{serum} Shearer	0.00178	0.00169	0.00160	0.00152	0.00143	0.00134
CF (ml/kg-day)	0.28	0.28	0.28	0.28	0.28	0.28
CSF_{intake} Vieira	0.00105	0.00100	0.00095	0.00090	0.00084	0.00079
CSF_{intake} Shearer	0.00637	0.00605	0.00573	0.00541	0.00509	0.00478
CSF_{intake} geometric mean	0.00259	0.00246	0.00233	0.00220	0.00207	0.00194
10^{-6} risk	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001
DWI (L/kg-day)	0.053	0.053	0.053	0.053	0.053	0.053
C (ng/L)	0.00729	0.00767	0.00810	0.00857	0.00911	0.00972
$C_{rounded}$ (ng/L)	0.007	0.008	0.008	0.009	0.009	0.010

Abbreviations: b, regression slope between serum PFOA (ng/ml) and RCC relative risk; C, cancer health protective concentration; CSF_{intake} cancer slope factor or excess RCC risk per ng/kg-day intake of PFOA; CSF_{serum}, cancer slope factor or excess RCC risk per ng/ml serum PFOA; DWI, drinking water intake; R_O, baseline risk (i.e., RCC risk in the "unexposed" group)